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Regulation of adiponectin receptor expression in human liver and a hepatocyte cell line

Akiko Shimizu[a], Toshinari Takamura[a,*], Naoto Matsuzawa[a], Seiji Nakamura[a], Satoko Nabemoto[a], Yumie Takeshita[a], Hirofumi Misu[a], Seiichiro Kurita[a], Masaru Sakurai[a], Masayoshi Yokoyama[a], Yoh Zen[b], Motoko Sasaki[b], Yasuni Nakanuma[b], Shuichi Kaneko[a]

[a]*Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa 920-8641, Japan*
[b]*Department of Human Pathology, Kanazawa University Graduate School of Medical Science, Kanazawa, Ishikawa 920-8641, Japan*

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is closely associated with obesity. An adipocyte-derived hormone, adiponectin, may play a role in the pathophysiology of NAFLD through insulin-sensitizing and antifibrotic effects. We found that hepatic expression of adiponectin receptor AdipoR2, but not AdipoR1, was down-regulated in 14 patients with NAFLD compared with 7 patients with a normal liver ($P < .05$). To investigate the significance of the adiponectin system in obesity and NAFLD, we examined the regulation of AdipoR2 expression in a nonmalignant human hepatocyte cell line, the THLE-5b cells. Insulin down-regulated the levels of AdipoR2 messenger RNA (mRNA) and protein, whereas an adipocytokine, tumor necrosis factor α, up-regulated them. A thiazolidinedione, pioglitazone, up-regulated the expression of AdipoR2 mRNA and protein in THLE-5b cells. The AdipoR2 mRNA level was decreased in fatty THLE-5b cells induced by coincubating with fatty acids. These findings suggest that down-regulation of AdipoR2 in the liver caused by hyperinsulinemia and steatosis may play a role in the development of NAFLD.

1. Introduction

Obesity and insulin resistance are closely associated with nonalcoholic fatty liver disease (NAFLD) [1], which ranges from simple fatty liver to fibrotic nonalcoholic steatohepatitis (NASH). The link between obesity and NAFLD is visceral adiposity, which provides an immediate source of free fatty acids (FFA), [2] and tumor necrosis factor (TNF) α [3] via the portal vein [2]. In addition, adipose tissue is considered a hormonally active system that plays a role in the control of whole-body metabolism [4,5].

Adiponectin is an adipocyte-derived hormone that has insulin-sensitizing effects [6-8] and alleviates alcoholic and nonalcoholic fatty liver disease [9]. Plasma adiponectin levels are decreased in patients with obesity, type 2 diabetes mellitus [10], and NAFLD [11,12].

Recently, 2 receptors for adiponectin—AdipoR1 and AdipoR2—were identified. AdipoR1 is expressed ubiquitously, most abundantly in skeletal muscle, whereas AdipoR2 is predominantly expressed in the liver [13]. In mice, AdipoR1 and AdipoR2 messenger RNA (mRNA) levels in skeletal muscle and liver are increased by fasting and decreased by refeeding [14], suggesting that various nutritional states may regulate adiponectin action not only by altering adiponectin levels but also by altering the expression of adiponectin receptors. However, in vivo and in vitro evidence of regulated expression of AdipoRs in human hepatocytes is still lacking.

Here we assayed the expression of AdipoRs in the livers of patients with NAFLD by using a real-time polymerase chain reaction (PCR) method and found that AdipoR2, but not AdipoR1, is down-regulated in the liver of patients with NAFLD. We further addressed the mechanisms underlying down-regulation of hepatic AdipoR2 expression in patients with NAFLD by using a nonmalignant human hepatocyte cell line, the THLE-5b cells.

* Corresponding author. Tel.: +81 76 265 2233; fax: +81 76 234 4250.
E-mail address: ttakamura@m-kanazawa.jp (T. Takamura).

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2. Materials and methods

2.1. Patients

The subjects included 21 patients with clinically suspected NAFLD in whom a liver biopsy was indicated recruited between 2000 and 2003 at Kanazawa University Hospital, Japan. Informed consent was obtained from all subjects, both for the study and for histologic examination for liver diseases including NAFLD, often complicated with insulin resistance. The study protocol was approved by the relevant ethics committee and was carried out in accordance with the Declaration of Helsinki.

