

## The role of PPAR $\gamma$ as a thrifty gene both in mice and humans

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The biological role of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) was investigated by gene targeting and case–control study of the Pro12Ala PPAR $\gamma$ 2 polymorphism. Homozygous PPAR $\gamma$ -deficient embryos died at 10.5–11.5 days post conception (dpc) due to placental dysfunction. Heterozygous PPAR $\gamma$ -deficient mice were protected from the development of insulin resistance due to adipocyte hypertrophy under a high-fat diet, whose phenotypes were abrogated by PPAR $\gamma$  agonist treatment. Heterozygous PPAR $\gamma$ -deficient mice showed over-expression and hypersecretion of leptin despite the smaller size of adipocytes and decreased fat mass, which may explain these phenotypes at least in part. This study reveals a hitherto unpredicted role for PPAR $\gamma$  in high-fat diet-induced obesity due to adipocyte hypertrophy and insulin resistance, which requires both alleles of PPAR $\gamma$ . A Pro12Ala polymorphism has been detected in the human PPAR $\gamma$ 2 gene. Since this amino acid substitution may cause a reduction in the transcriptional activity of PPAR $\gamma$ , this polymorphism may be associated with decreased insulin resistance and decreased risk of type 2 diabetes. To investigate this hypothesis, we performed a case–control study of the Pro12Ala PPAR $\gamma$ 2 polymorphism. In an obese group, subjects with Ala12 were more insulin sensitive than those without. The frequency of Ala12 was significantly lower in the diabetic group, suggesting that this polymorphism protects against type 2 diabetes. These results revealed that in both mice and humans, PPAR $\gamma$  is a thrifty gene mediating type 2 diabetes.

### Type 2 diabetes: Obesity: Thiazolidinediones

Hypertrophic obesity due to adipocyte hypertrophy is closely linked to major health issues such as diabetes, hyperlipidemia, hypertension, and cardiovascular diseases (Spiegelman & Flier, 1998). The prevalence of hypertrophic obesity, which often develops in adulthood, is increasing sharply under a high-fat diet and sedentary lifestyle in Western countries and Japan. Insulin resistance, which is usually associated with hypertrophic obesity, is believed to be the major underlying mechanism of these diseases. Although an excess of hormones and metabolic nutrients produced by hypertrophic adipocytes such as tumor necrosis factor  $\alpha$  (TNF $\alpha$ ) and free fatty acids have

been proposed to cause insulin resistance in peripheral tissues such as skeletal muscles and liver (Hotamisligil *et al.* 1993; Boden, 1997), the precise mechanism of insulin resistance remains unclear (Spiegelman & Flier, 1998).

Thiazolidinedione (TZD), a new class of anti-diabetic drugs which increase insulin sensitivity, can directly bind and activate PPAR $\gamma$  and can stimulate adipocyte differentiation (Spiegelman 1998; Spiegelman & Flier, 1998; Lehmann *et al.* 1995). Although TZD can ameliorate insulin resistance in both human and animal models of obese type 2 diabetes, the link between its potency to promote adipocyte differentiation and amelioration of

**Abbreviations:** BMI, body mass index; HOMA, homeostasis model assessment; PPAR $\gamma$ , peroxisome proliferator-activated receptor  $\gamma$ ; TZD, thiazolidinedione.

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insulin resistance has been largely elusive (Spiegelman & Flier, 1998; Saltiel & Olefsky, 1996). We have previously shown that treatment of Zucker fa/fa rats with TZD caused an increase in the number of small adipocytes, and a concomitant decrease in the number of large adipocytes which had produced an excess amount of TNF $\alpha$  and free fatty acids, thereby ameliorating insulin resistance (Okuno *et al.* 1998). These results led us to propose that stimulation of PPAR $\gamma$  with potent synthetic agonists such as TZD may result in adipocyte differentiation and a consequent increase in the number of small adipocytes, thereby rendering the body more sensitive to insulin (Okuno *et al.* 1998). Nevertheless, the physiological roles of PPAR $\gamma$  in mature adipocytes and regulation of insulin sensitivity *in vivo* remain largely unclear.

