Molecular Mechanism of Insulin Resistance and Obesity

TAKASHI KADOWAKI,*,[1] KAZUO HARA,*,† TOSHIMASA YAMAUCHI,* YASUO TERAUCHI,* KAZUYUKI TOBE,* AND RYOZO NAGAI†

Department of Metabolic Diseases, and †Department of Clinical Bioinformatics, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan

Obesity and insulin resistance have been recognized as leading causes of major health issues. We have endeavored to depict the molecular mechanism of insulin resistance, focusing on the function of adipocyte.

We have investigated a role of PPARγ on the pathogenesis of Type II diabetes. Heterozygous PPARγ-deficient mice were protected from the development of insulin resistance due to adipocyte hypertrophy under a high-fat diet. Moreover, a Pro12Ala polymorphism in the human PPARγ2 gene was associated with decreased risk of Type II diabetes in Japanese. Taken together with these results, PPARγ is proved to be a thrifty gene mediating Type II diabetes. Pharmacological inhibitors of PPARγ/RXR ameliorate high-fat diet-induced insulin resistance in animal models of Type II diabetes.

We have performed a genome-wide scan of Japanese Type 2 diabetic families using affected sib pair analysis. Our genome scan reveals at least 9 chromosomal regions potentially harbor susceptibility genes of Type II diabetes in Japanese. Among these regions, 3q26-q28 appeared to be very attractive one, because of the gene encoding adiponectin, the expression of which we had found enhanced in insulin-sensitive PPARγ-deficient mice. Indeed, the subjects with the G/G genotype of SNP276 in the adiponectin gene were at increased risk for Type II diabetes compared with those having the T/T genotype. The plasma adiponectin levels were lower in the subjects with the G allele, suggesting that genetically inherited decrease in adiponectin levels predispose subjects to insulin resistance and Type II diabetes. Our work also confirmed that replenishment of adiponectin represents a novel treatment strategy for insulin resistance and Type II diabetes using animal models. Further investigation will be needed to clarify how adiponectin exerts its effect and to discover the molecular target of therapies. Exp Biol Med 228:1111–1117, 2003

Key words: adiponectin; PPARγ; insulin resistance; obesity; adipocyte

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[1] To whom requests for reprints should be addressed at University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: kadowaki-3im@h.u-tokyo.ac.jp

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Obesity and insulin resistance have been quite well recognized as fundamental and leading causes of major health issues such as diabetes, hyperlipidemia, hypertension, and cardiovascular diseases. There is a plentiful body of evidence indicating high-fat diet is the major cause of obesity and insulin resistance. Indeed, concordant with a rapid increase in fat consumption in recent Japan, the number of people with diabetes is increasing rapidly to be estimated around 6.9 million by the report of the national survey in 1997 (1). However, the molecular mechanism of how high-fat diet induces obesity and how obesity causes insulin resistance and a wide range of metabolic disorders remains to be vigorously investigated. This article focuses on the role of adipocyte and its related molecules, which have essential roles in regulating insulin sensitivities. By our work and others, it has been widely accepted that adipose tissue is not a mere storage of fat but endocrine tissue, which secretes molecules regulating insulin sensitivities. We have also endeavored to depict genetic background predisposing people of Japan to Type II diabetes by molecular genetics using SNP (single nucleotide polymorphism).

The Role of PPARγ as a Thrifty Genotype

Clarifying the Role of PPARγ as a Thrifty Gene through Gene Targeting. PPARγ (peroxisome proliferative activated receptor) is a transcription factor that is abundantly expressed in adipose tissue, having a key role in adipocyte differentiation. Thiazolidinediones (TZD), an insulin-sensitizing drug, can stimulate adipocyte differentiation via binding directly to PPARγ and activate it. We have previously shown that treatment of Zucker fa/fa rats with TZD increased the number of small adipocytes and decreased the number of large adipocytes that produced a large amount of TNFα and free fatty acids, presumably leading to insulin resistance (2). These results led us to propose that stimulation of PPARγ with potent synthetic agonist increases the number of small adipocytes through adipocyte differentiation, ameliorating insulin resistance (2). However, it is generally accepted that adipocyte differ-