All subjects tested negative for hepatitis B and C viruses. Alcohol consumption was less than 20 g/d. The homeostasis model assessment of insulin resistance (HOMA-IR) method, which was used as a conventional index for insulin resistance, was calculated using the formula HOMA-IR = [fasting insulin (in microunits per milliliter) × fasting plasma glucose (in millimoles per liter)]/22.5 [15]. The quantitative insulin sensitivity check index (QUICKI), which was used as a conventional index for insulin resistance, was calculated using the formula QUICKI = 1/[(log(fasting plasma glucose) + log (immunoreactive insulin)] [16].

All liver biopsy specimens were immediately frozen in liquid nitrogen, stored at −80°C until use, and examined using hematoxylin-eosin and silver reticulin stain.

Each biopsy was scored histologically using the standard criteria for steatosis and grade and stage of NASH according to the modified criteria of Brunt et al [17] and Neuschwander-Tetri and Caldwell [18]. The liver steatosis was scored from 0 to 3 (0, none; 1, <33%; 2, 33%-66%; 3, >66% of the hepatocytes in the biopsy involved) [17]. The grade for NASH was based on the combination of features of hepatocellular steatosis, ballooning and disarray, and inflammation (acinar and portal) [18]. The severity of fibrosis was scored from 0 to 4, reflecting both the patterns of fibrosis and the increase in connective tissue deposition and architectural remodeling [18]. Patients with histologically typical livers were categorized into 3 groups: patients with a normal liver (n = 7), patients with a fatty liver (n = 7), and patients with NASH (n = 7). *Normal liver* was defined as a steatosis score ≤1 and a fibrosis score ≤1, *fatty liver* was defined as a steatosis score >2 and a fibrosis score ≤2, and *NASH* was defined as a fibrosis score ≥3 with the additional presence of ballooned hepatocytes [18].

2.2. Cell lines and culture conditions

We used a simian virus 40 large T-antigen–immortalized normal human hepatocyte cell line, the THLE-5b cells [19]. The third- to fourth-passage THLE-5b cells were seeded onto 6-well dishes (1.0×10^6 cells per well) and grown to confluence in the conditioned Pasadena Foundation for Medical Research Medium 4 (BioSource International, Camarillo, CA) containing 0.4 mmol/L calcium, 126 mg/dL glucose, and 896.3 mg/L amino acids supplemented with 10% fetal bovine serum (Gibco, Grand Island, NY), 100 U/mL penicillin, and 0.1 mg/mL streptomycin (Gibco). The cells were cultured at 37°C under a humidified atmosphere containing 5% CO_2, and the medium was changed 3 times a week. All studies were conducted using 80% to 90% confluent THLE-5b cells. The THLE-5b cells were cultured in medium with or without the agents recombinant human TNF-α (Pierce Biotechnology, Rockford, IL), insulin (Sigma, St. Louis, MO), and pioglitazone for 6 hours. Pioglitazone, the thiazolidinedione compound, was supplied by Takeda Chemical Industries, Osaka, Japan. A concentrated stock of pioglitazone was prepared at 1 mmol/L in ethanol. The long-chain fatty acids (2:1 oleate-palmitate) were purchased from Sigma and were dissolved with methanol and then diluted with the conditioned Pasadena Foundation for Medical Research Medium 4 in the presence of 2% FFA-free bovine serum albumin. The cell culture supernatant of each well was collected at the indicated times and stored at −20°C until assayed.

2.3. Real-time quantitative PCR

Total RNA was extracted using an RNeasy mini kit (QIAGEN, West Sussex, UK) according to the manufacturer's recommendations. The RNA was dissolved in 50 μL RNase-free water. Concentration of RNA was determined at 260 nm with a spectrophotometer. The complementary DNA (cDNA) was synthesized from 100 ng of total RNA as described previously [20] using a High-Capacity cDNA Archive Kit (Applied Biosystems, Foster City, CA). The cDNA was then used as a template. Real-time quantitative PCR was performed using an ABI Prism 7700 Sequence Detection System (Applied Biosystems) as described previously [21]. The set of primers and a TaqMan probe for AdipoR1 and AdipoR2 were proprietary to Applied Biosystems. To control for variation in the amount of DNA available for PCR in the different samples, AdipoR gene expression was normalized in relation to the expression of an endogenous control, the human β-actin (Human β-actin TaqMan Control Reagent Kit; Applied Biosystems). The PCR conditions were 1 cycle of 50°C for 2 minutes and 95°C for 10 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute.