To investigate the role of PPAR $\gamma$  *in vivo*, we have generated PPAR $\gamma$ -deficient mice by gene targeting (Kubota *et al.* 1999). Homozygous PPAR $\gamma$ -deficient mice were embryonic lethal due to placental dysfunction. Quite unexpectedly, heterozygous PPAR $\gamma$ -deficiency in mice prevented the development of adipocyte hypertrophy and insulin resistance under a high-fat diet. This effect was blocked by use of PPAR $\gamma$  agonist, indicating that decreased activity of PPAR $\gamma$  in PPAR $\gamma$  (+/–) mice indeed conferred protection from adipocyte hypertrophy and insulin resistance under a high-fat diet. Heterozygous PPAR $\gamma$ -deficient mice showed overexpression and hypersecretion of leptin despite the smaller size of adipocytes and decreased fat mass, which may explain these phenotypes at least in part. This study provides the evidence that both PPAR $\gamma$  alleles are required for high-fat diet-induced obesity due to adipocyte hypertrophy and insulin resistance, a hitherto unpredicted role of PPAR $\gamma$  in mature adipocytes (Kubota *et al.* 1999).

In humans, a Pro12Ala substitution has been detected in the PPAR $\gamma$ 2 gene (Yen *et al.* 1997). This amino acid is located in the PPAR $\gamma$ 2 domain that enhances ligand-independent activation. The non-conservative substitution of proline to alanine may cause a conformational change in the protein, which may affect its activity. Indeed, a mutant PPAR $\gamma$ 2 with Ala12 had reduced transactivation activity (Deeb *et al.* 1998). Based on the fact that this amino acid is highly conserved and that codon 12 in the mouse PPAR $\gamma$ 2 gene is also proline (Yen *et al.* 1997), we hypothesized that subjects with this polymorphism may be partially protected from type 2 diabetes. To test this hypothesis we performed an association study regarding this polymorphism. Frequency of subjects bearing Ala allele was significantly higher in non-diabetic subjects associated with the decreased risk of type 2 diabetes. In obese subjects, Ala allele bearers were more insulin sensitive than non-bearers.

These findings provide profound insights into the role of PPAR $\gamma$  mediating insulin resistance and type 2 diabetes.

#### **High-fat diet-induced adipocyte hypertrophy requires both PPAR $\gamma$ alleles**

Heterozygous PPAR $\gamma$ -deficient mice showed normal weight gain and insulin sensitivity under a standard diet. To determine the role of PPAR $\gamma$  in high-fat diet-induced obesity and insulin resistance, we studied body weight gain

and white adipose tissue mass of wild-type and heterozygous PPAR $\gamma$ -deficient mice under either a high-carbohydrate diet or a high-fat diet. Body weight gain under a high-carbohydrate diet for 15 weeks was not distinguishable between wild-type and heterozygous PPAR $\gamma$ -deficient mice. Under a high-fat diet, wild-type mice gained significantly more body weight than that under a high-carbohydrate diet. In contrast, heterozygous PPAR $\gamma$ -deficient mice had little weight gain under a high-fat diet. Although the total white adipose tissue mass of epididymal, retroperitoneal, and perirenal fat pads was significantly larger under a high-fat diet in wild-type mice, an increase in white adipose tissue mass under a high-fat diet was inhibited by more than 70 % in heterozygous PPAR $\gamma$ -deficient mice (Kubota *et al.* 1999).

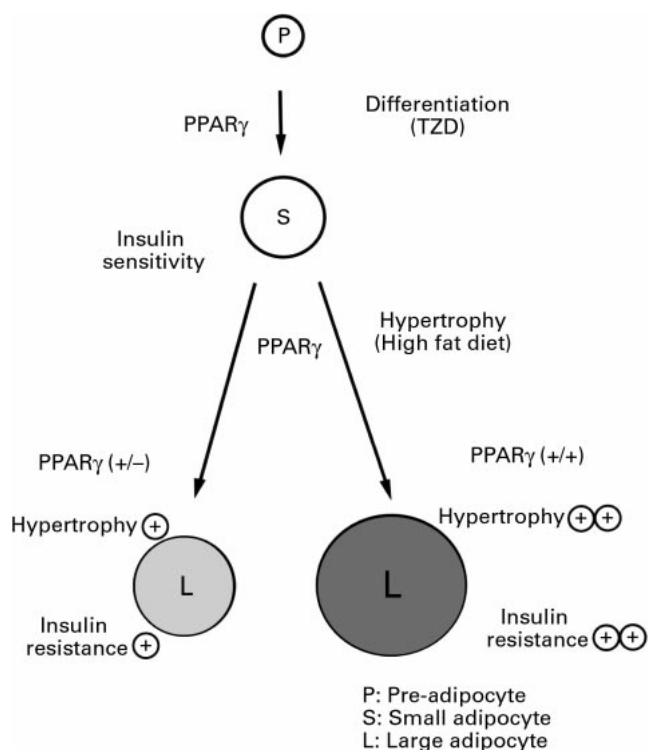
Histological analyses of epididymal fat pads after direct fixation and quantification of adipocyte size revealed that adipocytes from wild-type mice and heterozygous PPAR $\gamma$ -deficient mice under a high-fat diet were significantly larger than those from both types of mice under a high-carbohydrate diet. However, the size of adipocytes from heterozygous PPAR $\gamma$ -deficient mice was significantly smaller than that of adipocytes from wild-type mice under a high-fat diet (Kubota *et al.* 1999). The number of adipocytes from both types of mice under either a high-fat or a high-carbohydrate diet was similar.