entiation does not usually take place in adipose tissue of adulthood and adipocyte differentiation by TZD is far from a physiological event. To clarify the physiological role of PPARγ *in vivo*, we have generated PPARγ-deficient mice by gene targeting (3). Homozygous PPARγ-deficient mice were embryonic lethal due to placental dysfunction. To investigate the role of PPARγ, we studied the phenotypes of heterozygous PPARγ-deficient mice under a high-carbohydrate diet and a high-fat diet. Expectedly, under a high-fat diet, both wild-type and heterozygous PPARγ-deficient mice gained significantly more body weight than under a high-carbohydrate diet. However, unexpectedly, we found that weight gain and an increase in white adipose tissue mass under a high-fat diet was significantly less in heterozygous PPARγ-deficient mice than wild-type mice. Histological analyses revealed that under a high-fat diet, adipocytes from wild-type mice and heterozygous PPARγ-deficient mice were significantly larger than those under a high-carbohydrate diet. Very interestingly, the size of adipocytes from heterozygous PPARγ-deficient mice was significantly smaller than that of adipocytes from wild-type mice under a high-fat diet. Surprisingly, the glucose-lowering effect of insulin was larger in heterozygous PPARγ-deficient mice than in wild-type mice, indicating that under a high-fat diet, heterozygous PPARγ-deficient mice were more insulin sensitive than wild-type mice. We further investigated to clarify the mechanism of this unexpected phenotype of PPARγ-deficient mice under a high-fat diet. Food intake was significantly lower in heterozygous PPARγ-deficient mice than in wild-type mice. Rectal temperature of heterozygous PPARγ-deficient mice was significantly higher than that of wild-type mice and adipocytes in BAT in heterozygous PPARγ-deficient mice were smaller than those from wild-type mice, indicating that energy expenditure was higher in PPARγ-deficient mice than in wild-type mice. These results suggested that PPARγ is a thrifty gene mediating high-fat diet induced obesity, adipocyte hypertrophy, and insulin resistance. In wild-type mice, a high-fat diet promotes adipocyte hypertrophy, which converts small adipocytes into large adipocytes, which in turn induce factors such as TNFα and free fatty acids, thereby causing insulin resistance. In heterozygous PPARγ-deficient mice, adipocyte hypertrophy and development of insulin resistance under a high-fat diet are partially protected (3–5).

Clarifying the Role of PPARγ as a Type II-Diabetes Susceptibility Gene through Molecular Genetics. In humans, a Pro12Ala substitution has been detected in the PPARγ2 gene. This non-conservative substitution of proline to alanine reduces transactivational activity of PPARγ by 20% to 30% (6). Based on the results that PPARγ-deficient mice were more insulin sensitive compared with wild type, we hypothesized that subjects with this polymorphism may be protected from Type II diabetes. To test this hypothesis we compared the frequency of Ala12 bearer in the non-related Type II diabetic and non-diabetic subjects. 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%) ($P = 0.003$) (7). Indeed, subjects with the Ala12 allele had a decreased risk for Type II diabetes (OR = 0. 413, 95% CI; 0.220–0.735). After this work on PPARγ Pro12Ala polymorphism, we and 9 other groups in Japan performed a large and collaborative work to confirm the protective effect of this polymorphism; 2201 subjects with Type II diabetes and 1212 normal control subjects participated in this study (8). The allele frequency for the Ala12 variant was significantly lower in the Type II diabetic group than in the control group (2.39 vs 4.13%, $P = 0.000054$), consistent with the previous result. Meta-analysis of major published reports on the association between this polymorphism and Type II diabetes confirmed that the Ala12 allele is consistently associated with reduced risk of diabetes in several ethnic groups (9).

PPARγ: Therapeutic Target of Insulin Resistance and Type II Diabetes. The results in mice and humans indicate that partial decrease of PPARγ activity exerted protection from high-fat-diet induced adipocyte hypertrophy, obesity, and insulin resistance (3). These findings raise the possibilities that functional antagonism of PPARγ/RXR could serve as a fundamental treatment strategy for obesity and Type II diabetes especially in westernized countries. To address these issues, we investigated the effects of the RXR antagonist, HX531, on body weight, glucose, and insulin concentrations in an animal model of diabetes, KKAy mice (10), on HF diet. Untreated KKAy mice gained significantly more weight than the mice on the high-carbohydrate (HC) diet, whereas treatment with HX531 prevented an increase in weight on the high-fat (HF) diet. Treatment with HX531 also prevented HF diet-induced hyperglycemia and hyperinsulinemia. On the HF diet, the glucose-lowering effect of insulin was greater in mice treated with HX531 than in untreated mice. These findings clearly indicate that the functional antagonist can be an anti-obesity and anti-diabetic drug. Then, how far can we reduce the level of PPARγ activity to treat insulin resistance? To address this issue, we investigated the effects of HX531 on PPARγ- deficient mice, a state of already-reduced PPARγ activity. Administration of HX531 to heterozygous PPARγ-deficient mice for 4 weeks resulted in the disappearance of visible white adipose tissue (WAT), similar to lipoatrophy in humans (11). Like lipoatrophy, heterozygous PPARγ-deficient mice treated with HX531 had marked hyperglycemia and insulin resistance.