2.4. Western blot analysis

The THLE-5b cells were homogenized in lysis buffer containing 20 mmol/L Tris (pH 7.5), 5 mmol/L EDTA, 1% NP-40, and a protease inhibitor cocktail (Pierce Biotechnology). The homogenated protein(15 μg per lane) precipitates or the lysates were separated by 4% to 20% gradient sodium dodecyl sulfate polyacrylamide gels (Daiichi Pure Chemicals, Tokyo, Japan) and resolved at 130 V over 2 hours. Proteins were transferred to polyvinylidene difluoride membranes (Millipore, Billerica, MA) using a Transblot apparatus (Bio-Rad Laboratories, Hercules, CA). The membranes were blocked in a buffer containing 5% nonfat milk, 50 mmol/L Tris-HCl (pH 7.6), 150 mmol/L NaCl, and 0.1% Tween 20 (TBS-T) for 12 hours at 4°C. They

were then probed with goat polyclonal anti-AdipoR2 antibody (Santa Cruz Biotechnology, Santa Cruz, CA) at a 1:500 dilution in 5% bovine serum albumin TBS-T for 16 hours at 4°C. After the membranes had been washed in TBS-T, blots were incubated with horseradish peroxidase–linked secondary antibody (Cell Signaling Technology, Beverly, MA) at 1:2000 dilution in 5% nonfat milk TBS-T for 1 hour at room temperature. Immune complexes were detected with an ECL Plus Detection System (GE Healthcare Bio-Sciences, Piscataway, NJ). Densitometric analysis was conducted directly from the blotted membrane using a charge-coupled device camera system (LAS-3000 Mini; Fuji Film, Tokyo, Japan) and Scion Image software (Frederick, MD; http://www.scioncorp.com/).

2.5. Laboratory examinations

Serum adiponectin levels were measured using an enzyme-linked immunosorbent assay (Otsuka Pharmaceuticals, Tokushima, Japan). The detection limit of these assays is 0.96 μg/mL.

2.6. Evaluation of cellular triglyceride accumulation

To quantify cellular triglyceride levels, the cells were washed with phosphate buffered saline, lysed with the buffer in a commercially available test kit (Sanassay TG-N; Sanko Pure Chemical, Tokyo, Japan), and disrupted by sonication. The triglyceride content of the homogenate was then determined with the above kit using the colorimetric method as per the manufacturer's protocol and expressed as milligrams per milligram of protein.

2.7. Statistical analysis

Data are presented as mean ± SEM. The significance of differences between groups was assessed by the Mann-Whitney *U* test. $P < .05$ was considered statistically significant. All calculations were performed with the computer program StatView, version 5.0, for Windows (Abacus Concepts, Berkeley, CA).

3. Results

3.1. Expression of AdipoRs in the liver of patients with NAFLD

In total, 7 subjects each with fatty liver and NASH were studied and compared with 7 normal controls. The clinical and biochemical variables of individuals with NAFLD and controls are summarized in Table 1. Patients with NAFLD had a significantly higher body mass index (BMI) ($P < .05$) and liver steatosis scores ($P < .05$) than those with normal livers. In addition, QUICKI was significantly lower and HOMA-IR tended to be higher in patients with fatty liver and NASH. The serum adiponectin levels were significantly lower in patients with NAFLD than in those with a normal liver ($P < .05$).

Table 1
Clinical characteristics of the patients

	Normal liver	Fatty liver	NASH
Male-female	5:2	5:2	3:4
T2DM	3	5	5
Age (y)	51 ± 4.3	40 ± 13.1	53 ± 12.3
BMI (kg/m^2)	23.6 ± 3.2	27.2 ± 4.2 *	29.5 ± 3.1 *
HbA_{1c} (%)	6.8 ± 2.0	7.2 ± 2.8	6.4 ± 1.6
HOMA-IR	2.4 ± 1.5	4.3 ± 0.9	4.3 ± 1.0
QUICKI	0.35 ± 0.03	0.32 ± 0.02 *	0.32 ± 0.03 *
Adiponectin (U/mL)	7.6 ± 2.5	3.0 ± 1.0 *	4.7 ± 2.2 *
Histologic scores			
Stage	0.7 ± 0.5	1.1 ± 0.4	3.0 ± 0.8*,**
Grade	0.0	0.0	1.9 ± 1.2*,**
Steatosis	0.7 ± 0.4	2.4 ± 0.9 *	2.3 ± 0.8 *

P values were determined by Student *t* test. Data are expressed as mean ± SD. T2DM indicates number of patients with type 2 diabetes mellitus; HbA_{1c}, hemoglobin A_{1c}.

* $P < .05$ vs normal liver

** $P < .05$ vs fatty liver.

To identify how expression of AdipoRs is altered in patients with NAFLD, we performed a real-time PCR analysis using human liver biopsy specimens.

