Available online at www.sciencedirect.com

SCIENCE DIRECT

Metabolism Clinical and Experimental 54 (2005) 995–1001

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Physiological difference between obese (fa/fa) Zucker rats and lean Zucker rats concerning adiponectin

Fumiki Oana*, Hiroo Takeda, Kohichi Hayakawa, Akane Matsuzawa, Satoshi Akahane, Masayuki Isaji, Masuo Akahane

Division of Discovery Research, Kissei Pharmaceutical Co Ltd, 4365-1, Hotaka, Nagano 399-8304, Japan

Received 28 September 2004; accepted 17 February 2005

Abstract

Obese (fa/fa) Zucker rat is a spontaneous genetic obesity model and, by comparison with lean Zucker rat, exhibits hyperphagia, hyperinsulinemia, and hyperlipidemia. The aim of this study was to examine the physiological difference concerning adiponectin between obese (fa/fa) Zucker rats and control lean Zucker rats. We therefore measured plasma adiponectin level and analyzed adiponectin and adiponectin receptor 1 mRNA expression in retroperitoneal white adipose tissue (RT WAT), brown adipose tissue (BAT), liver, and soleus muscle. We also examined the tissue mRNA expression of peroxisome proliferator–activated receptor α (PPARα), PPARδ, and PPARγ, which regulate adiponectin expression sensitivity to a PPARγ agonist shown by brown adipocytes from obese (fa/fa) Zucker rats and lean Zucker rats, by measuring adiponectin release from these cells. Plasma adiponectin levels of obese (fa/fa) Zucker rats were significantly higher than those of lean Zucker rats. Adiponectin mRNA expression levels in RT WAT were lower in obese (fa/fa) Zucker rats than in lean Zucker rats, but those in BAT were higher. Adiponectin receptor 1 expression levels in RT WAT, BAT, and liver of obese (fa/fa) Zucker rats were lower than in lean Zucker rats. The expression level of PPARα, PPARδ, and PPARγ in BAT was lower in obese (fa/fa) Zucker rats than in lean Zucker rats. Moreover, the PPARγ agonist increased adiponectin release only from the brown adipocytes isolated from lean Zucker rats. It is the conclusive difference between obese (fa/fa) Zucker rats and lean Zucker rats that plasma adiponectin levels of obese (fa/fa) Zucker rats are significantly higher than those of lean Zucker rats. Moreover, we clarified that mRNA expression level of adiponectin receptor 1 in RT WAT, BAT, and liver of obese (fa/fa) Zucker rats is low despite high plasma adiponectin level, and low expression of PPARs in BAT leads to less sensibility of adiponectin release from brown adipocytes to a PPARγ agonist in obese (fa/fa) Zucker rats.

1. Introduction

Adipose tissue acts not only as an energy store, but also as part of the endocrine system, secreting a wealth of different protein factors into the circulation. In human beings, obesity and fat accumulation are accompanied by higher plasma levels of plasminogen activator inhibitor 1, tumor necrosis factor α, and leptin, but by a lower plasma levels of adiponectin, an adipocytokine [1-7]. Adiponectin, an insulin-sensitive hormone that plays central role in glucose and lipid metabolism [8], also inhibits the tumor necrosis factor α–induced expression of endothelial adhesion molecules [9-12]. Furthermore, transgenic mice lacking adiponectin manifest diet-induced insulin resistance [13,14], and the plasma level of adiponectin is lower than normal in obese and diabetic rhesus monkeys and in patients with human type 2 diabetes [15,16]. In addition, adiponectin regulates glucose metabolism and insulin sensitivity by activating AMP-activated protein kinase (AMPK) in peripheral tissues [17]. By comparison with lean Zucker rats, obese (fa/fa) Zucker rats exhibit hyperphagia, hyperinsulinemia, and hyperlipidemia and thus represent a spontaneous genetic obesity model [18]. Moreover, by the age of 18 weeks, obese (fa/fa) Zucker rats have become severely hyperinsulinemic by comparison with lean Zucker rats without a severe increase in their plasma glucose level [19]. The mechanism by which fat accumulation and metabolic abnormality lead to insulin resistance in obese (fa/fa) Zucker rats is not yet clear. However, Zucker diabetic fatty (ZDF) rats exhibit hyperglycemia [20], and both the plasma adiponectin level and the adiponectin expression level in white adipose tissue (WAT) are higher in ZDF-lean

* Corresponding author. Tel.: +81 263 82 8820; fax: +81 263 82 8827.
E-mail address: fumiki_oana@pharm.kissei.co.jp (F. Oana).

0026-0495/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2005.02.016

than in ZDF rats [21]. Moreover, the existence of adiponectin receptors was apparent in recent years [22]. There are reports that adiponectin receptor 1 mRNA was highly expressed in human skeletal muscle [23,24]. Moreover, this receptor was expressed in human macrophages [25].

