Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 946–953
Metabolism
Clinical and Experimental
www.metabolismjournal.com

# Adiponectin receptors: expression in Zucker diabetic rats and effects of fenofibrate and metformin

Coralie Metais[a], Fabien Forcheron[a], Pauline Abdallah[b], Alexandra Basset[b], Peggy Del Carmine[b], Giampiero Bricca[a], Michel Beylot[a,*]

[a]*EA 4173-ERI 22 Agressions vasculaires et réponses tissulaires, Faculté Rockefeller, UCBLyon1, 69008 Lyon, France*
[b]*ANIPHY, Faculté Rockefeller, UCBLyon1, 69008 Lyon, France*

Received 28 August 2007; accepted 6 February 2008

**Abstract**

The insulin-sensitizing adipokine, adiponectin, acts through 2 receptors, AdipoR1 and AdipoR2. A decreased expression of these receptors could contribute to insulin resistance and diabetes. We determined if the expression of adiponectin receptors is decreased in an experimental model, the Zucker diabetic rat (ZDF), and if a peroxisome proliferator–activated receptor α agonist, fenofibrate, and metformin could increase these expressions. The ZDF and control (L) rats were studied at 7, 14, and 21 weeks. After initial study at 7 weeks, ZDF received no treatment (n = 10), metformin (n = 10), or fenofibrate (n = 10) until final studies at 14 or 21 weeks. The L rats received no treatment. AdipoR1 and R2 expressions were measured in liver, muscle, and white adipose tissue (WAT). As expected, ZDF rats were insulin resistant at 7 weeks, had type 2 diabetes mellitus at 14 weeks, and had diabetes with insulin deficiency at 21 weeks. Compared with L rats, AdipoRs messenger RNA was decreased only in the WAT ($P < .05$) of 7-week–old ZDF rats, but was unchanged in muscle and increased in liver. Metformin and fenofibrate decreased plasma triacylglycerols ($P < .01$) as expected. The only effect of fenofibrate on AdipoRs was a moderate increase ($P < .01$) of both receptors' messenger RNA in liver. Metformin increased AdipoR1 and R2 expression in muscle ($P < .01$) and AdipoR1 ($P < .01$) in WAT. These results do not support an important role for decreased AdipoRs expression in the development of insulin resistance and diabetes. Parts of the actions of fenofibrate and of metformin could be mediated by a stimulation of the expression of these receptors in liver and in insulin-sensitive, glucose-utilizing tissues (muscle, WAT), respectively.


## 1. Introduction

Adiponectin is one of the many proteins secreted by adipocytes that play a role in the regulation of glucose and lipids metabolism and can modify insulin sensitivity and energy balance [1,2]. It improves the sensitivity of tissues to insulin, has protective effects against the development of type 2 diabetes mellitus [3-5], and has some anti-inflammatory and antiatherogenic properties [6,7]. Adiponectin acts at least in part through a stimulation of adenosine 5′-monophosphate–activated protein kinase, which increases glucose uptake and fatty acid oxidation [8,9], and of the nuclear receptor peroxisome proliferator–activated receptor (PPAR) α [2], which stimulates lipid oxidation. All these effects are considered to be mediated by 2 related receptors, AdipoR1 and AdipoR2 [10], expressed in most tissues, including liver, adipose tissue, skeletal muscles [10], pancreatic β-cells [11], and macrophages [12]. Impairing the expression of these receptors impacts on whole-body metabolism and on the metabolic effects of adiponectin [13-15]. Therefore, the expression level of AdipoR1 and R2 in target tissues may play a role in the control of metabolism and insulin sensitivity. Expression of adiponectin receptors was found reduced in *ob/ob* and *db/db* mice [16,17] as well as in skeletal muscle of subjects with a family history of type 2 diabetes mellitus [18]. However, these expression levels were not found decreased in obese Zucker rats [19] or in skeletal muscle of type 2 diabetes mellitus patients [20]. Therefore, the exact role of AdipoRs in insulin-resistant states remains debated and may be different dependent upon the model studied and the cause of insulin resistance.

C Metais and F Forcheron contributed equally to this work.

* Corresponding author. Tel.: +1 33 426688221; fax: +1 33 478777517.
*E-mail address:* beylot@sante.univ-lyon1.fr (M. Beylot).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2008.02.010

Whatever their exact expression level during insulin resistance, it is generally considered that increasing the expression of adiponectin receptors could improve insulin sensitivity and reduce the risk of appearance of type 2 diabetes mellitus and also of complications such as atheroma. This general idea could be modified by the recent demonstration that AdipoR1 and AdipoR2 have some opposed effects on metabolism [13]: invalidation of AdipoR1 induces obesity and glucose intolerance, whereas invalidation of AdipoR2 results in resistance to diet-induced obesity and improved glucose tolerance. These results suggest that the effects of adiponectin on insulin sensitivity depend not only on the expression levels of adiponectin itself and of its receptors but also on the balance between the expression of AdipoR1 and AdipoR2, a point not considered in previous studies. They suggest also that an efficient modulation of AdipoRs expression for the prevention of insulin resistance should indeed stimulate the expression of AdipoR1 but inhibit on the contrary AdipoR2 expression.

Previous studies suggested that AdipoRs expression is controlled by nutritional, hormonal, and metabolic factors, particularly insulin and the fasted or fed state [16,17], although discordant results have been reported [19,21]. Fatty acids [11], liver X receptor, PPAR-α and PPAR-γ agonists [12], adiponectin itself [22], and growth hormone [23] have also been reported to modify AdipoRs expression. Several studies showed some selectivity of the effects on one of the 2 receptors: only AdipoR2 expression was stimulated by PPAR-α and PPAR-γ agonists in macrophages [12] and growth hormone in 3T3-L1 adipocytes [23], and was inhibited by adiponectin [22]. These results suggest that it could be possible to act selectively and/or differently on the expression of the 2 AdipoRs.