As shown in Fig 1B, hepatic expression of AdipoR2 was significantly down-regulated in patients with NAFLD, especially in patients with fatty liver ($P = .05$ vs normal liver and $P = .03$ vs NASH). Conversely, hepatic expression of AdipoR1 did not differ significantly among the 3 groups (Fig. 1A). No significant difference in AdipoR2 mRNA levels was observed between patients with fatty liver and NASH (Fig. 1B).

As previously reported [10], serum adiponectin levels showed a significant inverse correlation with BMI (Fig. 2A; $r = -0.46$, $P = .02$). Conversely, there was no significant correlation between the BMI and the hepatic mRNA expression of AdipoR2 (Fig. 2B; $r = -0.14$, $P = .51$). These findings suggest that other factors besides the higher BMI regulate the mRNA expression of AdipoR2 in the liver.

3.2. Effect of insulin and TNF-α on AdipoR2 expression in THLE-5b cells

Because our patients with NAFLD had a higher BMI, insulin resistance, and liver steatosis score, the pathophysiology that determines the expression of AdipoRs still remains unclear.

To investigate how AdipoRs are regulated in the condition of insulin resistance, we examined the effect of insulin and TNF-α on AdipoRs' expression in a human hepatocyte cell line, the THLE-5b cells [19].

In THLE-5b cells, mRNAs for both AdipoR1 and AdipoR2 were abundantly expressed. The quantities of mRNAs for AdipoR1 and AdipoR2 relative to those of β-actin were 75 ± 16 and 69 ± 25 arbitrary units (AU), respectively. As shown in Fig. 3B, insulin down-regulated the expression of AdipoR2 mRNA significantly (61 ± 11 AU at 2 μg/mL vs 69 ± 10 AU control, $P < .01$), whereas TNF-α dose-dependently (0.1-10 ng/mL) up-regulated it (at 2 ng/mL

of TNF-α, 76 ± 9 AU, $P = .03$; Fig. 3D). In addition, TNF-α reversed the insulin-induced decrease in AdipoR2 expression (Fig. 4A). In contrast, AdipoR1 mRNA expression was not affected by insulin or TNF-α in the THLE-5b cells (Fig. 3A and C).

The same results were obtained in commonly used human hepatoma cell lines such as HepG2 cells and Alex cells (data not shown).

3.3. Effect of pioglitazone on AdipoR2 expression in THLE-5b cells

Next, we examined the effect of a thiazolidinedione, pioglitazone, on the expression of AdipoRs in human hepatocytes. As shown in Fig. 4A, the expression of AdipoR2 mRNA was significantly up-regulated by pioglitazone (at 2 μmol/L, 82 ± 11 AU, $P < .0001$). Pioglitazone additively up-regulated TNF-α–induced AdipoR2 expression (Fig. 4A). Interestingly, pioglitazone canceled the insulin-induced down-regulation of AdipoR2 (Fig. 4A), suggesting that a thiazolidinedione might ameliorate adiponectin resistance in insulin resistance [22]. AdipoR1 mRNA expression was not altered by pioglitazone (data not shown).

This regulation of the AdipoR2 mRNA levels accompanied the changes in protein levels analyzed by Western blotting (Fig. 4B, lower panel). In concert with the regulation of AdipoR2 mRNA expression, pioglitazone and TNF-α significantly increased and insulin significantly decreased the protein levels of AdipoR2 in THLE-5b cells (Fig. 4B).

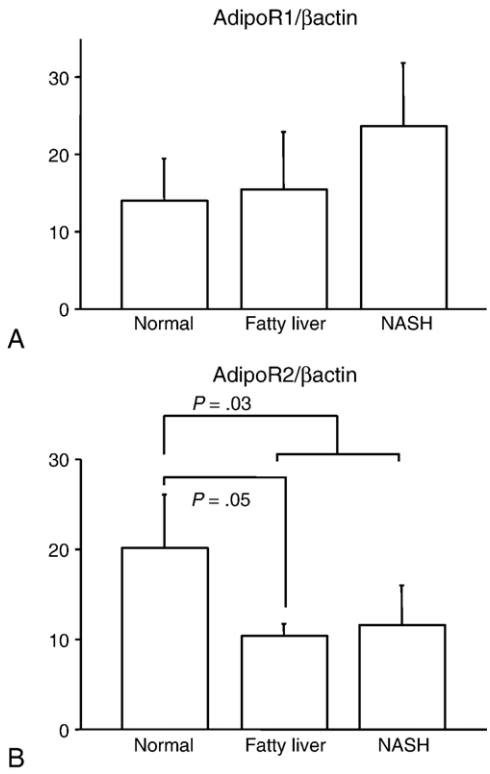

Fig. 1. Real time PCR analyses of AdipoR1 and AdipoR2 in human liver biopsy specimens. AdipoR1 (A) and AdipoR2 (B) gene expression levels in patients with normal liver, fatty liver, and NASH. Expression levels were normalized to β-actin and expressed relative to arbitrary units. Values are mean ± SEM.