It has recently become apparent that peroxisome proliferator–activated receptor (PPAR)–responsive element (PPRE) is present in the human adiponectin promoter region [26]. Pioglitazone, a PPARγ agonist, may exert an antiatherosclerotic effect by increasing the serum adiponectin level [27,28]. Fibrates are widely used to ameliorate lipid metabolism by activating PPARα [29,30], whereas PPARδ, which exists ubiquitously throughout the body, is preferentially activated by unsaturated fatty acids [31]. In 12-week-old male obese (fa/fa) Zucker rats, PPARα and PPARγ have been found to be highly expressed in brown adipose tissue (BAT), but their expression levels were not significantly different between fa/fa and lean rats [32].

The aim of this study was to examine the physiological difference concerning adiponectin between obese (fa/fa) Zucker rats and control lean Zucker rats. We therefore measured plasma adiponectin level and analyzed adiponectin and adiponectin receptor 1 mRNA expression in retroperitoneal (RT) WAT, BAT, liver, and soleus muscle. We also examined the tissue mRNA expression of PPARα, PPARδ, and PPARγ, which are considered to influence adiponectin mRNA expression. In addition, to examine the sensitivity to a PPARγ agonist shown by brown adipocytes isolated from obese (fa/fa) Zucker rats and lean Zucker rats, we measured adiponectin release from these cells.

2. Materials and methods

2.1. Animal studies

Five male obese (fa/fa) Zucker rats and 5 age-matched male lean Zucker rats were purchased at the age of 6 weeks from Charles River Japan, Inc (Tokyo, Japan). These rats were allowed an animal chow, CE-2 (Oriental Yeast, Tokyo), and water ad libitum while residing in sterile cages in a barrier animal facility at a 12-hour light/dark cycle. Up to 18 weeks of age, the 2 groups were kept under the same conditions. To determine plasma chemical parameters, blood samples were collected from the tail vein into Heparinized Calibrated Pipets (Drummond Scientific Company, Broomall, Pa, USA), and the plasma was prepared by centrifugation at 3000 rpm for 10 minutes. The plasma insulin level was determined using a Morinaga insulin assay kit (Morinaga Bioscience Laboratory, Yokohama, Japan), the plasma triglyceride level using a Triglyceride E Test (Wako Pure Chemical Industries, Osaka, Japan), the plasma glucose level using a Glucose C-II Test (Wako Pure Chemical Industries), the plasma free fatty acids (FFAs) level using a nonesterified fatty acids C-Test (Wako Pure Chemical Industries), and the plasma adiponectin levels using a mouse/rat adiponectin enzyme-linked immunosorbent assay kit (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan). All procedures involving the use of laboratory animals, including blood sampling from veins and killing by diethyl ether overdosing, were conducted according to guidelines approved by the Laboratory Animal Committee of Kissei Pharmaceutical Co Ltd.

2.2. Tissue RNA isolation

To quantify the expression levels of the mRNAs for adiponectin, adiponectin receptor 1, and PPARs, obese (fa/fa) Zucker rats and lean Zucker rats were killed at 18 weeks. Then, RT WAT, BAT, liver, and soleus muscle were excised, and the weighed tissues stored at -80°C. Each tissue was homogenized for 1 minute in TRIZOL Reagent (Invitrogen, Carlsbad, Calif, USA) using an ULTRA-TURRAX T8 homogenizer (IKA-Werke GmbH & Co. KG, Staufen, Germany). The subsequent procedures were carried out according to the manufacturer's instructions, unless otherwise specified. Isolated total RNAs were cleaned up using RNeasy Mini Kit (QIAGEN, Valencia, Calif, USA). Concentrations of total RNA were determined using a RiboGreen Kit (Molecular Probes, Eugene, Ore, USA).

2.3. Reverse transcription and real-time quantitative polymerase chain reaction

Reverse transcription of 1 μg total RNA to cDNA was performed using a Super Script First-Strand Synthesis System (Invitrogen) for reverse transcriptase–polymerase chain reaction (PCR) according to the manufacturer's instructions. The forward primer, reverse primer, and TaqMan probe were designed using Primer Express software version 1.0 (Applied Biosystem, Foster City, Calif, USA). Their sequences are shown in Table 1, but TaqMan Rodent glyceraldehye-3-phosphate dehydrogenase (GAPDH) Control Reagents (Applied Biosystems) were used as primers of rat GAPDH. A real-time PCR-based 5′ nuclease assays (TaqMan assays) was performed using a Gene Amp 5700 Sequence Detection System (Applied Biosystem). A number of cDNA copies for each tissue were assayed on one 96-well plate (Applied Biosystem) together with a nontemplate control that did not contain cDNA and standards consisting of 10-fold serial dilutions of the pCR-Blunt 2 TOPO vector (Invitrogen) in which was inserted the PCR product to be amplified by the forward primer and reverse primer for the analyzed gene. For each reaction, 2 μL cDNA for each tissue was mixed with 25 μL of Platinum Quantitative PCR SuperMix-UDG (Invitrogen), 1 μL of primer mix containing 20 μmol/L each of forward primer and reverse primer, 0.25 μL of 20 μmol/L TaqMan probe, and 19.75 μL of diethyl pyrocarbonate (DEPC)-treated water (Nacalai tesque, Kyoto, Japan). All reactions were carried out by PCR amplification using a program consisting of 40 cycles of 95°C for 5 minutes, 60°C for 90 seconds for each cycle. At the end of the reaction, the cycle threshold (Ct) value (ie, the cycle numbers at which fluorescent signals were obtained) was noted for standard and