In the present study, we studied the expression level of AdipoR1 and R2 in insulin-sensitive tissues of Zucker diabetic rats (ZDF) throughout the development of insulin resistance and diabetes compared with control Zucker rats (L), with special attention to the relative expression of the 2 receptors. We tested also whether a PPAR-α agonist (fenofibrate) and metformin, an insulin-sensitizing molecule widely used in the treatment of type 2 diabetes mellitus, modify these expressions in ZDF rats.

## 2. Materials and methods

### 2.1. Protocols

Male ZDF rats (*fa/fa*) and their control littermates (L, +/+) were from Charles River (L'Arbresle, France). They were housed at arrival (6 weeks old for all rats) in an animal facility with controlled temperature (22°C ± 1°C) and lighting (12-hour light/dark cycle with light on at 7:00 AM). They had free access to water and food during the whole study. All rats were fed a high-fat diet (Purina 5008, protein 26.8%, carbohydrate 56.4%, fat 16.7% of caloric value; IPS, London, United Kingdom) as recommended because this diet is necessary for the appearance of diabetes in male ZDF rats. Food intake and body weight were recorded 5 times per week. A first metabolic investigation was performed in all rats after 1 week of acclimation, at the age of 7 weeks. Thereafter, 5 rats of the control group and 5 of the ZDF group were killed for blood collection and tissue sampling. The remaining control rats were divided into 2 groups (5 rats each): one group was killed at the age of 14 weeks after a second metabolic investigation, and the other group had a metabolic investigation at the age of 14 and 21 weeks before being killed at 21 weeks. The ZDF rats were divided into 3 groups of 10 rats. One group received only the high-fat diet (ZDF group), whereas the other groups were given also fenofibrate (ZDF + F group; 100 mg/[kg d], mixed with diet) or metformin (ZDF + M group; 300 mg/[kg d], mixed with diet). This administration of fenofibrate or metformin started once the first metabolic investigation (7-week–old rats) was completed and was continued until the final sacrifice. Five rats of each group were killed at 14 weeks after a second metabolic investigation, and the remaining 5 were investigated at 14 and 21 weeks before sacrifice at 21 weeks. All experiments were conducted in agreement with the French regulation for experimentation in animals.

### 2.2. Metabolic investigations

Each metabolic investigation comprised sampling in the fed state of blood (250 μL, tail vein) for measurement of blood glucose (One Touch Ultra; Life Technology, Issy-Les-Moulineaux, France) and plasma insulin. An insulin tolerance test was performed also during the initial metabolic investigation (7 weeks) in the postabsorptive state. Food was removed at 7:00 AM, and insulin (1 U/kg) was injected intraperitoneally (IP) at 1:00 PM after an initial blood glucose measurement. Glucose level was measured again 15, 30, 45, 60, 90, and 120 minutes after the injection of insulin.

Five rats of each group were killed for tissue sampling at the age of 7, 14, and 21 weeks. Food was removed at 8:00 AM, and the rats were anesthetized at 2:00 PM (pentobarbital, 60 mg/kg IP). Blood (inferior vena cava) was collected and centrifuged, and plasma was stored at −20°C until analysis (nonesterified fatty acids [NEFA] and triacylglycerols [TAG]). Adipose tissue (epididymal), skeletal muscle (flexor digitorum superficialis), and liver were quickly removed, washed with cold isotonic saline, snap frozen in liquid nitrogen, and stored at −80°C until analysis (determination of messenger RNA [mRNA] level).

### 2.3. Analytical procedures

Plasma NEFA and TAG were measured by enzymatic methods [24] and insulin by enzyme-linked immunosorbent assay (Crystal Chem, Downers Grove, IL). Tissues total mRNAs were purified using TRIZOL reagent (Invitrogen, Cergy-Pontoise, France) with the addition of treatment with DNAse. Concentrations and purity were verified by

measuring optical density at 230, 260, and 280 nm; and integrity was checked by agarose gel electrophoresis. For measurements of individual mRNA levels, total mRNA was reverse transcripted using Superscript II (Invitrogen) and random hexamers. Real-time polymerase chain reaction was performed in a MyIQ thermal cycler (Biorad, Marnes La Coquette, France) using iQ SYBR Green Supermix (Biorad). All samples were run in duplicate along with dilutions of known amounts of target sequence for quantification of initial complementary DNA copies. Results are expressed as the target over 18S RNA concentration ratio (nanograms per microgram). Primer sequences were as follows: for AdipoR1—forward, ctggactattcagggattg; reverse, acacagacgatggagaggta; for AdipoR2—forward, atgtttgccacccctcagta; reverse, agcctatctgccctatggt; and for 18S—forward, tgaggccatgattaagaggg; reverse, agtcggcatcgtttatggtc.

### 2.4. Statistics

All results are shown as mean and SEM. Intragroup comparisons of the values obtained in the various groups of rats (control, ZDF, ZDF + M, ZDF + F) at 7, 14, and 21 weeks were performed by 1-way analysis of variance followed by the Newman-Keuls procedure to locate the differences, or by 2-tailed Student *t* test for unpaired values when data were available only at 14 and 21 weeks (ZDF + M and ZDF + F groups). Between-group comparisons of the values obtained for each metabolic investigation (at 7, 14, or 21 weeks) were also performed by 1-way analysis of variance followed by the Newman-Keuls test. $P < .05$ was considered as indicating a significant difference. Statistical analysis was performed with GraphPad Prism 4.02 software (GraphPad, San Diego, CA).