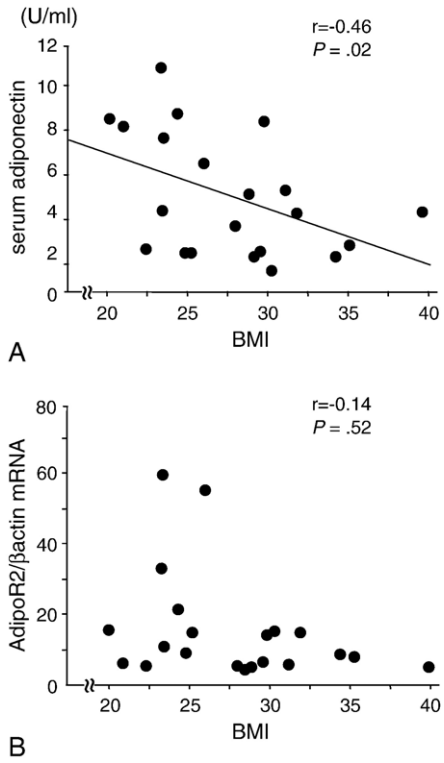

Fig. 2. The relationship between BMI and serum adiponectin (A) or hepatic AdipoR2 mRNA expression (B). No significant correlation was detected between the BMI and the hepatic AdipoR2 mRNA expression (B, $r = -0.14$, $P = .51$), whereas the serum adiponectin levels showed a significant inverse correlation with BMI (A, $r = -0.14$, $P = .51$).

3.4. Alteration of AdipoR2 expression during steatosis of THLE-5b cells

To address the mechanism underlying the in vivo finding that hepatic expression of AdipoR2 was down-regulated in patients with NAFLD (Fig. 1), we assayed the expression of AdipoRs in fatty hepatocytes in vitro. We induced steatosis in THLE-5b cells by incubating them with fatty acids for 24 hours. As shown in Fig. 5A, fatty acids dose-dependently increased the intracellular content of triglyceride in THLE-5b cells as reported previously [23]; fatty acids at 0.5 mmol/L increased the triglyceride content approximately 7-fold (1336 ± 77 mg/g·protein) the control levels. AdipoR2 mRNA levels were decreased in fat containing THLE-5b cells to 89% ± 3% (at 0.5 mmol/L FFA, 52 ± 2 AU vs control 69 ± 10 AU, $P < .001$; Fig. 5C). In contrast, AdipoR1 was not altered during steatosis of the THLE-5b cells (Fig. 5B).

4. Discussion

In this study, patients with fatty liver and NASH had significantly lower serum adiponectin levels together with