Table 1
Oligonucleotide sequences of gene-specific primers and probes for TaqMan analysis of rat adiponectin, adiponectin receptor 1, and PPAR mRNAs

Genes	Accession no. with amplicon position	Primer type or direction	Sequences
Adiponectin	NM_144744 (119-190)	Forward	5′ -CCCTCCACCCAAGGAAACTT-3′
		Reverse	5′ -GGTATCCCATTGTGACCAGGA-3′
		Fluoregenic probe	FAM-5′ -AGGTTGGATGGCAGGCATCCCA-3′ -TAMRA
Adiponectin receptor 1	BC061838 (537-638)	Forward	5′ -ATGACTACCTGCTACATGGCCA-3′
		Reverse	5′ -GTGTCCAGATGTTGCCAGTTTC-3′
		Fluoregenic probe	FAM-5′ -CTTCAAGAGCATCTTCCGCATCCACA-3′ -TAMRA
PPARα	NM_013196 (1421-1542)	Forward	5′ -GAAGCCATTCTGCGACATCA-3′
		Reverse	5′ -GCCGATCTCCACAGCAAATT-3′
		Fluoregenic probe	FAM-5′ -AGTTCAATGCCCTCGGAACTGGATGACA-3′ -TAMRA
PPARδ	NM_013141 (1181-1310)	Forward	5′ -TACGAGAAGTGCGATCGGATCT-3′
		Reverse	5′ -TCCAAAGCGGATAGCGTTGT-3′
		Fluoregenic probe	FAM-5′ -ACCGCAACAAGTGTCAGT-3′ -TAMRA
PPARγ	AB011365 (495-614)	Forward	5′ -CTGTCGGTTTCAGAAGTGCCTT-3′
		Reverse	5′ -AGCTGGTCGATATCACTGGAGA-3′
		Fluoregenic probe	FAM-5′ -TCTCACAATGCCATCAGGTTTGGGC-3′ -TAMRA

unknown samples. A standard curve was constructed by plotting the Ct values as a function of the log concentration of standard cDNA. On the basis of the Ct values of unknown samples, their relative mRNA concentrations were deduced, with GAPDH mRNA being used as the internal standard.

2.4. Tissue DNA isolation and quantitation

Each tissue was homogenized in 1 mL of ice-cold homogenization buffer (100 mmol/L potassium phosphate, 250 mmol/L sucrose, 2 mmol EDTA, 1 mmol/L HEPES, pH 7.0) using ULTRA-TURRAX T8 homogenizer for 1 minute. The homogenate was extracted by 3 cycles of freeze-thawing (liquid nitrogen/37°C) and centrifuged at 4000 rpm for 5 minutes at 4°C. The cleared supernatant was collected and stored at −80°C until analysis could be performed for DNA. Concentration of DNA in each tissue was determined with CyQUANT Cell Proliferation Kit (Molecular Probes).

2.5. Adiponectin release from brown adipocytes of obese (fa/fa) Zucker rats and lean Zucker rats

Obese (fa/fa) Zucker rats and lean Zucker rats were killed at 18 weeks of age, and BAT was excised from each animal. The BAT was dispersed in Krebs-Henseleit solution (Sigma-Aldrich, Inc, St Louis, Mo) containing 3% bovine serum albumin (Sigma) and collagenase (Roche, Basel, Switzerland) for 1 hour at 37°C. Isolated brown adipocytes (2×10^4 cells per well) were incubated with pioglitazone synthesized at Kissei Pharmaceutical Laboratory for 24 hours at 37°C. The adiponectin concentration of the supernatant was quantitated using a mouse/rat adiponectin enzyme-linked immunosorbent assay kit from Otsuka Pharmaceutical Co Ltd.

2.6. Statistics

Statistical analysis of data was performed by means of an unpaired t test using Prism software (GraphPad Software, Inc, San Diego, Calif, USA). Data are expressed as the mean ± SE, and P values $<.05$ were considered to indicate statistical significance.

3. Results

3.1. Body weight and plasma adiponectin

As shown in Fig. 1, body weight of obese (fa/fa) Zucker rats was significantly heavier than that of lean Zucker rats from age of 13 to 17 weeks. Plasma adiponectin concen-