#### **Heterozygous deficiency of PPAR $\gamma$ protects against high-fat diet-induced insulin resistance**

We next investigated whether heterozygous PPAR $\gamma$ -deficient mice were associated with insulin resistance or insulin sensitivity. Before a high-fat diet, the glucose lowering effect of insulin was similar between wild-type and heterozygous PPAR $\gamma$ -deficient mice. Surprisingly, after a 15-week high-fat diet, the glucose lowering effect of insulin was markedly larger in heterozygous PPAR $\gamma$ -deficient mice than in wild-type mice. Thus, heterozygous PPAR $\gamma$ -deficient mice showed insulin sensitivity rather than insulin resistance as compared to wild-type mice under a high-fat diet (Kubota *et al.* 1999).

#### **Mechanism for protection from high-fat diet-induced adipocyte hypertrophy and insulin resistance-role of leptin**

To determine the mechanism for the protection against increased fat mass due to adipocyte hypertrophy and development of insulin resistance under a high-fat diet in heterozygous PPAR $\gamma$ -deficient mice, we first studied food intake. Food intake after a high-fat diet was significantly lower in heterozygous PPAR $\gamma$ -deficient mice than in wild-type mice. We next studied energy expenditure. Rectal temperature of heterozygous PPAR $\gamma$ -deficient mice was significantly higher than that of wild-type mice under a high-fat diet, indicating that energy expenditure was increased. The total brown adipose tissue mass was approximately 40 % smaller in heterozygous PPAR $\gamma$ -deficient mice than in wild-type mice under a high-fat diet. Importantly, the adipocytes in brown adipose tissue in heterozygous PPAR $\gamma$ -deficient mice were approximately



**Fig. 1.** PPAR $\gamma$  mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance.

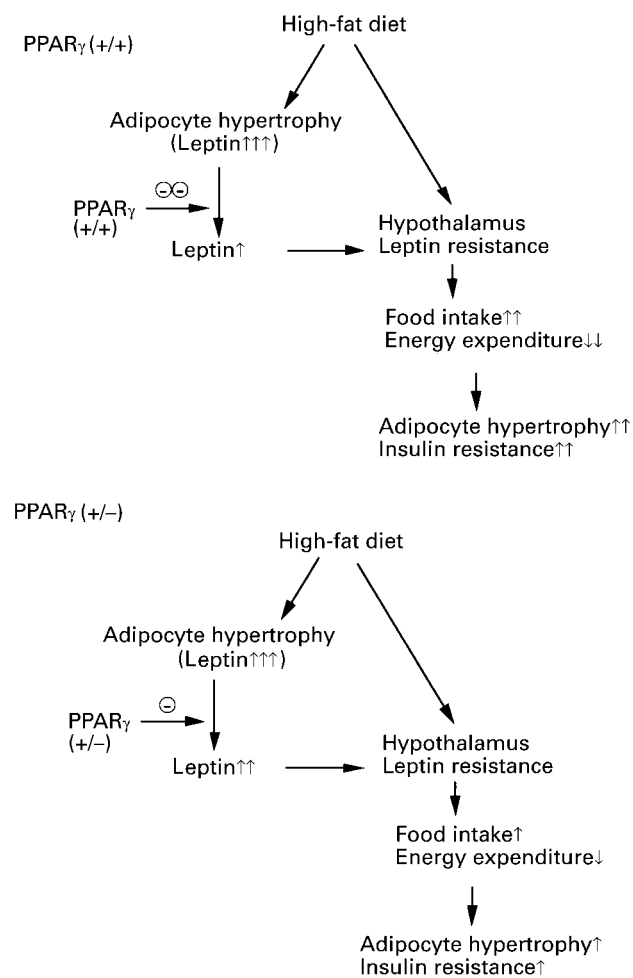
36 % smaller than those from wild-type mice, which may be consistent with the higher energy expenditure in heterozygous PPAR $\gamma$ -deficient mice under a high-fat diet (Kubota *et al.* 1999).