PPARγ and Adipocytekines. Recognizing PPARγ as a key molecule mediating high-fat-diet induced obesity and insulin resistance prompted us to investigate the expression profile of PPARγ-deficient mice to elucidate how PPARγ mediates high-fat diet-induced obesity and insulin resistance (Fig. 1). Leptin, abundantly expressed in adipocyte, regulates energy balance by decreasing food intake and increasing energy expenditure (12). Under a high-fat diet, expression of leptin was increased only modestly in wild-type mice. Expression levels of leptin and serum leptin

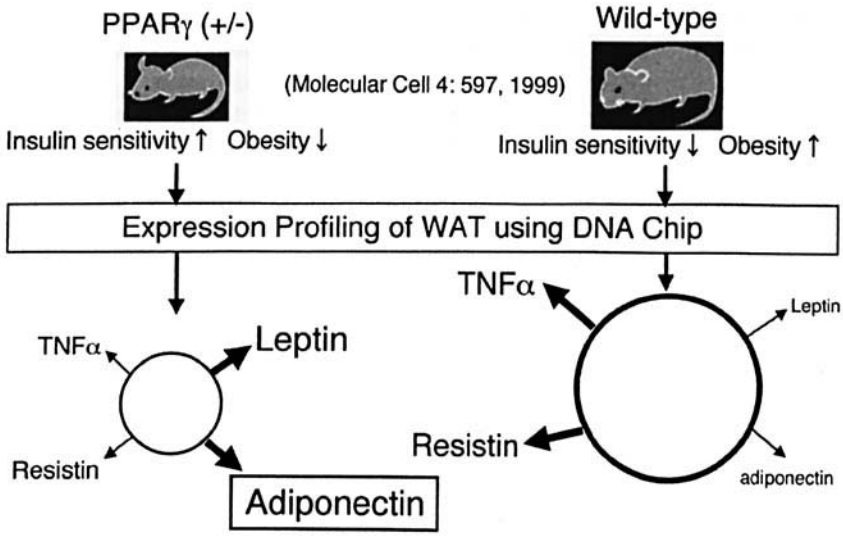

Figure 1. An approach for discovering noel adipocytekines regulating insulin sensitivity.

levels were more markedly increased in heterozygous PPARγ-deficient mice despite the fact that adipocytes under a high-fat diet were significantly smaller than in wild-type mice despite the fact that mass of WAT under a high-fat diet was significantly less than in wild-type mice (3). These results suggested that PPARγ mediate high-fat-diet induced obesity and insulin resistance at least in part through the depression of leptin. Among molecules whose expression was enhanced in PPARγ-deficient mice, we particularly paid attention to adiponectin because of several distinct features. Details of why and how we have investigated adiponectin are described later in this article.

PPARγ-Dependent and Independent Pathway Mediating Adipocyte Hypertrophy

The CBP protein (cAMP response element binding protein (CREB) binding protein) (13) is a co-activator (14) for several transcription factors such as sterol regulatory element binding proteins (SREBPs) (15), CCAAT/enhancer-binding proteins (C/EBPs) (16), nuclear receptors (17,18) including peroxisome proliferator–activated receptors, PPARs (19), and signal transducers and activators of transcription (STATs) (20). These transcription factors are well known to have important biological functions regarding glucose and lipid metabolism, which prompted us to investigate the physiologic role of CBP *in vivo* using *Crebbp*$^{+/-}$ mice generated previously. Fat mass of *Crebbp*$^{+/-}$ mice was significantly decreased compared with that of wild-type mice on a high-carbohydrate (HC) diet (3). Reduction of adipose tissue was mainly attributed to the loss of white adipose tissue (WAT). The size of adipocytes from *Crebbp*$^{+/-}$ mice was markedly smaller than that of adipocytes from wild-type mice on an HC diet. Amounts of adipose tissue in *Crebbp*$^{+/-}$ mice were almost normal at 3 days post natum (21,22), suggesting that markedly reduced WAT mass in *Crebbp*$^{+/-}$ mice was due to the inhibition of triglyceride accumulation in WAT, not due to the inhibition of adipocyte differentiation. Despite the phenotype of lipodystrophy, which exhibits severe insulin resistance in human disease, *Crebbp*$^{+/-}$ mice had a higher insulin sensitivity and increased glucose tolerance compared with wild-type mice on both the HC and HF diet. Expression levels of neuropeptide regulating appetite, such as neuropeptide Y and hypocretin, were comparable in *Crebbp*$^{+/-}$ mice and food intake was not significantly lower in *Crebbp*$^{+/-}$ mice. In contrast, resting oxygen consumption was significantly increased in *Crebbp*$^{+/-}$ mice. In *Crebbp*$^{+/-}$ mice, serum leptin (23) levels were increased in spite of extremely decreased WAT mass. Moreover, the serum levels and mRNA levels in WAT of the insulin-sensitizing hormone adiponectin (24) in *Crebbp*$^{+/-}$ mice were significantly higher than those of wild-type mice, despite their markedly lower WAT mass (25). These data suggested that protection of *Crebbp*$^{+/-}$ from insulin resistance was at least in part mediated by 2 major adipocytekines, leptin and adiponectin. These data suggest that heterozygous *Crebbp* deficiency prevented adipocyte hypertrophy, which finally led to the alleviation of insulin resistance through the diminution of molecules causing insulin resistance, such as FFA and TNFα, as well as through increased effects of insulin-sensitizing hormones secreted from WAT (23, 25, 26). The important roles of adiponectin in regulating insulin sensitivity are described in the next section.