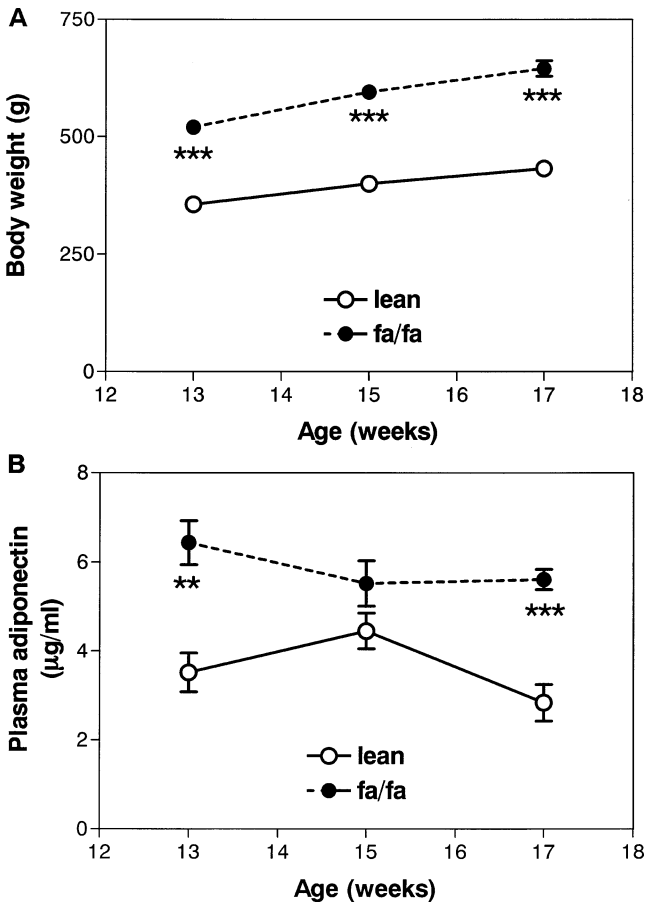

Fig. 1. Body weight (A) and plasma adiponectin (B) level from the age of 13 to 17 weeks. **P < .01, ***P < .001, lean versus obese rats (n = 5).

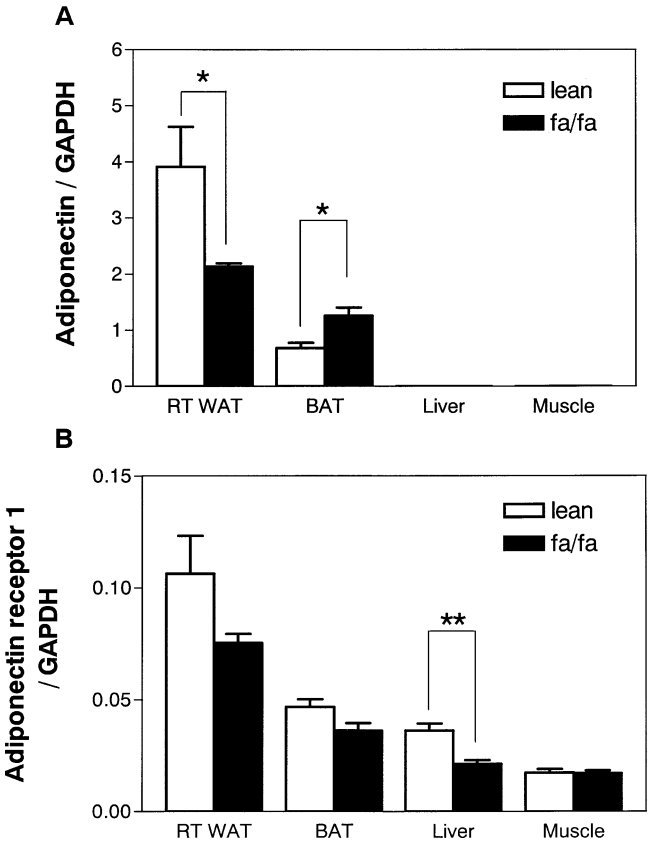

Fig. 2. Adiponectin and adiponectin receptor 1 mRNA expressions in retroperitoneal (RT) WAT, brown WAT (BAT), liver, and muscle. A, Adiponectin expression; B, Adiponectin receptor 1 expression. *$P < .05$, **$P < .01$, lean versus obese rats (n = 4-5).

tration of obese (fa/fa) Zucker rats at the age of 13 weeks (6.44 ± 0.49 μg/mL) was significantly higher than that of lean Zucker rats (3.52 ± 0.44 μg/mL). Until they are 17 weeks, plasma adiponectin concentration of obese (fa/fa) Zucker rats was higher than that of lean Zucker rats. The concentration of obese (fa/fa) Zucker rats at the age of 17 weeks (5.61 ± 0.41 μg/mL) was significantly higher than that of lean Zucker rats (2.84 ± 0.41 μg/mL).

3.2. Physiological and biochemical parameters

The plasma insulin, triglyceride, and FFA of obese (fa/fa) Zucker rats were significantly higher than that of lean Zucker rats, but the plasma glucose levels between 2 groups were not significant. The tissue weights of RT WAT and BAT of obese (fa/fa) Zucker rats were significantly heavier than that of lean Zucker rats.