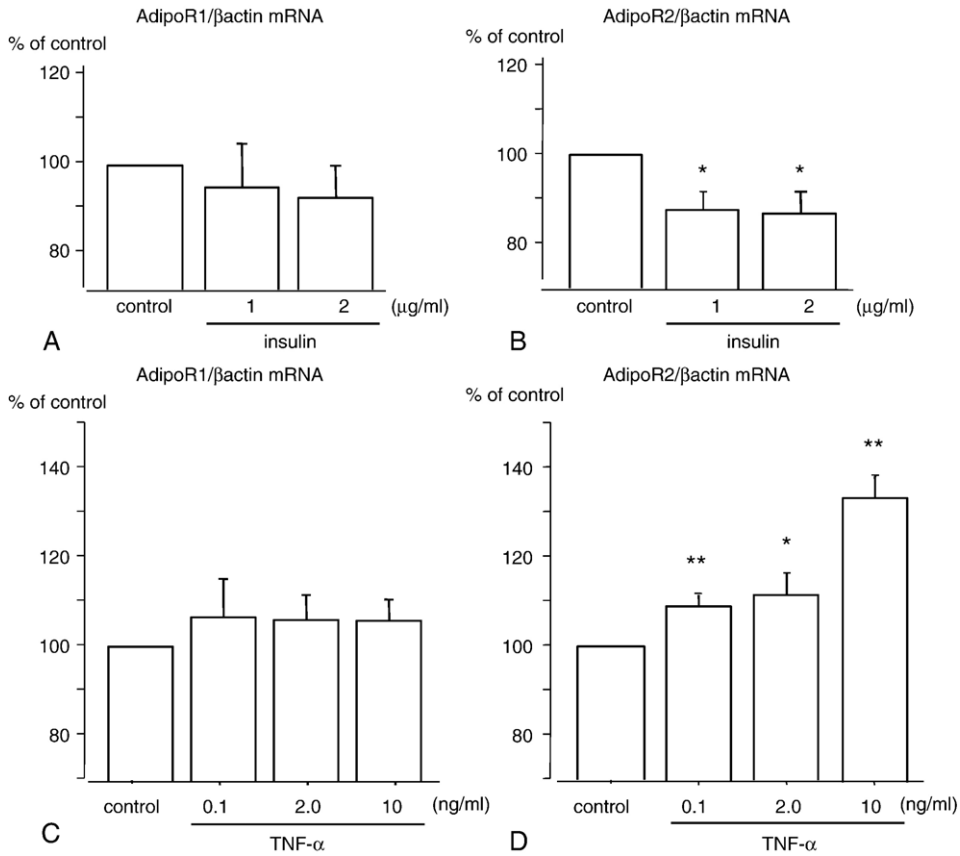

Fig. 3. Dose-dependent effects of insulin (A, B) and TNF-α (C, D) on AdipoR1 (A, C) and AdipoR2 (B, D) gene expression in THLE-5b cells. AdipoR mRNA levels were normalized to β-actin and were expressed relative to the control levels. Values are mean ± SEM of more than 3 independent experiments. *$P < .05$; **$P < .01$ vs control.

higher BMI and liver steatosis scores than those with normal liver, as has been observed in recent reports [24,25]. We demonstrated that hepatic expression of AdipoR2 was significantly down-regulated in patients with NAFLD, especially in those with fatty liver. No significant difference in AdipoR2 mRNA levels was observed between patients with fatty liver and NASH, which contradicts a recent study reporting reduced hepatic expression of AdipoR2 in patients with NASH compared with those with simple fatty liver using a similar steatosis score [26]. In addition, no significant correlation was detected between the BMI and hepatic mRNA expression of AdipoR2 (Fig. 2B; $r = -0.14$, $P = .51$), whereas the serum adiponectin levels showed a significant inverse correlation with BMI, as reported previously [10]. These findings suggest that liver steatosis is a major determinant for regulation of AdipoR2 expression in patients with NAFLD. Considering the possible protective effect of adiponectin against the development of NAFLD [9] and liver fibrosis [27], reduced serum adiponectin levels and reduced hepatic expression of AdipoR2 might be of pathophysiologic relevance in NAFLD.

To address the mechanism underlying down-regulation of hepatic AdipoR2 expression in patients with NAFLD, we examined the regulation of AdipoR2 expression by nutritional mediators, such as insulin, TNF-α, and FFA, in a human hepatocyte cell line, the THLE-5b cells [19]. Most in vitro studies of hepatocytes have been performed using cell lines derived from hepatomas, such as the HepG2, Huh7, and Alex cell lines. We previously observed, however, that gene expression profiles differed among hepatocyte cell lines, especially with respect to α-fetoprotein production [19]. Therefore, we used THLE-5b cells, a nonmalignant human hepatocyte cell line immortalized with simian virus 40 T antigen. These cells maintain a nontumorigenic phenotype and do not have telomerase activity or oncogenic ras [19]. In addition, angiogenic and growth factors, such as ephrin-A1 and TGF-β2, are not up-regulated in THLE-5b cells as they are in hepatoma cell lines [19], suggesting that THLE-5b cells are a suitable hepatocyte cell line for the study of gene expression stimulated by nutrients and cytokines.

Insulin resistance may be associated with hypoadiponectinemia [28,29], and fasting insulin concentrations are negatively correlated with plasma concentrations of adiponectin. Long-term treatment with insulin down-regulates adiponectin expression in adipocytes in vitro [30]. Conversely, it still remains unclear how adiponectin signaling is