## 3. Results

### 3.1. Food intake and body weights

Food intake was higher in ZDF rats than in control rats throughout the study ($P < .001$) and was not modified by fenofibrate or metformin administration (Table 1). At 7 weeks, ZDF rats were heavier than control rats ($P < .001$); and there was no difference in body weight between ZDF rats that received thereafter fenofibrate or metformin and those that received no treatment. Control rats gained weight between 7 and 14 weeks ($P < .01$) and between 14 and 21 weeks ($P < .01$). The ZDF rats also gained weight between 7 and 14 weeks ($P < .01$), but less than that of control rats, without further gain at 21 weeks. Their body weight was comparable to that of control rats at 14 weeks and lower at 21 weeks ($P < .01$). Rats receiving fenofibrate gained less weight than the untreated ZDF group; and at 21 weeks, their weight was less than that in the control and ZDF groups ($P < .05$). Metformin had no effect on body weight.

Table 1
Evolution of food intake and of body weight in control rats and in ZDF rats receiving or not fenofibrate or metformin

| | Control | ZDF | ZDF + F | ZDF + M |
|---|---|---|---|---|
| Food intake (g/d) | | | | |
| 7 wk | 18.5 ± 0.4 | 27.5 ± 0.7*** | 26.7 ± 0.7*** | 26.3 ± 0.6*** |
| 14 wk | 26.5 ± 0.9 | 46.9 ± 2.4*** | 43.5 ± 2.4*** | 47.1 ± 2.1*** |
| 21 wk | 26.6 ± 1.0 | 46.5 ± 3.0*** | 46.9 ± 3.7*** | 43.8 ± 2.2*** |
| Body weight (g) | | | | |
| 7 wk | 188 ± 4 | 231 ± 5*** | 253 ± 11*** | 228 ± 8*** |
| 14 wk | 325 ± 14 | 345 ± 5 | 314 ± 5$^{\dagger}$ | 360 ± 13 |
| 21 wk | 409 ± 9 | 361 ± 6** | 326 ± 8***,$^{\dagger}$ | 398 ± 25 |

Results shown are mean and SEM.
**$P < .01$ and ***$P < .001$ vs the corresponding control group.
$^{\dagger}P < .05$ vs the corresponding ZDF group.

Table 2
Evolution of blood glucose, plasma insulin, TAG, and NEFA in control rats and in ZDF rats receiving or not fenofibrate or metformin

| | Control | ZDF | ZDF + F | ZDF + M |
|---|---|---|---|---|
| Glucose (mmol/L) | | | | |
| 7 wk | 7.35 ± 0.29 | 7.26 ± 0.32 | 7.65 ± 0.24 | 7.35 ± 0.30 |
| 14 wk | 6.97 ± 0.39 | 30.15 ± 0.87*** | 31.47 ± 0.48*** | 31.64 ± 0.56*** |
| 21 wk | 7.57 ± 0.28 | 30.24 ± 0.20*** | 33.20 ± 0.18*** | 34.21 ± 0.42*** |
| Insulin (mU/L) | | | | |
| 7 wk | 4.7 ± 0.7 | 16.1 ± 2.9** | 12.7 ± 0.8** | 12.4 ± 1.1** |
| 14 wk | 6.7 ± 1.2 | 4.2 ± 0.9 | 5.2 ± 0.7 | 6.2 ± 0.7 |
| 21 wk | 8.4 ± 0.5 | <0.5** | <0.5** | <0.5** |
| TAG (mmol/L) | | | | |
| 7 wk | 0.53 ± 0.06 | 4.12 ± 0.48*** | | |
| 14 wk | 0.53 ± 0.07 | 3.78 ± 0.39*** | 2.97 ± 0.77**,$^{\dagger}$ | 1.49 ± 0.42*,$^{\ddagger}$ |
| 21 wk | 0.59 ± 0.05 | 5.85 ± 1.03*** | 3.26 ± 0.40***,$^{\dagger}$ | 2.39 ± 0.49**,$^{\dagger}$ |
| NEFA (mmol/L) | | | | |
| 7 wk | 0.61 ± 0.07 | 0.49 ± 0.03 | | |
| 14 wk | 0.55 ± 0.06 | 1.15 ± 0.09** | 0.99 ± 0.07** | 1.05 ± 0.06** |
| 21 wk | 0.54 ± 0.0 | 1.49 ± 0.15*** | 1.31 ± 0.15*** | 1.16 ± 0.12*** |

Results shown are mean and SEM. Blood glucose and plasma insulin were measured in the fed state. Plasma TAG and NEFA were measured only in rats killed in the postabsorptive state for tissue sampling (n = 5 for each group); therefore, they were measured at 7 weeks only in a group of control and ZDF rats.
*$P < .05$, **$P < .01$, and ***$P < .001$ vs the corresponding control group.
$^{\dagger}P < .05$ and $^{\ddagger}P < .01$ vs the corresponding ZDF group.