3.3. Adiponectin and adiponectin receptor 1 expression in obese (fa/fa) Zucker rats and lean Zucker rats

To quantify the adiponectin and adiponectin receptor 1 expression level in RT WAT, interscapular BAT, soleus muscle, and liver, the relative abundance of adiponectin and adiponectin receptor 1 mRNA was determined by real-time quantitative PCR. As shown in Fig. 2, the adiponectin expression level in obese (fa/fa) Zucker rats was significantly higher than that in lean Zucker rats in BAT. In RT WAT, that level in obese (fa/fa) Zucker rats was significantly lower than that in lean Zucker rats. An expression level of adiponectin was not detectable in liver and soleus muscle in either obese (fa/fa) or lean Zucker rats. The adiponectin receptor 1 expression level in RT WAT, BAT, and liver of obese (fa/fa) Zucker rats was lower than that of lean Zucker rats. In soleus muscle, there is no difference of its expression between obese (fa/fa) and lean Zucker rats.

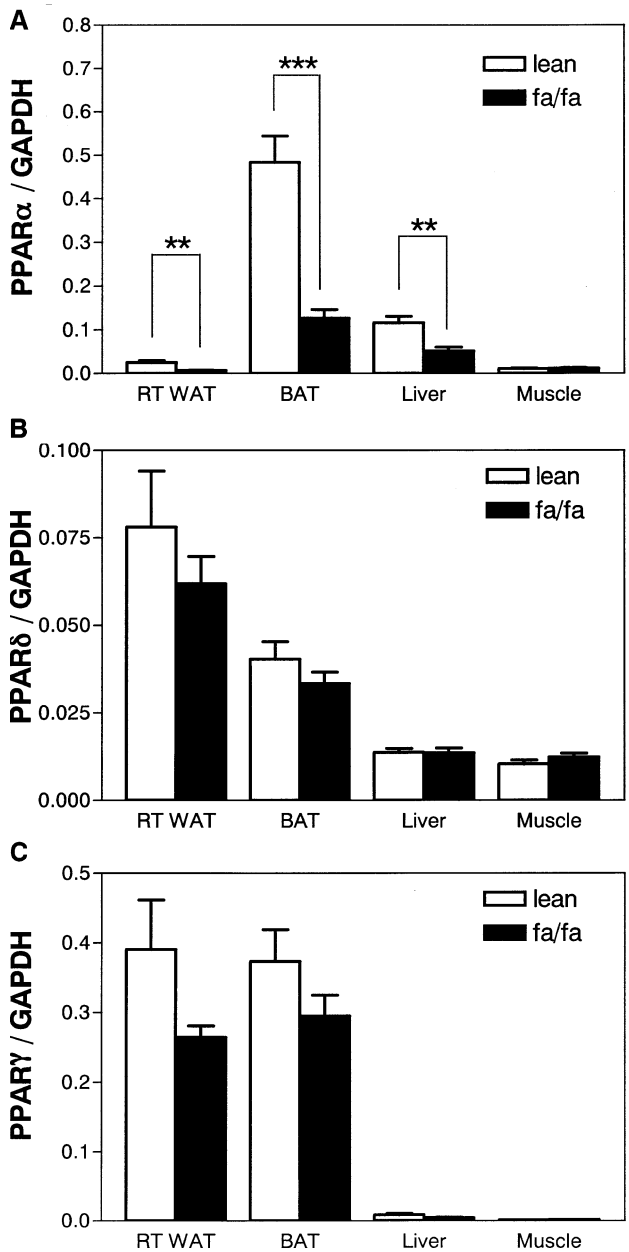

Fig. 3. PPARα, PPARδ, and PPARγ expressions in retroperitoneal (RT) WAT, brown WAT (BAT), liver and muscle. A, PPARα expression; B, PPARδ expression; C, PPARγ expression. **$P < .01$, ***$P < .001$, lean versus obese rats (n = 4-5).

Table 2
Physiological and biochemical parameters in obese (fa/fa) Zucker rats and lean Zucker rats

	Lean Zucker rats (n=5)	Obese (fa/fa) Zucker rats (n=5)
Body weight (g)	432.5 ± 8.9	645.7 ± 16.6***
Daily food intake (g/body)	22.8 ± 0.5	36.1 ± 1.9***
Plasma glucose (mg/dL)	95.2 ± 2.0	208.6 ± 61.7
Plasma insulin (ng/mL)	0.92 ± 0.07	30.6 ± 2.79**
Plasma triglyceride (mg/dL)	236 ± 5.4	1201 ± 230.5**
Plasma FFAs (mEq/L)	0.36 ± 0.05	0.74 ± 0.11*
RT WAT weight (g)	7.309 ± 0.898	31.202 ± 1.462***
RT WAT total DNA (μg)	8.328 ± 1.372	62.585 ± 5.757***
BAT weight (g)	0.829 ± 0.072	2.303 ± 0.087***
BAT total DNA (μg)	3.907 ± 0.548	10.457 ± 1.487**

Data are the mean ± SE of 5 animals. Body weight and food intake were measured under fed conditions. Blood was collected in feeding. Tissue weights are at the age of 18 weeks; another parameters are at the age of 17 weeks.

* $P < .05$, the mark on fa/fa rats in comparison with lean rats by unpaired Student t test.