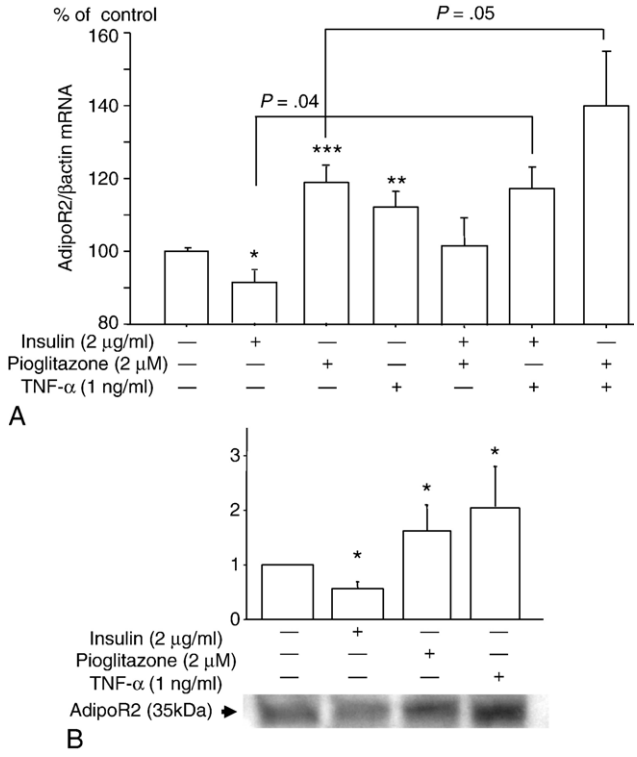

Fig. 4. Effect of insulin (2 μg/mL), pioglitazone (2 μmol/L), and TNF-α (1 ng/mL) on mRNA (A) and protein (B) expression of AdipoR2 in THLE-5b cells. A, Messenger RNA levels of AdipoR2 were normalized to β-actin and expressed relative to the control levels. Values are mean ± SEM of 3 independent experiments. *P = .03; **P = .01; ***P < .0001 vs control. B, The levels of AdipoR2 protein were analyzed by Western blotting with antibody for AdipoR2 and quantified as described in Materials and methods. A representative blot is shown in the lower panel. Values are the mean ± SEM of 3 independent experiments. *P < .05.

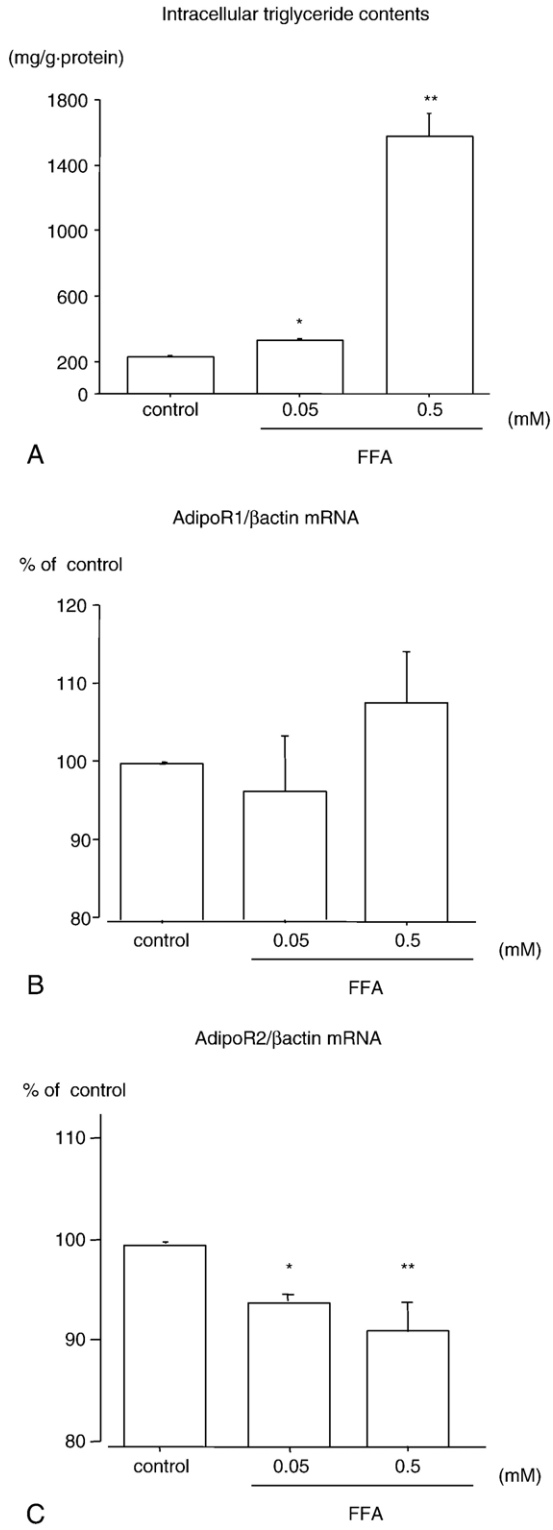

Fig. 5. A, Dose-dependent effects of FFA on intracellular triglyceride content in THLE-5b cells. The cellular protein content was determined as described. Values are mean ± SEM of 3 independent experiments. *P = .05; **P = .0008 vs control. B and C, Dose-dependent effects of FFA on AdipoR1 (B) and AdipoR2 (C) mRNA expression in THLE-5b cells. AdipoR mRNA levels were normalized to β-actin and expressed relative to the control levels. Values are mean ± SEM of 3 independent experiments. *P = .05; **P < .01 vs control.