Adiponectin: Key Molecule Mediating Insulin Resistance in Obesity

Adiponectin: Susceptibility Gene of Insulin Resistance and Type II Diabetes in Japanese. Type II diabetes is a complex disorder where multiple genes having weak or moderate genetic effects on the susceptibility to diabetes interact with each other to develop this disease. Combination of susceptibility genes may be different among different families of diabetes. Thus, conservative linkage analysis has no power to detect susceptibility genes of diabetes. It is also possible that there may be different genes in different ethnic backgrounds. This prompted us to

perform a genome-wide scan of Japanese Type II diabetic families using affected sib pair analysis, which requires no specific model of inheritance in disease. Our Japanese genome scan reveals that at least 9 chromosomal regions (1p36-p32, 2q34, 3q26-q28, 6p23, 7p22-p21, 9p, 15q13-q21, and 20q12-q13) potentially harbor susceptibility genes of Type II diabetes in Japanese. Among these chromosomal regions, 3q26-q28 appears to be very attractive one. These data were replicated by another genome-wide scan in French Caucasians and in Caucasians living in the United States. The former detected the strongest linkage to diabetes in 3q27-qter, and the latter found several significant QTL (quantitative trait loci) associated with metabolic traits (BMI, leptin, insulin, and anthropometric measurements) in the same region. Moreover, the gene encoding adiponectin, expression of which we had found enhanced in insulin sensitive PPARγ deficient mice, is located in 3q26-q28. Adiponectin is abundantly expressed in adipose tissue (27–31), but plasma adiponectin levels are reduced in patients with obesity (31), Type II diabetes (32), and coronary artery diseases (32), all of which are closely related to insulin resistance. We were excited by the coincidence to scan the adiponectin gene polymorphism and perform the association study using discovered SNPs to elucidate the role of adiponectin as a susceptibility gene of Type II diabetes. We detected 10 relatively frequent polymorphisms in the adiponectin gene. For SNPs at positions 276 (SNP276), statistically significant differences in the distribution of genotypes ($P = 0.007$) between Type II diabetic and nondiabetic subjects were detected. The subjects with the G/G genotype of SNP276 were at increased risk for Type II diabetes (OR 2.16, 95% CI 1.22–3.95) compared with those having the T/T genotype. After adjusting for possible confounding effects of age, sex, and body mass index (BMI), we found significant associations between SNP276 and the insulin-resistance index. Moreover, the plasma adiponectin levels were lower in the subjects with the G allele, suggesting that genetically inherited decreases in adiponectin levels predispose subjects to insulin resistance and Type II diabetes.

Adiponectin: Adipocyte-Derived Insulin-Sensitizing Hormone. Our work on genetic study on adiponectin gene, and previous reports that plasma adiponectin levels were decreased in Type II diabetes and obesity, prompted us to investigate the role of adiponectin *in vivo* using animal models. Adiponectin expression correlates impressively with insulin sensitivity and adiponectin is decreased in obese mice and depleted in lipoatrophic mice. We investigated whether adiponectin reverses insulin resistance in those mice. Continuous systemic infusion of a physiological dose of recombinant adiponectin significantly ameliorated hyperglycemia and hyperinsulinemia. Insulin resistance in lipoatrophic mice is completely reversed by a combination of physiological doses of adiponectin and leptin but only partially by either adiponectin or leptin alone (24), suggesting that adiponectin/leptin deficiency explains insulin resistance in lipoatrophic mice. We next studied whether adiponectin can improve insulin resistance and diabetes in wild-type mice under high-fat diet, *db/db*, and KKA^y mice (KK mice overexpressing agouti). Continuous systemic infusion of low doses of recombinant adiponectin significantly ameliorated hyperglycemia and hyperinsulinemia in those mice, indicating that high-fat feeding, leptin-receptor deficiency, or agouti overexpression causes insulin resistance, partially through decreases in adiponectin (24). Adiponectin increased expression of molecules involved in fatty-acid transport, combustion and energy dissipation such as CD36 (33, 34), acyl-CoA oxidase (ACO) (35, 36), and uncoupling protein (UCP) 2 (37, 38), indicating that adiponectin acts primarily on skeletal muscle to increase influx and combustion of FFA, thereby reducing muscle triglyceride content. As a consequence of decreased serum FFA and triglyceride levels, hepatic triglyceride content is decreased. Decreased triglyceride content accounts for the improving insulin signaling in both organs. Adiponectin is composed of an N-terminal collagen-like sequence (cAd) and a C-terminal globular region (gAd) (39, 40). Full-length adiponectin undergo proteolytic processing and a small amount of gAd exists in plasma. Interestingly, gAd ameliorated hyperglycemia and hyperinsulinemia much more potently than full-length adiponectin.