Leptin may be a candidate molecule to account for the reduced food intake and increased energy expenditure in heterozygous PPAR $\gamma$ -deficient mice (Friedman & Halaas, 1998). We thus studied the expression of leptin mRNA in white adipose tissue. Expression levels of leptin were similar between wild-type and heterozygous PPAR $\gamma$ -deficient mice under a high-carbohydrate diet. Under a high-fat diet, expression of leptin was increased only modestly in wild-type mice. Surprisingly, expression levels of leptin were more markedly increased in heterozygous PPAR $\gamma$ -deficient mice despite the fact that adipocytes under a high-fat diet were significantly smaller than in wild-type mice. Moreover, serum leptin levels were approximately 1-8-fold higher in heterozygous PPAR $\gamma$ -deficient mice under a high-fat diet than those in wild-type mice despite the fact that mass of white adipose tissue under a high-fat diet was significantly less than in wild-type mice. We next addressed whether this increase in leptin expression levels reflected an increased ability of heterozygous PPAR $\gamma$ -deficient adipocytes to express and secrete leptin. Thus, we measured leptin mRNA levels by ribonuclease protection assay of EF cells during *in vitro* adipocyte differentiation experiments from wild-type, heterozygous and homozygous PPAR $\gamma$ -deficient embryos. Interestingly, while leptin mRNA in homozygous mutants was completely abrogated, it was increased in heterozygous mutants (data not shown) despite the decreased triglyceride

contents. Moreover, secretion of leptin into the medium was significantly higher with EF cells from heterozygous PPAR $\gamma$ -deficient embryos than those from wild-type embryos. We next examined the leptin sensitivity in wild-type and heterozygous PPAR $\gamma$ -deficient mice under a high-fat diet. Leptin administered as a daily intraperitoneal injection of 5 mg/g body weight per day caused a similar degree of reduction in food intake and body weight in the two mouse genotypes. These results indicated that the leptin sensitivity was similar between the two mouse genotypes (Kubota *et al.* 1999).

### Roles of PPAR $\gamma$ in the regulation of adipocyte size and insulin sensitivity: a novel model

Based upon the experimental results we propose the following model with respect to the roles of PPAR $\gamma$  in the regulation of adipocyte size and insulin resistance under a high-fat diet. In wild-type mice, a high-fat diet promotes adipocyte hypertrophy that converts small adipocytes (S) into large adipocytes (L), which in turn



**Fig. 2.** Suppression of leptin expression by PPAR $\gamma$  plays a role in high-fat diet-induced adipocyte hypertrophy and insulin resistance.

**Table 1.** Genotypic and allelic distribution of the PPAR $\gamma$ 2 Pro12Ala polymorphism in type 2 diabetic and non-diabetic subjects

Subjects	Pro/Pro	Pro/Ala + Ala/Ala	Allelic frequency of Ala12
Type 2 diabetic subjects ( <i>N</i> = 415)	400 (96.4 %)	15 (3.6 %)**	0.018**
Non-diabetic subjects ( <i>N</i> = 541)	496 (91.7 %)	45 (8.3 %)**	0.043**

Pro/Pro, homozygous for Pro12 allele; Pro/Ala, heterozygous for Pro12 and Ala12 alleles; Ala/Ala, homozygous for Ala12 allele.  
\*\**P* < 0.005 by  $\chi^2$  test.

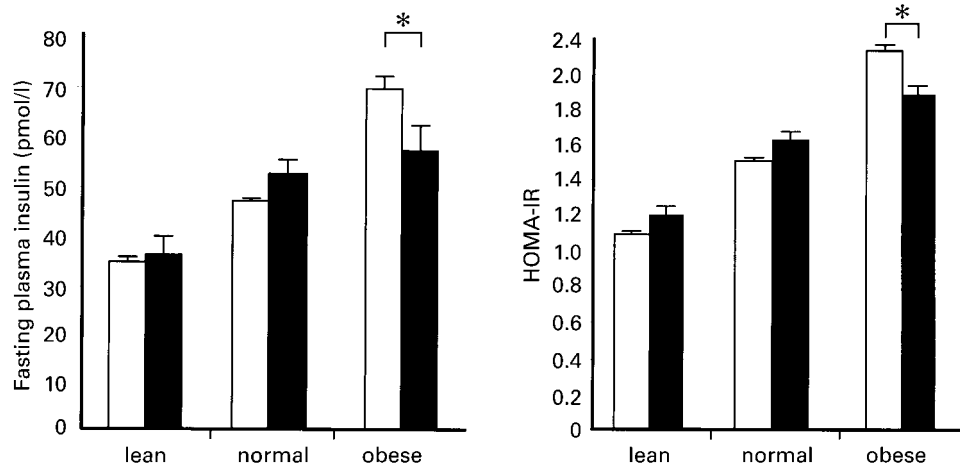
induce factors such as TNF $\alpha$  and free fatty acids thereby causing insulin resistance. In heterozygous PPAR $\gamma$  deficient mice, adipocyte hypertrophy and development of insulin resistance under a high-fat diet are partially protected.