### 3.2. Insulin and metabolites concentrations

#### 3.2.1. Glucose and insulin levels

At 7 weeks, ZDF and control rats had comparable blood glucose concentrations; but plasma insulin was higher in ZDF rats ($P < .01$) (Table 2), showing the presence of insulin resistance. This resistance to insulin was confirmed by the insulin tolerance test (Fig. 1). Diabetes developed in all ZDF rats around the 10th week. In all ZDF rats, glucose was greater than 25 mmol/L at 14 and 21 weeks. Plasma insulin decreased to values comparable to those of control rats at 14 weeks and less than the detection level at 21 weeks. These evolutions are comparable to those previously reported in ZDF rats [25]. Neither fenofibrate nor metformin modified these evolutions. Previous studies [26,27] found that metformin reduced hyperglycemia in ZDF rats; however, these studies used higher doses of metformin.

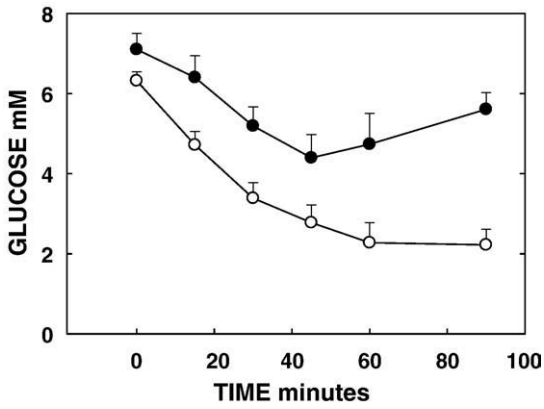


Fig. 1. Insulin tolerance test performed in 7-week–old control (open circles) and ZDF rats (closed circles). Insulin was injected (IP) at time 0.

*3.2.2. Plasma lipid concentrations*

At 7 weeks, L and ZDF rats had comparable NEFA concentrations; but TAG were higher in ZDF rats. Plasma lipid concentrations were unchanged at 14 and 21 weeks in control rats. The NEFA concentrations were much higher in ZDF rats, at 14 or 21 weeks, than both in corresponding control rats at 14 or 21 weeks and in ZDF rats before the appearance of diabetes ($P < .01$). Neither fenofibrate nor metformin modified plasma NEFA levels. Plasma TAG were always higher in ZDF than in control rats at 14 and 21 weeks. Fenofibrate and metformin decreased plasma TAG, with a more marked effect of metformin particularly at 14 weeks.

*3.3. AdipoR mRNA levels*

*3.3.1. Control rats*

AdipoR1 and R2 mRNA concentrations were unchanged with aging in muscles despite a trend for higher values for both receptors at 14 weeks. On the contrary, there was in white adipose tissue (WAT) and liver a decrease in both AdipoR1 and R2 expression at 14 and at 21 weeks, except for AdipoR2 at 21 weeks in liver (Fig. 2). The ratio of AdipoR1 over R2 mRNA concentration was at 7 weeks higher in adipose tissue (5.10 ± 1.82) and muscle (2.40 ± 0.19) than in liver (1.40 ± 0.07, $P < .05$) (Fig. 3). These ratios were unchanged at 14 and 21 weeks except for a moderate increase at 14 weeks in muscle and were always lower in liver than in muscle or WAT ($P < .05$).

*3.3.2. Untreated ZDF rats*

In 7-week–old ZDF rats, mRNA concentrations of adiponectin receptors were comparable to values of control rats in muscle and were increased for AdipoR2 in the liver ($P < .01$), with a lower R1/R2 mRNA ratio than in control rats, but decreased for both receptors in WAT ($P < .05$). These mRNA concentrations did not change after the appearance of diabetes (14- and 21-week–old ZDF rats) in muscle and WAT, despite a trend for higher values of WAT AdipoR1 and R2 at 14 weeks (Fig. 2). The only significant modification was an increase in liver AdipoR1 in 21-week–old ZDF rats ($P < .05$ vs 7-week–old ZDF rats). As a result, the AdipoR1 over R2 mRNA ratio increased in liver (Fig. 3) ($P < .05$ and $P < .01$ at 14 and 21 weeks, respectively). This ratio was unchanged in muscle and decreased in WAT of diabetic (14 and 21 weeks) compared with obese, insulin-resistant (7 weeks) ZDF rats ($P < .05$).

Compared with the corresponding control rats, 14- and 21-week–old diabetic ZDF rats had no decrease in the expression of adiponectin receptors. On the contrary, mRNA levels of AdipoR1 and R2 were increased in liver ($P < .05$ to $P < .001$) (Fig. 2). The AdipoR1 over R2 ratios were identical in muscles of control and ZDF rats, whatever the age. In liver, this ratio was lower in 7-week–old ZDF rats ($P < .01$), but increased in 14- ($P < .05$) and 21-week–old ($P < .01$) ZDF rats (Fig. 3). In adipose tissue, this ratio was lower ($P < .05$) in ZDF rats at 14 and 21 weeks.

*3.3.3. Effects of fenofibrate and metformin administration to ZDF rats*

Fenofibrate and metformin had metabolic actions as shown by the decrease in plasma TAG levels. Fenofibrate had no effect on AdipoRs expression in muscle or WAT. Its only effect was an increase of AdipoR1 and R2 mRNA levels in liver at 14 weeks ($P < .01$, Fig. 2). The ratios R1/R2 were unchanged. On the contrary, metformin induced a large increase in AdipoR1 and R2 expression in muscle (14 and 21 weeks). This stimulation was more important for AdipoR1 than R2, and the ratio R1/R2 increased ($P < .05$). The effects of metformin was different in liver and WAT. In liver, the only effect was a moderate stimulation of AdipoR2 expression in 21-week–old ZDF rats. In WAT, AdipoR1 expression was stimulated as in muscle; but AdipoR2 expression was on the contrary reduced (Fig. 2). Therefore, the R1/R2 ratio, already high in WAT of control and untreated ZDF rats, was dramatically increased ($P < .01$, Fig. 3).