** $P < .01$, the mark on fa/fa rats in comparison with lean rats by unpaired Student t test.

*** $P < .001$, the mark on fa/fa rats in comparison with lean rats by unpaired Student t test.

3.4. PPARs expression in obese (fa/fa) and lean Zucker rats

As shown in Fig. 3A, the PPARα expression level in BAT was significantly lower in obese (fa/fa) Zucker rats, the level being only 26% of that in lean Zucker rats. In RT WAT and liver, that expression level in obese (fa/fa) Zucker rats was significantly lower than that in lean Zucker rats. As shown in Fig. 3B, the PPARδ expression level in RT WAT and BAT of obese (fa/fa) Zucker rats was lower than that of lean Zucker rats. Its expression level in liver and soleus muscle between obese (fa/fa) and lean Zucker rats was the same. As shown in Fig. 3C, the PPARγ expression level in RT WAT and BAT of obese (fa/fa) Zucker rats was lower than that of lean Zucker rats. In liver and soleus muscle, its expression was scarcely detectable in either obese (fa/fa) or lean Zucker rats.

3.5. Tissue DNA concentration

In Table 2, the total DNA concentration of RT WAT and BAT of obese (fa/fa) Zucker rats was significantly higher than that of lean rats.

3.6. Adiponectin release from brown adipocytes isolated from obese (fa/fa) Zucker rats and lean Zucker rats

As shown in Fig. 4, the basal concentration of adiponectin released from BAT isolated from obese (fa/fa) Zucker rats was 22.2 ± 1.42 ($\times 10^{-5}$ ng per cell), and no increase was observed on addition of an PPARγ agonist at the concentrations used. The corresponding value for lean Zucker rats was only 7.04 ± 0.31 ($\times 10^{-5}$ ng per cell), and this was increased by the PPARγ agonist in a dose-dependent manner.

4. Discussion

As described in the Introduction, obese (fa/fa) Zucker rats represent a spontaneous genetic obesity model in which the individuals exhibit hyperphagia, hyperinsulinemia, and hyperlipidemia [18], the hyperinsulinemia being severe at the age of 18 weeks without an increased plasma glucose level [19]. In this study, we showed the plasma insulin, triglyceride, and FFA of obese (fa/fa) Zucker rats were higher than that of lean Zucker rats at the age of 17 weeks, but the plasma glucose levels were not significant. Moreover, the body weight of obese (fa/fa) Zucker rats was significantly heavier than that of lean Zucker rats until the age of 17 weeks. However, we have shown that the plasma adiponectin level is significantly higher in obese (fa/fa) Zucker rats than in lean Zucker rats until the age of 17 weeks. In ZDF rats, on the other hand,

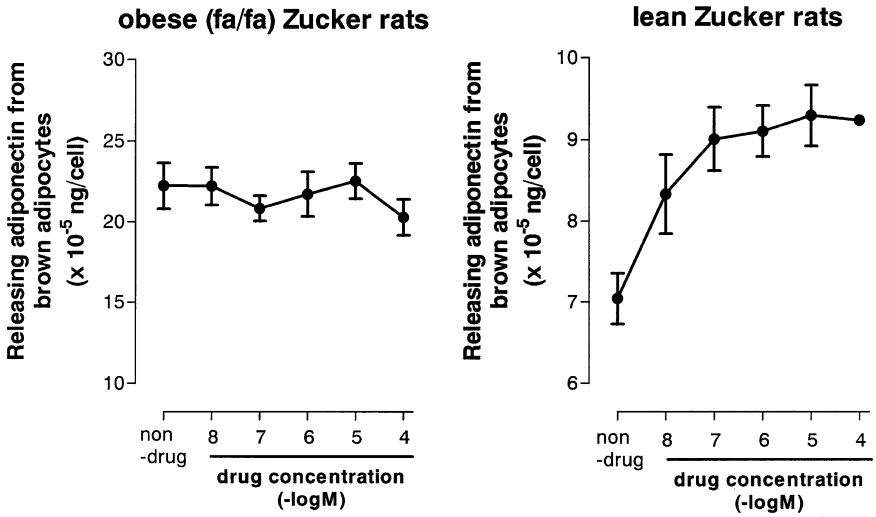

Fig. 4. Effect of PPARγ agonist on adiponectin release from isolated brown adipocytes in obese and lean rats. Mean ± SE (n = 3).

the plasma adiponectin level is reportedly significantly lower than that found in ZDF-lean rats [21]. This would appear to represent a difference between ZDF rats and obese (fa/fa) Zucker rats. The fact that the disease status differs between obese (fa/fa) Zucker rats and ZDF rats might be responsible for the difference described above. However, this is merely speculative, and further work needs to be done on this point.

The adiponectin mRNA expression level in RT WAT of obese (fa/fa) Zucker rats was significantly lower than lean Zucker rats, but that expression level in BAT of obese (fa/fa) Zucker rats was significantly higher than that of lean Zucker rats. This result suggests that the adiponectin released from BAT was part of high plasma adiponectin level in obese (fa/fa) Zucker rats. Moreover, the fact that the adiponectin expression level in liver and soleus muscle was scarcely detectable in either obese (fa/fa) or lean Zucker rats suggests that the plasma adiponectin released from liver and soleus muscle was little. Although the adiponectin mRNA expression in WAT was low in obese (fa/fa) Zucker rats, the high levels of total DNA concentration of WAT in those animals might contribute the high plasma adiponectin.