Thus, it seems likely that PPAR $\gamma$  plays dual roles in the regulation of insulin sensitivity. In the presence of potent synthetic ligands such as TZD, and presumably in childhood, the differentiation of adipocytes and hence the number of adipocytes are dependent upon activation of PPAR $\gamma$ . PPAR $\gamma$  stimulation results in adipocyte differentiation to generate small adipocytes and insulin sensitivity. In adults under a high-fat diet, however, adipocyte hypertrophy and hence the size of adipocytes is dependent upon the amount of PPAR $\gamma$ . PPAR $\gamma$  stimulation results in adipocyte hypertrophy to generate large adipocytes and insulin resistance.

Fig. 2 shows our model for the role of leptin in relation to adipocyte size and insulin sensitivity. In wild-type mice, high-fat diet-induced adipocyte hypertrophy and insulin resistance may be caused by impaired leptin effects due to central leptin resistance (Frederich *et al.* 1995) and relative suppression of leptin expression by two alleles of PPAR $\gamma$ . In heterozygous PPAR $\gamma$ -deficient mice, on the other hand, high-fat diet-induced adipocyte hypertrophy and insulin resistance may partially be protected by a relative preservation of leptin effects due to a partial release from suppression of leptin expression by loss of one PPAR $\gamma$  allele.

### Pro12Ala polymorphism in PPAR $\gamma$ is associated with type 2 diabetes and insulin resistance

To investigate whether PPAR $\gamma$  plays a role in insulin resistance and onset of type 2 diabetes in humans, we compared the frequency of Ala12 bearer in the non-related type 2 diabetic and non-diabetic subjects. Non-diabetic subjects who were over the age of 60, had HbA1c values lower than 6.0 %, and no family history of type 2 diabetes were recruited from an unselected population who underwent a routine health check-up at the Hiroshima Atomic Bomb Casualty Council Health Management Center. The diabetic subjects were recruited from the outpatient clinic of the Institute for Diabetes Care and Research, Asahi Life Foundation and the Department of Metabolic Diseases, Tokyo University. This study was performed under informed consent from all subjects and was approved by the Ethics Committee of Tokyo University. The frequency of subjects bearing the Ala12 allele was significantly lower in the diabetic group (3.6 %) than in the non-diabetic group (8.3 %, *P* = 0.003) see Table 1 (Hara *et al.* 2000). Subjects with the Ala12 allele had a decreased risk for type 2 diabetes [odds ratio (OR) = 0.413, 95 %CI: 0.220–0.735] (Hara *et al.* 2000). After adjustment for age, gender, and BMI, this reduced risk was still observed (OR = 0.324, 95 %CI: 0.152–0.658) (Hara *et al.* 2000). To assess the insulin resistance and  $\beta$ -cell function of subjects, we used the homeostasis model assessment (HOMA-IR and HOMA- $\beta$ , respectively) (Matthews *et al.* 1985). Neither



**Fig. 3.** Influence of the PPAR $\gamma$  Pro12Ala polymorphism on fasting plasma insulin (left panel) and insulin resistance index (right panel), in non-diabetic subjects with (solid bars) or without (open bars) the Ala12 allele. \**P* < 0.05.