## 4. Discussion

The first aim of the present study was to determine whether insulin resistance and diabetes are associated with modifications of the expression of adiponectin receptors that could play a role in the development of these metabolic abnormalities. To achieve this aim, we measured AdipoRs expression in major insulin-responsive tissues (liver, muscle, WAT) of ZDF rats at 3 different steps of the development of their metabolic abnormalities (insulin resistance without diabetes, overt diabetes, late diabetes with insulin deficiency) and compared these expressions with those of control rats of same ages. The ZDF rats are characterized by insufficient leptin signaling. Leptin has been shown to stimulate the expression of AdipoR1, but not of AdipoR2, in myotubes from control human subjects [28]. However, this effect was lost in myotubes from obese or diabetic subjects. Therefore, although the possibility cannot be fully excluded, it seems unlikely

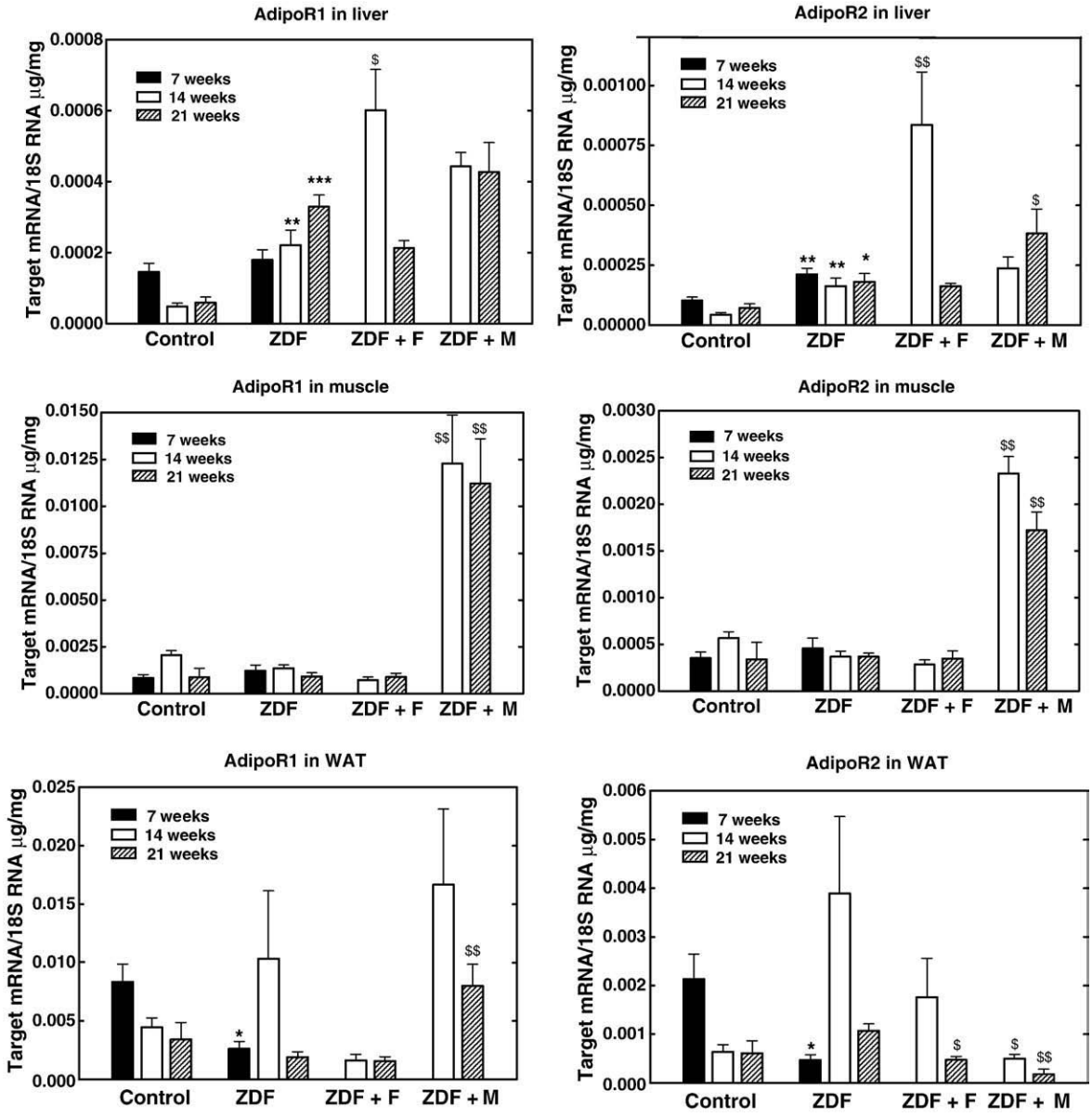


Fig. 2. Concentrations of mRNA for AdipoR1 and AdipoR2, expressed as nanogram target mRNA per microgram of 18S RNA, in liver, skeletal muscle, and WAT of 7-, 14-, and 21-week–old control and Zucker diabetic fatty rats. Zucker diabetic fatty rats received, starting at 7 weeks, no treatment (ZDF), fenofibrate (ZDF + F), or metformin (ZDF + M). Results shown are mean and SEM. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ vs the corresponding control rat of same age. $^{\$}P < .05$ and $^{\$\$}P < .01$ vs the corresponding untreated ZDF rat group of same age. For the clarity of figure, symbols for modifications with age of AdipoRs expression are not indicated.

that the loss of leptin signaling in ZDF rats modified by itself AdipoRs expression.