In recent years, the existence of adiponectin receptors was apparent [22]. There are reports that adiponectin receptor 1 mRNA was highly expressed in human skeletal muscle [23,24]. Moreover, this receptor was expressed in human macrophages [25]. In this study, adiponectin receptor 1 mRNA was expressed in RT WAT, BAT, and liver of obese (fa/fa) and lean Zucker rats. Moreover, its expression level in liver of obese (fa/fa) Zucker rats was significantly lower than that of lean Zucker rats. These results demonstrate that the adiponectin receptor 1 mRNA expression level in RT WAT, BAT, and liver was lower than that of lean Zucker rats despite high plasma adiponectin of obese (fa/fa) Zucker rats. Moreover, the possibility exists that RT WAT and BAT have autocrine system concerning adiponectin in obese (fa/fa) and lean Zucker rats.

Several pieces of evidence link PPARs with adiponectin and with lipid metabolism. Recently, PPRE has been identified in the human adiponectin promoter region, and adiponectin is induced by nuclear receptors [26]. Pioglitazone, a PPARγ agonist, may have an antiatherosclerotic effect by increasing serum adiponectin levels [27,28]. Fibrates are widely used to ameliorate lipid metabolism by inducing gene expressions of lipoprotein lipase and of apolipoprotein A-I and A-II via PPARα activation [29,30]. Transgenic mice lacking PPARα exhibit increased plasma lipids and increased body weight [33]. In addition, phosphorylation of PPARα is insulin-dependent [34]. PPARδ, which is expressed ubiquitously, is preferentially activated by unsaturated fatty acids [31]. In contrast, PPARγ is expressed predominantly in adipose tissue, and activation of PPARγ improves insulin sensitivity and decreases both the plasma levels of FFA level and blood pressure [27]. PPARγ phosphorylation is dependent on MAP kinase and leads to a decreased differentiation of PPARγ-mediated preadipocytes into adipocytes [35]. In our study, adiponectin expression level in BAT was significantly lower in obese (fa/fa) Zucker rats than in lean Zucker rats. We therefore analyzed the expressions of PPARα, PPARδ, and PPARγ in BAT and found that these expression levels were all lower in obese (fa/fa) Zucker rats than in lean Zucker rats. This result tempts us to think that transcriptional stimulation by PPAR via PPRE may be weaker in obese (fa/fa) Zucker rats than in lean Zucker rats.

Finally, we analyzed adiponectin release from isolated brown adipocytes. The basal release into the medium from BAT was 3 times higher in obese (fa/fa) Zucker rats than in lean Zucker rats. However, addition of a PPARγ agonist did not increase adiponectin release into the medium from the BAT isolated from obese (fa/fa) Zucker rats. This result accords well with our finding of a decreased expression of the mRNA for PPARγ in this tissue in obese (fa/fa) Zucker rats. Although the basal release of adiponectin into the medium from BAT was lower for lean Zucker rats than for obese (fa/fa) Zucker rats, the release was increased by the PPARγ agonist dose-dependently in the former group. These results show that only in lean Zucker rats is BAT sensitive to PPAR. Although the stimulatory effect of the PPARγ agonist on adiponectin synthesis may have been weak in the brown adipocytes of obese (fa/fa) Zucker rats, their brown adipocytes clearly produce a rich secretion of adiponectin under basal conditions. In contrast, in lean Zucker rats, the basal secretion of adiponectin from brown adipocytes seems to be relatively sparse, but clearly, it can be enhanced via PPARγ stimulation.

In this study, it is the conclusive difference between obese (fa/fa) Zucker rats and lean Zucker rats that plasma adiponectin level of obese (fa/fa) Zucker rats is significantly higher than that of lean Zucker rats. Moreover, we clarified that mRNA expression level of adiponectin receptor 1 in RT WAT, BAT, and liver of obese (fa/fa) Zucker rats is low despite high plasma adiponectin level, and low expression of PPARs in BAT leads to less sensibility of adiponectin release from brown adipocytes to a PPARγ agonist in obese (fa/fa) Zucker rats.

References

[1] Weyer C, Funahashi T, Tanaka S, et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 2001;86:1930-5.
[2] Steppan CM, Lazar MA. Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 2002;13:18-23.
[3] Havel PJ. Control of energy homeostasis and insulin action by adipocyte hormones: leptin, acylation, stimulating protein, and adiponectin. Curr Opin Lipidol 2002;13:51-9.
[4] Shimomura I, Funahashi T, Takahashi M, et al. Enhanced expression of PAI-1 in visceral fat: possible contributor to vascular disease in obesity. Nat Med 1996;2:800-3.
[5] Arita Y, Kihara S, Ouchi N, et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 1999;257:79-83.