HOMA-IR nor HOMA-b was different between subjects with or without the Ala12 allele (data not shown). Since it is well known that obesity has a confounding effect on variables relevant to insulin resistance, we subdivided non-diabetic subjects into three groups according to body mass index (BMI) (lean, BMI <22 kg/m<sup>2</sup>: normal, 22 kg/m<sup>2</sup>  $\leq$  BMI < 25 kg/m<sup>2</sup> : obese, 25 kg/m<sup>2</sup>  $\leq$  BMI). In lean and normal groups, neither fasting plasma insulin nor HOMA-IR were different between subjects with and without the Ala12 allele. However in the obese group, both fasting plasma insulin ( $57.4 \pm 5.23$  v.  $70.6 \pm 2.53$  pmol/l,  $P = 0.03$ ) and HOMA-IR ( $1.89 \pm 0.18$  v.  $2.35 \pm 0.09$ ,  $P = 0.03$ ) were lower in subjects with the Ala12 allele than those without (Fig. 3) (Hara *et al.* 2000). Since the average BMI of subjects with the Ala12 allele was comparable to that of those without, these results suggest that having the Ala12 allele may protect against insulin resistance which would normally arise in obese subjects. The plasma leptin levels tended to be higher in subjects with the Ala12 allele ( $8.02 \pm 1.04$  ng/ml) than in those without ( $6.27 \pm 0.20$  ng/ml) (Hara *et al.* 2000). Thus it is possible that higher levels of leptin might be associated with a relatively increased insulin sensitivity in subjects bearing the Ala12 allele. These findings suggest that the Ala12 allele of the PPAR $\gamma$ 2 gene may protect subjects with this polymorphism from type 2 diabetes.

In conclusion, PPAR $\gamma$  has the pivotal role in adipocyte hypertrophy and insulin resistance induced by high-fat diet, which requires two alleles of wild type PPAR $\gamma$ . Partial reduction in dose or activity of PPAR $\gamma$ , due to genetically inherited or functional antagonist, leads to the protection against obesity and type 2 diabetes induced by a high-fat diet. Both in mice and humans, PPAR $\gamma$  is an important thrifty gene mediating insulin resistance and type 2 diabetes.

## References

- Boden G (1997) Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes* **46**, 3–10.
- Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W & Auwerx J (1998) A Pro12Ala substitution in PPAR $\gamma$ 2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. *Nature Genetics* **20**, 284–287.
- Frederich RC, Hamann A, Anderson S, Lollmann B, Lowell BB & Flier JS (1995) Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. *Nature Medicine* **1**, 1311–1314.
- Friedman JM & Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* **395**, 763–770.
- Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C & Kadowaki T (2000) The Pro12Ala polymorphism in PPAR $\gamma$ 2 may confer resistance to type 2 diabetes. *Biochemical and Biophysical Research Communications* **271**, 212–216.
- Hotamisligil GS, Shargill NS & Spiegelman BM (1993) Adipose expression of tumor necrosis factor  $\alpha$ : direct role in obesity-linked insulin resistance. *Science* **259**, 87–91.
- Kubota N, Terauchi Y, Miki H, Tamemoto H, Yamauchi T, Komeda K, Satoh S, Nakano R, Ishii C, Sugiyama T, Eto K, Tsubamoto Y, Okuno A, Murakami K, Sekihara H, Hasegawa G, Naito M, Toyoshima Y, Tanaka S, Shiota K, Kitamura T, Fujita T, Ezaki O, Aizawa S & Kadowaki T (1999) PPAR $\gamma$  mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. *Molecular Cell* **4**, 597–609.
- Lehmann JM, Moore LB, Smith-Oliver TA, Wilkison WO, Willson TM & Kliewer SA (1995) An antidiabetic thiazolidinedione is a high affinity ligand of peroxisome proliferator-activated receptor  $\gamma$ . *Journal of Biological Chemistry* **270**, 12953–12956.
- Matthews DR, Rudenski AS, Naylor BA, Treacher DF & Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412–419.
- Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y & Kadowaki T (1998) Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. *Journal of Clinical Investigation* **101**, 1354–1361.
- Saltiel AR & Olefsky JM (1996) Thiazolidinediones in the treatment of insulin resistance and type II diabetes. *Diabetes* **54**, 1661–1669.
- Spiegelman BM & Flier JS (1998) Adipogenesis and obesity: rounding out the big picture. *Cell* **87**, 377–389.
- Spiegelman BM (1998) PPAR $\gamma$  adipogenic regulator and thiazolidinedione receptor. *Diabetes* **47**, 507–514.
- Yen CJ, Beamer BA, Negri C, Silver K, Brown KA, Yarnall DP, Burns DK, Roth J & Shuldiner AR (1997) Molecular scanning of the human peroxisome proliferator activated receptor  $\gamma$  (hPPAR $\gamma$ ) gene in diabetic Caucasians: identification of a Pro12Ala PPAR $\gamma$ 2 missense mutation. *Biochemical and Biophysical Research Communications* **241**, 270–274.