There was in control rats a clear decrease with increase in age of the expression of adiponectin receptors in adipose tissue and liver, but not in muscle. We are aware of no other longitudinal studies of AdipoR1 or R2 expression in animals or in human beings. If aging is indeed associated with a progressive decline in the expression of adiponectin receptors, this could have a role in the reduced sensitivity to insulin of aged subjects. This will need further studies. At 7 weeks, ZDF rats were insulin resistant. In this situation, expression of adiponectin receptors was not decreased in muscle or in liver. AdipoR2 mRNA levels were on the contrary higher in liver. The only decreases found were in adipose tissue. These results in adipose tissue agree with previous reports in *ob/ob* mice [17] and in obese Zucker rats [19]. Data in liver and muscles agree also with our previous results in obese Zucker rats [19]. In insulin-resistant *ob/ob* mice, Tsuchida et al [17] found on the contrary a reduced expression in liver and muscles. Therefore, there may be species differences in the regulation of the expression of adiponectin receptors. Once diabetes was present in ZDF rats (14- and 21-week–old rats), we found no decrease in any tissue of either AdipoR1 or R2. Their expression was even in

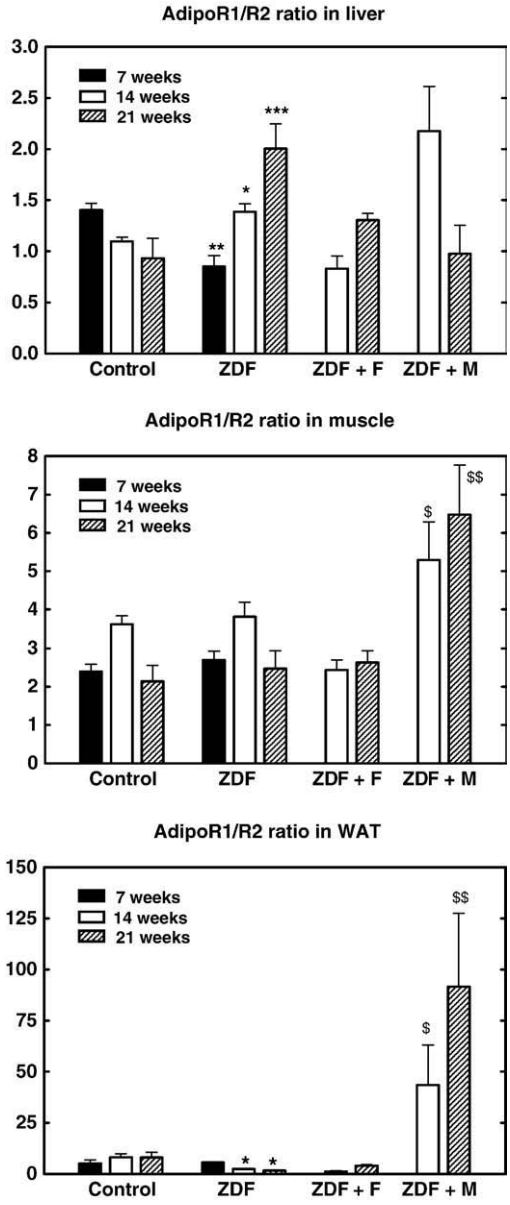


Fig. 3. Ratios of AdipoR1 over AdipoR2 mRNA concentrations in liver, skeletal muscles, and adipose tissue of 7-, 14-, and 21-week–old control and Zucker diabetic fatty rats. Zucker diabetic fatty rats received, starting at 7 weeks, no treatment (ZDF), fenofibrate (ZDF + F), or metformin (ZDF + M). Results shown are mean and SEM. $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$ vs the corresponding control rat of same age. $^{\$}P < .05$ and $^{\$\$}P < .01$ vs the corresponding untreated ZDF rat group of same age. For the clarity of figure, symbols for modifications with age of AdipoRs mRNA ratios are not indicated.

liver higher than that of the corresponding control rats. These results disagree with those reported in diabetic KKAy mice (decreased expression of both receptors in adipose tissue) [29] and in part with those obtained in *db/db* mice (decreased expression of AdipoR1 in liver, muscle, and adipose tissue). Again, this may be linked to species differences. In humans, conflicting data have been reported. Citivarese et al [18] reported that AdipoR1 and R2 expression was reduced in people with a family history of type 2 diabetes mellitus. On the contrary, Debard et al [20] found no decrease of AdipoR1 or R2 mRNA levels in the skeletal muscle of type 2 diabetes mellitus patients compared with healthy subjects. Therefore, a role for decreased expression of adiponectin receptors in the pathogenesis of diabetes remains controversial; and our present results do not bring support to this possibility.