[6] Stefan N, Stumvoll M. Adiponectin—its role in metabolism and beyond. Horm Metab Res 2002;34:469-74.
[7] Frühbeck J, Gómez-Ambrosi J, Muruzábal FJ, et al. The adipocyte: a model for integration of endocrine and metabolic signaling in energy metabolism regulation. Am J Physiol Endocrinol Metab 2001;280: E827-47.
[8] Berg AH, Combs TP, Scherer PE. ACRP30/adiponectin: an adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 2002;13:84-9.
[9] Ouchi N, Kihara S, Arita Y, et al. Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-κB signaling through a cAMP-dependent pathway. Circulation 2000;102:1296-301.
[10] Matsuda M, Shimomura I, Sata M, et al. Role of adiponectin in preventing vascular stenosis. The missing link of adipo-vascular axis. J Biol Chem 2002;277:37487-91.
[11] Okamoto Y, Arita Y, Nishida M, et al. An adipocyte-derived plasma protein, adiponectin, adheres to injured vascular walls. Horm Metab Res 2000;32:47-50.
[12] Ouchi N, Kihara S, Arita Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473-6.
[13] Maeda N, Shimomura I, Kishida K, et al. Diet-induced insulin resistance in mice lacking adiponbectin/ACRP30. Nat Med 2002; 8:731-7.
[14] Kubota N, Terauchi Y, Yamauchi T, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 2002;277:25863-6.
[15] Hotta K, Funahashi T, Bodkin NL, et al. Circulating concentrations of adipocyte protein adiponectin are decreased in parallel with reduced insulin sensitivity during the progression to type 2 diabetes in rhesus monkeys. Diabetes 2001;50:1126-33.
[16] Hotta K, Funahashi T, Arita Y, et al. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol 2000;20:1595-9.
[17] Yamauchi T, Kamon J, Minokoshi Y, et al. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.
[18] Kobatake T, Matsuzawa Y, Tokunaga K, et al. Metabolic improvements associated with a reduction of abdominal visceral fat caused by a new α-glucosidase inhibitor, AO-128, in Zucker fatty rats. Int J Obes 1988;13:147-54.
[19] Shimoike T, Yanase T, Umeda F, et al. Subcutaneous or visceral adipose tissue expression of the PPARγ gene is not altered in the fatty (fa/fa) Zucker rat. Metabolism 1998;47:1494-8.
[20] Liu X, Pérusse F, Bukowiecki LJ. Mechanism of the antidiabetic effects of the β_3-adrenergic agonist CL-316243 in obese Zucker-ZDF rats. Am J Physiol 1998;274:R1212-19.
[21] Altomonte J, Harbaran S, Richter A, et al. Fat depot–specific expression of adiponectin is impaired in Zucker fatty rats. Metabolism 2003;52:958-63.
[22] Yamauchi T, Kamon J, Ito Y, et al. Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. Nature 2003;423:762-9.
[23] Debard C, Laville M, Berbe V, et al. Expression of key genes of fatty acids oxidation, including adiponectin receptors, in skeletal muscle of type 2 diabetic patients. Diabetologia 2004;47:917-25.
[24] Civitarese AE, Jenkinson CP, Richardson D, et al. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of type 2 diabetes. Diabetologia 2004;47:816-20.
[25] Chinetti G, Zawadski C, Fruchart JC, et al. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPARalpha, PPARgamma, and LXR. Biochem Biophys Res Commun 2004;314:151-8.
[26] Iwaki M, Matsuda M, Maeda N, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. Diabetes 2003;52:1655-63.
[27] Hirose H, Kawai T, Yamamoto Y, et al. Effects of pioglitazone on metabolic parameters, body fat distribution, and serum adiponectin levels in Japanese male patients with type 2 diabetes. Metabolism 2002;51:314-7.
[28] Maeda N, Takahashi M, Funahashi T, et al. PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 2001;50:2094-9.
[29] Vosper H, Khoudoli GA, Graham TL, et al. Peroxisome proliferators–activated receptor agonists, hyperlipidaemia, and atherosclerosis. Pharmacol Ther 2002;95:47-62.
[30] Fruchart JC, Staels B, Duriez P. The role of fibric acids in atherosclerosis. Curr Atheroscler Rep 2001;3:83-92.
[31] Shin HD, Park BL, Kim LH, et al. Genetic polymorphisms in peroxisome proliferators–activated receptorδ associated with obesity. Diabetes 2004;53:847-51.
[32] Gorla-Bajszczak A, Siegrist-Kaiser C, Boss O, et al. Expression of peroxisome proliferator–activated receptors in lean and obese Zucker rats. Eur J Endocrinol 2000;142:71-8.
[33] Costet P, Legendre C, More J, et al. Peroxisome proliferator–activated receptor α-isoform deficiency leads to progressive dyslipidemia with sexually dimorphic obesity and steatosis. J Biol Chem 1998; 273:29577-85.
[34] Shalev A, Siegrist-Kaiser CA, Yen PM, et al. The peroxisome proliferator–activated receptor alpha is a phosphoprotein: regulation by insulin. Endocrinology 1996;137:4499-502.
[35] Ristow M, Muller-Wieland D, Pfeiffer A, et al. Obesity associated with a mutation in a genetic regulator of adipocyte differentiation. N Engl J Med 1998;339:953-9.