We further investigated the role of adiponectin by generating adiponectin-deficient mice. Heterozygous adiponectin-deficient ($adipo^{+/-}$) mice showed mild insulin resistance, while homozygous adiponectin-deficient ($adipo^{-/-}$) mice showed moderate insulin resistance and glucose intolerance despite a body weight gain similar to that of wild-type mice. This study provides the first direct evidence that adiponectin plays a protective role against insulin resistance and atherosclerosis *in vivo*. These observations clearly indicated that adiponectin is indeed an insulin-sensitizing hormone and exerts a protective role against insulin resistance *in vivo*.

Molecular Mechanism of Insulin-Sensitizing Effect by Adiponectin. What are the signaling molecules that mediate the metabolic effects of adiponectin? The activation of 5′-AMP-activated protein kinase (AMPK) by muscle contraction has been reported to increase fatty-acid oxidation (31, 41, 42) and glucose uptake in skeletal muscle (43). Activation of AMPK reduces expression levels of phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase), key molecules of gluconeogenesis in hepatocytes (44). AMPK is a molecular candidate mediating the insulin-sensitizing effect of exercise (45–47) and the antidiabetic drug metformin (48–49). We investigated the role of AMPK in the signal transduction of adiponectin in the liver and skeletal muscle. The administration of gAd or full-length Ad in mice increased AMPK phosphorylation in the soleus muscle in a dose-dependent fashion. Globular Ad had a more pronounced effect on AMPK activation in skeletal muscle. In contrast to muscle, only full-length Ad, which has a higher binding affinity to the membrane fractions of the liver, was capable of stimulating phosphorylation and activation of AMPK in the liver. Both globular and

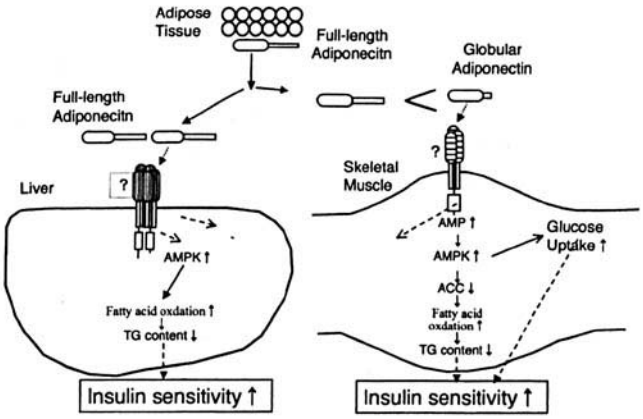

Figure 2. Mechanism of sensitizing effect of adiponectin in liver and muscle.

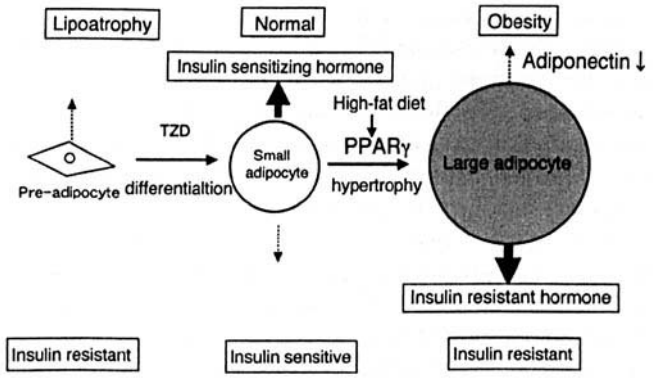

Figure 3. Molecular mechanism of insulin resistance induced by hypertrophic obesity ("small adipocyte" hypothesis).

full-length Ad induced an increase in ACC phosphorylation, fatty-acid oxidation, glucose uptake, and lactate production in C2C12 myocytes. These effects were blocked by the retrovirus- or adenovirus-mediated expression of dominant negative (DN) AMPK. Thus, the activation of AMPK is necessary for the Ad-induced stimulation of ACC phosphorylation, fatty-acid oxidation, and glucose uptake and lactate production in muscle cells. We next studied the mechanisms by which the expression of DN AMPK in the liver reduced the glucose-lowering effect of Ad. Full-length Ad reduced expression levels of molecules involved in gluconeogenesis such as PEPCK and G6Pase in the liver as reported (7). Expression of DN-1AMPK in the liver blocked these effects of Ad, which was consistent with a previous report that activation of AMPK reduced expression levels of PEPCK and G6Pase (44). Thus, activation of AMPK in the liver is necessary for Ad to reduce expression levels of molecules involved in gluconeogenesis in the liver, and trigger an *in vivo* reduction in glucose levels. These results indicate that AMPK is requires for adiponectin to exert insulin-sensitizing effects in the liver and skeletal muscle (Fig. 2).