Whatever the exact status of adiponectin receptors in insulin resistance and diabetes, a possibility to ameliorate the insulin-sensitizing action of adiponectin is to stimulate the expression of these receptors. Several investigators explored the possibility that part of the effects of thiazolidinediones on insulin sensitivity could be linked to such a stimulation. The PPAR-$\gamma$ agonists stimulated indeed the expression of AdipoR2 in macrophages [12], cardiomyocytes [30], and HepG2 cells [31] in vitro and in mouse liver in vivo [31], as well as the expression of AdipoR1 in WAT [32]. However, they had in other studies no effect on adipose tissue [29,33] and did not modify [33,34] or decreased [32] adiponectin receptors in muscle. Therefore, the effects of thiazolidinediones do not seem to be mediated by modifications of AdipoRs expression. In the present report, we investigated the effects of fenofibrate, a PPAR-$\alpha$ agonist that has been reported to improve insulin sensitivity in rodents [35,36], and of metformin. The PPAR-$\alpha$ agonists have also been reported to stimulate the expression of AdipoR2 in macrophages [12] and of both receptors in adipose tissue of KKAy mice [29]. No effects in muscle were found by Kaltenbach et al [34]. In the ZDF rats we investigated, the only positive effect of fenofibrate was a transient increase of AdipoR1 and R2 mRNA in liver, a glucose-producing tissue; there was no effect in muscle and a rather inhibitory one in adipose tissue. Therefore, overall, there is no clear stimulatory action of fenofibrate on AdipoRs expression in insulin-sensitive, glucose-utilizing tissues and only moderate action in a glucose-producing tissue. Metformin had on the contrary a net stimulatory effect on both adiponectin receptors in muscle, with a more marked action on AdipoR1, and a mild stimulatory action on liver AdipoR2 and WAT AdipoR1, with on the contrary an inhibitory effect on WAT AdipoR2. Therefore, metformin modifies clearly AdipoRs expression; and this action takes place mainly in glucose-utilizing tissues. The differences in action on AdipoR1 and R2 on one hand and between tissues on the other show, as previously stated, that the regulation of these receptors has some tissue specificities and that their expression can be regulated at least in part independently.

This possibility of acting selectively on the expression of one or the other of the 2 adiponectin receptors rather than stimulating both would be all the more important if AdipoR1 and AdipoR2 have different, and possibly antagonist, actions. This is strongly suggested by recent reports [13,14] showing that invalidation of AdipoR1 reduces glucose tolerance, whereas AdipoR2 invalidation improves glucose tolerance and insulin sensitivity. Therefore, AdipoR1 would have a protective action against insulin

resistance; and AdipoR2 on the contrary would have unfavorable effects. This needs to be confirmed. In particular, it remains to be determined whether these favorable or unfavorable actions are a general property or are dependent upon the tissue investigated. For example, AdipoR2, which appears to have unfavorable action at the whole-body level on insulin sensitivity and glucose tolerance, could have a beneficial, stimulating role in the control of insulin secretion because its invalidation results in a decreased capacity of pancreatic $\beta$-cells to compensate for insulin resistance [14]. If AdipoR1 is indeed a "good" receptor and AdipoR2 is a "bad" receptor, the ability of metformin to raise the R1/R2 mRNA ratio in 2 main glucose-utilizing tissues, muscle and WAT, could indeed participate in its beneficial effects in the treatment.

In conclusion, we found a decrease of AdipoRs expression only in WAT of obese, insulin-resistant ZDF rats. Therefore, the present results do not support an important role for decreased AdipoRs expression in the development of insulin resistance and diabetes. The observed effects of fenofibrate and metformin on AdipoRs expression suggest that part of their beneficial actions could be mediated, particularly for metformin, by modifications of these expressions.

## References


[1] Guerre-Millo M. Adipose tissue and adipokines: for better or worse. Diabetes Metab 2004;30:13-9.

[2] Kadowaki T, Yamauchi T. Adiponectin and adiponectin receptors. Endocrine Rev 2005;26:439-51.

[3] Hotta K, Funahashi T, Bodkin N, Ortmeyer H, Arita Y, Hansen B, et al. Circulating concentrations of the 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.

[4] Lindsay R, Funahashi T, Hanson R, Matsuzawa Y, Tanaka S, Tataranni P, et al. Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 2002;360:57-8.

[5] Yamauchi T, Kamon J, Waki H, Teraudi Y, Kubota N, Hara K, et al. The fat-derived hormone adiponectin reverses insulin-resistance associated with both lipoatrophy and obesity. Nat Med 2001;7: 941-6.

[6] Ouchi N, Kihara S, Funahashi T, Matsuzawa Y, Walsh K. Obesity, adiponectin and vascular inflammatory disease. Curr Opin Lipidol 2003;14:561-6.

[7] Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. Clin Chim Acta 2004;344:1-12.

[8] Tomas E, Tsao T, Saha A, Murrey H, Zhang C, Itani S, et al. Enhanced muscle fat oxidation and glucose transport by ACRP30 globular domain: acetyl-CoA carboxylase inhibition and AMP-activated protein kinase activation. Proc Natl Acad Sci U S A 2002;99:16309-13.

[9] Yamauchi T, Kamon J, Minokoshi Y, Ito Y, Kaki H, Uchida S, et al. Adiponectin stimulates glucose utilization and fatty acid oxidation by activating AMP-activated protein kinase. Nat Med 2002;8:1288-95.

[10] Yamauchi T, Kamon J, Iot Y, Tsuchida A, Yokomizo T, Kita S, et al. Cloning of adiponectin receptors that mediate antidiabetic effects. Nature 2003;423:762-9.

[11] Kharroubi I, Rasschaert J, Eizirik D, Cnop M. Expression of adiponectin receptors in pancreatic beta cell. Biochem Biophys Res Com 2004;312:1118-22.

[12] Chinetti G, Zawadski C, Fruchart J, Staels B. Expression of adiponectin receptors in human macrophages and regulation by agonists of the nuclear receptors PPAR alpha, PPAR gamma and LXR. Biochem Biophys Res Com 2004;314:151-8.

[13] Bjursell M, Ahnmark A, Bohlooly-Y M, William-Olsson L, Rhedin M, Peng XR, et al. Opposing effects of adiponectin receptors 1 and 2 on energy metabolism. Diabetes 2007;56:583-93.

[14] Liu Y, Michael M, Kash S, Bensch W, Monia B, Murray S, et al. Deficiency of adiponectin receptor 2 reduces diet-induced insulin resistance but promotes type 2 diabetes. Endocrinology 2007;148:683-92.