altered in the target organs of adiponectin, such as the liver, at the level of the receptor in insulin resistance. In addition, hepatic expression of both TNF-α and TNF receptors p55 and p75 has been reported to be up-regulated in the liver of patients with NASH [31], suggesting that the TNF-α system is involved in the pathogenesis of NASH.

In this study, insulin down-regulated the expression of AdipoR2 mRNA, whereas TNF-α up-regulated it in the cultured THLE-5b cells. Furthermore, TNF-α reversed the insulin-induced decrease in AdipoR2 expression. Contrary to the previous findings that mRNAs for both AdipoR1 and R2 are inversely regulated by insulin in mouse primary cultured hepatocytes [14], only AdipoR2 may be regulated in human hepatocytes.

A thiazolidinedione controls the abundance of genes relevant for glucose and fatty acid metabolism and ameliorates insulin resistance and liver pathology in both experimental models [32] and patients [33,34] with NASH. It is also known to increase adiponectin production via enhancing adiponectin promoter activity in adipocytes [35]. Previous reports indicate that circulating adiponectin concentrations are increased after rosiglitazone treatment in

patients with type 2 diabetes mellitus [36], in those with glucose intolerance [37], and in normal subjects [38]. On the other hand, in vitro regulation of AdipoRs by a thiazolidinedione is controversial. One report found that thiazolidinediones do not regulate mRNA expression of AdipoR1 and AdipoR2 in human skeletal muscle cells [39]. This is in apparent contrast to other published data showing induction of AdipoR2, but not AdipoR1, by rosiglitazone in human macrophages [40]. In this study, pioglitazone additively up-regulated TNF-α–induced AdipoR2 expression. Interestingly, pioglitazone canceled the insulin-induced down-regulation of AdipoR2, suggesting that a thiazolidinedione might ameliorate adiponectin resistance in the state of insulin resistance [22]. These findings are in agreement with the regulation of AdipoRs by a thiazolidinedione in the macrophages [40].

These findings suggest that the liver might compensate for the excessive action of insulin and for the TNF-α–induced insulin resistance by altering AdipoR2 expression. Up-regulation of AdipoR2 by a thiazolidinedione mediated its known insulin-sensitizing and anti-inflammatory effects in an animal model [32,33,41] and in patients with NAFLD [32,33].

Although circulating adiponectin levels inversely correlate with hepatic fat content in patients with type 2 diabetes mellitus and NAFLD [11,12], regulation of AdipoRs by fat accumulation in hepatocytes remains unclear. In addition, whether hepatic steatosis itself or pathophysiology causing hepatic steatosis regulates the expression of AdipoRs also remains unclear. We found that AdipoR2, but not AdipoR1, was down-regulated during steatosis of human hepatocytes in vitro. Thus, together with the finding of impaired expression of AdipoR2 in the patients with NAFLD, deterioration of adiponectin action at the level of its receptor might play a role in the pathogenesis of NAFLD such as hepatic insulin resistance and steatosis of the liver per se.

Kaser et al [26] reported that liver adiponectin protein expression was mainly found in endothelial cells of portal vessels and liver sinusoids, whereas AdipoR2 was exclusively detected in hepatocytes. This suggests that the adiponectin-AdipoR system may function in a paracrine way in the liver and may be impaired in patients with NAFLD in the presence of insulin resistance and nutrient factors causing obesity.

In conclusion, hepatic expression of AdipoR2, but not AdipoR1, is down-regulated in patients with NAFLD. Our in vitro study suggests that high insulin levels and liver steatosis contribute to down-regulation of AdipoR2 in hepatocytes. We also found that TNF-α and a thiazolidinedione up-regulate AdipoR2 in hepatocytes. These findings suggest that regulated adiponectin receptor expression plays a role in the development of NAFLD. Further studies will be needed to determine the causal relationship between the adiponectin-AdipoR system and the development of NAFLD in patients with obesity.

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