Conclusions

It is evident from the preceding results that PPARγ is a key molecule to mediate high-fat-diet induced obesity and that depression of adiponectin action have crucial roles in insulin resistance induced by obesity. Figure 3 summarizes our understanding of the mechanism of high-fat-diet induced obesity and insulin resistance ("small adipocyte" hypothesis). Small adipocyte differentiated from pre-adipocyte and accumulation of lipid is small, secreted insulin-sensitizing hormone, adiponectin, and leptin. Pre-adipocyte is unable to produce insulin-sensitizing hormone, a leading cause of insulin resistance in lipoatrophic diabetes. High-fat-diet induced adipocyte hypertrophy (large adipocyte), which causes decrease in expression and secretion of insulin-sensitizing hormone and increase in insulin-resistant hormone, leading to insulin resistance in obesity. Partial reduction in dose or activity of PPARγ, due to genetically inherited or functional antagonist, leads to the protection against obesity and Type II diabetes induced by high-fat diet. Both in mice and humans, PPARγ is an important thrifty gene mediating insulin resistance and Type II diabetes. It is quite interesting that the frequency of Pro12 allele of PPARγPro12Ala

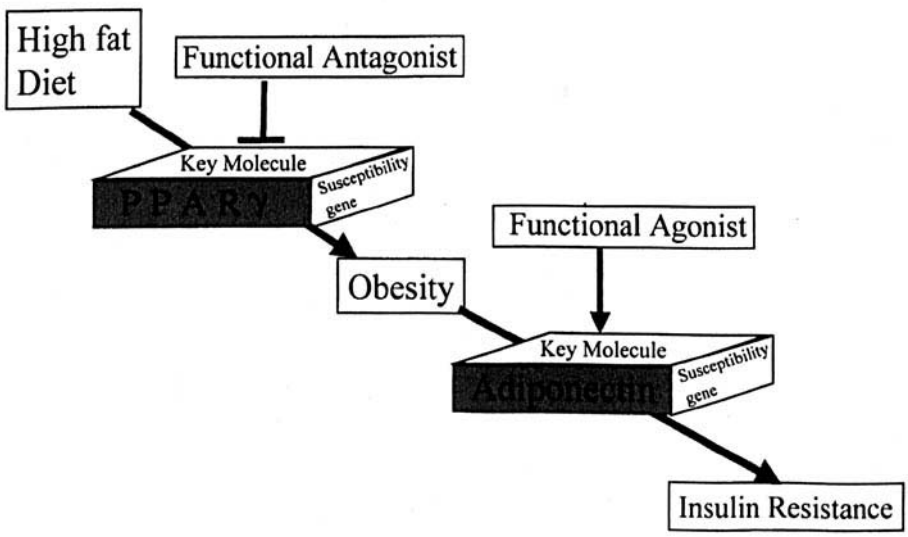

Figure 4. Fundamental therapies targeting key molecules involved in obesity-induced insulin resistance.

polymorphism, which raises the risk of diabetes, is much higher in Japanese people than in Caucasians. This means that the Japanese may be more prone to diabetes under westernized lifestyle, accounting for the rapid increase in the number of cases of diabetes in Japan. Genetical and/or environmental depression of adiponectin leads to insulin resistance and Type II diabetes. It is of note that more than 40% of Japanese have genotype that makes subjects prone to genetically decreased adiponectin levels and thus susceptible to Type II diabetes. This may be an alert to our society, because high-fat diet may have a disastrous effect on glucose metabolism to further decrease the adiponectin levels in those subjects. Our work confirmed that replenishment of adiponectin represents a novel treatment strategy for insulin resistance and Type II diabetes (Fig. 4). Because supplement of adiponectin itself is not practical due to its large molecular weight, an agonist of adiponectin receptor or a compound-inactivating molecule that disturbs adiponectin action should be developed. Further investigation will be needed to clarify how adiponectin exerts its effect and to discover the molecular target of therapies.