[15] Yamauchi T, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, et al. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. Nat Med 2007:332-9.

[16] Inukai K, Nakashima Y, Watanabe M, Takata N, Sawa T, Kurihara S, et al. Regulation of adiponectin receptor gene expression in diabetic mice. Am J Physiol 2005;288:E876-82.

[17] Tsuchida A, Yamauchi T, Ito Y, Hada Y, Maki T, Takakawa S, et al. Insulin/Foxo1 pathway regulates expression levels of adiponectin receptors and adiponectin sensitivity. J Biol Chem 2004;279:30817-22.

[18] Civitarese A, Jenkinson C, Richardson D, Bajaj M, Cusi K, Kashyap S, et al. Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican American with or without a family history of type 2 diabetes. Diabetologia 2004;47:816-20.

[19] Beylot M, Pinteur C, Peroni O. Expression of the adiponectin receptors AdipoR1 and AdipoR2 in lean rats and in obese Zucker rats. Metabolism 2006;55:396-401.

[20] Debard C, Laville M, Berbe V, Loizon E, Guillet C, Morio-Liondore B, et al. Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of type 2 diabetic patients. Diabetologia 2004;47:917-25.

[21] Staiger H, Kaltenbach S, Staiger K, Stefan N, Frische A, Guirguis A, et al. Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to in vivo parameters of glucose and lipid metabolism. Diabetes 2004;53:2195-201.

[22] Bauche I, Ait El Mkadem S, Rezsohazy R, Funahash iT, Maeda N, Miranda L, et al. Adiponectin downregulates its own production and the expression of its AdipoR2 receptor in transgenic mice. Biochem Biophys Res Commun 2006;345:1414-24.

[23] Fasshauer M, Klein J, Kralisch S, Klier M, Lossner U, Bluher M, et al. Growth hormone is a positive regulator of adiponectin receptor 2 in 3T3-L1 adipocytes. FEBS Letters 2004;28035:27-32.

[24] Forcheron F, Cachefo A, Thevenon S, Pinteur C, Beylot M. Mechanisms of the triglyceride and cholesterol-lowering effect of fenofibrate in hyperlipidemic type 2 diabetic patients. Diabetes 2002; 51:3486-91.

[25] Etgen G, Oldham B. Profiling of Zucker diabetic fatty rats in their progression to the overt diabetic state. Metabolism 2000;49:684-8.

[26] Smith AC, Mullen KL, Junkin KA, Nickerson J, Chabowski A, Bonen A, et al. Metformin and exercise reduce muscle FAT/CD36 and lipid accumulation and blunt the progression of high-fat diet–induced hyperglycemia. Am J Physiol 2007;293:E172-81.

[27] Sreenan S, Sturis J, Pugh W, Burant C, Polonsky K. Prevention of hyperglycemia in the Zucker diabetic fatty rat by treatment with metformin or troglitazone. Am J Physiol 1996;34:E742-7.

[28] McAinch A, Steinberg G, Mollica J, O'Brien P, Dixon J, Macaulay S, et al. Differential regulation of adiponectin receptor gene expression by adiponectin and leptin in myotubes derived from obese and diabetic individuals. Obesity 2006;14:1898-904.

[29] Tsuchida A, Yamauchi T, Takekawa S, Hada Y, Ito Y, Maki T, et al. Peroxisome proliferator-activated receptor (PPAR)alpha activation increases adiponectin receptors and reduces obesity-related inflammation in adipose tissue: comparison of activation of PPARalpha, PPARgamma, and their combination. Diabetes 2005;54:3358-70.

[30] Ding G, Qin Q, He N, Francis-David S, Hou J, Liu J, et al. Adiponectin and its receptors are expressed in adult ventricular cardiomyocytes and upregulated by activation or PPAR gamma. J Mol Cell Cardiol 2007; 43:73-84.

[31] Sun X, Han R, Wang Z, Chen Y. Regulation of adiponectin receptors in hepatocytes by the peroxisome proliferator–activated receptor–gamma agonist rosiglitazone. Diabetologia 2006;49:1303-10.

[32] Tan G, Debard C, Funahashi T, Humphreys S, Matsuzawa Y, Frayn K, et al. Changes in adiponectin receptor expression in muscle and adipose tissue of type 2 diabetic patients during rosiglitazone therapy. Diabetologia 2005;48:1585-9.

[33] Li W, Tonelli J, Kishore P, Owen R, Goodman E, Scherer P, et al. Insulin-sensitizing effects of thiazolidinediones are not linked to adiponectin receptor expression in human, fat or muscle. Am J Physiol Endocrinol Metab 2007;292:E1301-7.

[34] Kaltenbach S, Staiger H, Weisser M, Hass C, Stumvoll M, Machicao F, et al. Adiponectin receptor gene expression in human skeletal muscle cells is not regulated by fibrates and thiazolidinediones. Int J Obes Relat Metab Disord 2005;29:760-5.

[35] Guerre-Millo M, Gervois P, Raspé E, Madsen L, Poulain P, Derudas B, et al. PPAR alpha activators improve insulin sensitivity and reduce adiposity. J Biol Chem 2000;275:16638-42.

[36] Srivastava R, Jahagirdar R, Azhar S, Sharma S, Bisgaier C. Peroxisome proliferator–activated receptor–alpha selective ligand reduces adiposity, improves insulin sensitivity and inhibits atherosclerosis in LDL receptor-deficient mice. Mol Cell Biochem 2006;285:35-50.