1. Ministry of Health and Welfare, Government of Japan. Office for Lifestyle-Related Disease Control. Tokyo, Japan: Diabetes Survey, 1999.
2. Okuno A, Tamemoto H, Tobe K, Ueki K, Mori Y, Iwamoto K, Umesono K, Akanuma Y, Fujiwara T, Horikoshi H, Yazaki Y, Kadowaki T. Troglitazone increases the number of small adipocytes without the change of white adipose tissue mass in obese Zucker rats. J Clin Invest **101:**1354–1361, 1998.
3. 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. PPAR gamma mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance. Mol Cell **4:**597–609, 1999.
4. Hara K, Kubota N, Tobe K, Terauchi Y, Miki H, Komeda K, Tamemoto H, Yamauchi T, Hagura R, Ito C, Akanuma Y, Kadowaki T. The role of PPARgamma as a thrifty gene both in mice and humans. Br J Nutr **84:**S235–S239, 2000.
5. Kadowaki T, Hara K, Kubota N, Tobe K, Terauchi Y, Yamauchi T, Eto K, Kadowaki H, Noda M, Hagura R, Akanuma Y. The role of PPARgamma in high-fat diet-induced obesity and insulin resistance. J Diabetes Complications **16:**41–45, 2002.
6. Deeb SS, Fajas L, Nemoto M, Pihlajamaki J, Mykkanen L, Kuusisto J, Laakso M, Fujimoto W, Auwerx J. A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat Genet **20:**284–287, 1998.
7. Hara K, Okada T, Tobe K, Yasuda K, Mori Y, Kadowaki H, Hagura R, Akanuma Y, Kimura S, Ito C, Kadowaki T. The Pro12Ala polymorphism in PPAR gamma2 may confer resistance to type 2 diabetes. Biochem Biophys Res Commun **271:**212–216, 2000.
8. Mori H, Ikegami H, Kawaguchi Y, Seino S, Yokoi N, Takeda J, Inoue I, Seino Y, Yasuda K, Hanafusa T, Yamagata K, Awata T, Kadowaki T, Hara K, Yamada N, Gotoda T, Iwasaki N, Iwamoto Y, Sanke T, Nanjo K, Oka Y, Matsutani A, Maeda E, Kasuga M. The Pro12 →Ala substitution in PPAR-gamma is associated with resistance to development of diabetes in the general population: Possible involvement in impairment of insulin secretion in individuals with type 2 diabetes. Diabetes **50:**891–894, 2001.
9. Altshuler D, Hirschhorn JN, Klannemark M, Lindgren CM, Vohl MC, Nemesh J, Lane CR, Schaffner SF, Bolk S, Brewer C, Tuomi T, Gaudet D, Hudson TJ, Daly M, Groop L, Lander ES. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat Genet **26:**76–80, 2000.
10. Masuzaki H, Ogawa Y, Aizawa-Abe M, Hosoda K, Suga J, Ebihara K, Satoh N, Iwai H, Inoue G, Nishimura H, Yoshimasa Y, Nakao K. Glucose metabolism and insulin sensitivity in transgenic mice overexpressing leptin with lethal yellow agouti mutation: Usefulness of leptin for the treatment of obesity-associated diabetes. Diabetes **48:** 1615–1622, 1999.
11. Moitra J, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C. Life without white fat: A transgenic mouse. Genes Dev **12:**3168–3181, 1998.
12. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature **395:**763–770, 1998.
13. Goodman RH, Smolik S. CBP/p300 in cell growth, transformation, and development. Genes Dev **14:**1553–1577, 2000.
14. Westin S, Rosenfeld MG, Glass CK. Nuclear receptor coactivators. Adv Pharmacol **47:**89–112, 2000.
15. Brown MS, Ye J, Rawson RB, Goldstein JL. Regulated intramembrane proteolysis: a control mechanism conserved from bacteria to humans. Cell **100:**391–398, 2000.
16. Darlington GJ, Ross SE, MacDougald OA. The role of C/EBP genes in adipocyte differentiation. J Biol Chem **273:**30057–30060, 1998.
17. Perlmann T, Evans RM. Nuclear receptors in Sicily: all in the famiglia. Cell **90:**391–397, 1997.
18. Mark M, Ghyselinck NB, Wendling O, Dupe V, Mascrez B, Kastner P, Chambon P. A genetic dissection of the retinoid signalling pathway in the mouse. Proc Nutr Soc **58:**609–613, 1999.
19. Kersten S, Desvergne B, Wahli W. Roles of PPARs in health and disease. Nature **405:**421–424, 2000.
20. Horvath CM. STAT proteins and transcriptional responses to extracellular signals. Trends Biochem Sci **25:**496–502, 2000.
21. Houstek J, Kopecky J, Rychter Z, Soukup T. Uncoupling protein in embryonic brown adipose tissue—existence of nonthermogenic and thermogenic mitochondria. Biochim Biophys Acta **935:**19–25, 1988.
22. Ailaud G, Hauner H. Development of white adipose tissue. In: Bray GA, Bouchard C, James WPT. Handbook of Obesity. Marcel Dekker, Inc.; New York. pp359–378, 1998.
23. Friedman JM. Obesity in the new millennium. Nature **404:**632–634, 2000.
24. Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, Nagai R, Kimura S, Tomita M, Froguel P, Kadowaki T. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med **7:**941–946, 2001.
25. Yamauchi T, Kamon J, Waki H, Murakami K, Motojima K, Komeda K, Ide T, Kubota N, Terauchi Y, Tobe K, Miki H, Tsuchida A, Akanuma Y, Nagai R, Kimura S, Kadowaki T. The mechanisms by which both heterozygous peroxisome proliferator-activated receptor gamma (PPARgamma) deficiency and PPARgamma agonist improve insulin resistance. J Biol Chem **276:**41245–41254, 2001.
26. Berg AH, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. Nat Med **7:**947–953, 2001.
27. Maeda K, Okubo K, Shimomura I, Funahashi T, Matsuzawa Y, Matsubara K. cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1 (AdiPose Most abundant Gene transcript 1). Biochem Biophys Res Commun **221:**286–289, 1996.
28. Nakano Y, Tobe T, Choi-Miura NH, Mazda T, Tomita M. Isolation and characterization of GBP28, a novel gelatin-binding protein purified from human plasma. J Biochem (Tokyo) **120:**803–812, 1996.
29. Hu E, Liang P, Spiegelman BM. AdipoQ is a novel adipose-specific gene dysregulated in obesity. J Biol Chem **271:**10697–10703, 1996.

30. Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF. A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem **270**:26746–26749, 1995.
31. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun **257**:79–83, 1999.
32. Hotta K, Funahashi T, Arita Y, Takahashi M, Matsuda M, Okamoto Y, Iwahashi H, Kuriyama H, Ouchi N, Maeda K, Nishida M, Kihara S, Sakai N, Nakajima T, Hasegawa K, Muraguchi M, Ohmoto Y, Nakamura T, Yamashita S, Hanafusa T, Matsuzawa Y. Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. Arterioscler Thromb Vasc Biol **20**:1595–1599, 2000.
33. Motojima K, Passilly P, Peters JM, Gonzalez FJ, Latruffe N. Expression of putative fatty acid transporter genes are regulated by peroxisome proliferator-activated receptor alpha and gamma activators in a tissue- and inducer-specific manner. J Biol Chem **273**:16710–16714, 1998.
34. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell **93**:241–252, 1998.
35. Murakami K, Tobe K, Ide T, Mochizuki T, Ohashi M, Akanuma Y, Yazaki Y, Kadowaki T. A novel insulin sensitizer acts as a coligand for peroxisome proliferator-activated receptor-alpha (PPAR-alpha) and PPAR-gamma: effect of PPAR-alpha activation on abnormal lipid metabolism in liver of Zucker fatty rats. Diabetes **47**:1841–1847, 1998.
36. Kersten S, Seydoux J, Peters JM, Gonzalez FJ, Desvergne B, Wahli W. Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J Clin Invest **103**:1489–1498, 1999.
37. Lowell BB, Spiegelman BM. Towards a molecular understanding of adaptive thermogenesis. Nature **404**:652–660, 2000.
38. Kelly LJ, Vicario PP, Thompson GM, Candelore MR, Doebber TW, Ventre J, Wu MS, Meurer R, Forrest MJ, Conner MW, Cascieri MA, Moller DE. Peroxisome proliferator-activated receptors gamma and alpha mediate in vivo regulation of uncoupling protein (UCP-1, UCP-2, UCP-3) gene expression. Endocrinology **139**:4920–4927, 1998.
39. Shapiro L, Scherer PE. The crystal structure of a complement-1q family protein suggests an evolutionary link to tumor necrosis factor. Curr Biol **8**:335–338, 1998.
40. Eggleton P, Reid KB, Tenner AJ. C1q—how many functions? How many receptors? Trends Cell Biol **8**:428–431, 1998.
41. Hardie DG, Carling D, Carlson M. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Annu Rev Biochem **67**:821–855, 1998.
42. Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes. Am J Physiol **277**:E1–10, 1999.
43. Mu J, Brozinick JT Jr, Valladares O, Bucan M, Birnbaum MJ. A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle. Mol Cell **7**:1085–1094, 2001.
44. Lochhead PA, Salt IP, Walker KS, Hardie DG, Sutherland C. 5-aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes **49**:896–903, 2000.
45. Merrill GF, Kurth EJ, Hardie DG, Winder WW. AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. Am J Physiol **273**:E1107–E1112, 1997.
46. Bergeron R, Russell RR 3rd, Young LH, Ren JM, Marcucci M, Lee A, Shulman GI. Effect of AMPK activation on muscle glucose metabolism in conscious rats. Am J Physiol **276**:E938–E944, 1999.
47. Ruderman NB, Saha AK, Vavvas D, Witters LA. Malonyl-CoA, fuel sensing, and insulin resistance. Am J Physiol **276**:E1–E18, 1999.
48. Zhou G, Myers R, Li Y, Chen Y, Shen X, Fenyk-Melody J, Wu M, Ventre J, Doebber T, Fujii N, Musi N, Hirshman MF, Goodyear LJ, Moller DE. Role of AMP-activated protein kinase in mechanism of metformin action. J Clin Invest **108**:1167–1174, 2001.
49. Fryer LG, Parbu-Patel A, Carling D. The Anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways. J Biol Chem **277**:25226–25232, 2002.