Fat Depot-Specific Expression of Adiponectin Is Impaired in Zucker Fatty Rats

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Adiposity, particularly increased intra-abdominal fat, is a predisposing factor for the development of insulin resistance in obesity and type 2 diabetes. Visceral fat seems to differ from subcutaneous adipose tissue in adipocytokine production. This fat depot-related difference has been viewed as an important mechanism by which adipose tissue exerts its paracrine/autocrine effects on peripheral tissue in modulating insulin sensitivity. We have studied the relative expression of adiponectin in visceral versus subcutaneous fat in Zucker fatty versus lean rats. Visceral fat, as opposed to subcutaneous fat, exhibited relatively higher levels of adiponectin production in lean animals. However, in Zucker fatty rats, adiponectin expression in visceral fat was suppressed to basal levels, which correlated with significantly reduced plasma adiponectin concentrations and increased insulin resistance. These results suggest that an impaired depot-specific expression of adiponectin is a contributing factor for the development of insulin resistance in Zucker fatty rats.

ADIPOSE TISSUE, long considered a passive fat depot, is an endocrine organ that plays a pivotal role in energy homeostasis. Adipocytes not only secrete free fatty acid (FFA), but also release a large variety of different protein factors, including leptin, tumor necrosis factor alpha (TNF-α), plasminogen activator inhibitor 1 (PAI-1), angiotensin II, acylating stimulating protein (ASP), interleukin-6 (IL-6), adiposin, resistin, and adiponectin (also known as Acrp30, AdipoQ, or apM1), which are collectively termed adipocytokines.[1-5] These factors in circulation perform different paracrine/autocrine functions in controlling energy expenditure by modulating the whole-body insulin sensitivity. Dysregulated secretion of adipocytokines, eg, leptin, adiponectin, IL-6, or TNF-α, has been shown to result in metabolic perturbations associated with the pathophysiology of obesity and type 2 diabetes.[5-7]

Both obesity and type 2 diabetes are characterized by insulin resistance, a significantly diminished state of tissue responsiveness to normal plasma insulin levels. Recent preclinical and clinical studies have implicated adiponectin as an important mediator of insulin sensitivity in peripheral tissue.[2,5] Plasma adiponectin concentrations are markedly reduced with increasing insulin resistance in obese patients with type 2 diabetes.[8-15] Transgenic mice lacking adiponectin exhibit diet-induced insulin resistance.[16] Antihyperglycemia drug therapy with thiazolidinediones or substantial body weight reduction in obesity and type 2 diabetes improves insulin sensitivity and also results in increased adiponectin production.[17-21] Furthermore, adiponectin infusion enhances insulin action in the liver and increases FFA oxidation in the muscle, improving glucose and fat metabolism and ameliorating insulin resistance in type 2 diabetic mice.[22-24] It appears that adiponectin can act as an insulin-sensitizing factor to improve insulin sensitivity by augmenting the responsiveness of peripheral tissues, at least for the liver and muscle, to insulin action.[2]

Increased adiposity is a risk factor for the development of hyperinsulinemia, hyperlipidemia, and type 2 diabetes, but recent studies suggest that intra-abdominal fat, as opposed to subcutaneous fat, is closely associated with these obesity-related metabolic syndromes. Visceral fat cells are smaller in size and less responsive to insulin action, displaying a relatively higher basal lipolytic rate than subcutaneous fat cells.[25,26] Since visceral fat is directly drained into the portal venous system, increased adiposity in the visceral depot tends to increase FFA flux through the portal system, resulting in inhibition of insulin action via the Randle's effect in peripheral tissue.[27,28]

In this study, we have examined the expression of adiponectin in Zucker diabetic fatty (ZDF) rats, demonstrating that plasma adiponectin levels correlated inversely with the degree of insulin resistance and percentage of visceral fat mass. Furthermore, we showed that adiponectin expression in visceral fat was significantly higher than in subcutaneous fat in lean animals. However, in ZDF rats, this depot-related difference in adiponectin production was severely impaired, resulting in a much greater reduction in adiponectin production in visceral fat.

MATERIALS AND METHODS

Animal Studies

Inbred obese male ZDF rats and lean heterozygous littermates at the age of 8 weeks were purchased from Charles River Laboratory (Wilmington, MA) and fed with the Purina LabDiet 5058 (Purina Mills, St Louis, MO) and water ad libitum in sterile cages in a barrier animal facility with a 12-hour light/dark cycle. Blood glucose levels were monitored using the Glucometer Elite (Bayer, IN). To determine plasma chemistries, blood samples were collected from tail vein into capillary microvette containing Potassium-EDTA (Sarstedt, Germany) and plasma was prepared by centrifugation at 6,000 rpm for 5 minutes. Plasma triglyceride levels were measured using the Sigma Diagnostics kit (Sigma, St Louis, MO). Plasma FFA in serum was determined using the Wako NEFA C test kit (Wako Chemicals, Richmond, VA). Plasma insulin levels were quantified using the ultrasensitive rat insulin enzyme-linked immunosorbent assay (ELISA) kit (Crystal Chem, Chicago, IL). Plasma adiponectin levels were determined using the radioimmunoassay kit for rat adiponectin (Linco Research, St Charles, MI). Plasma levels of TNF-α were measured using the rat TNF-α ELISA kit (Alpco, Windham, NH).

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Tissue RNA Isolation

To study the relative contribution of depot-specific expression of adiponectin to the overall plasma adiponectin concentrations, animals were killed at age 5 months. Both subcutaneous and visceral adipose tissues were extracted from dead animals and used for RNA isolation using the RNeasy Mini Kit (Qiagen, Valencia, CA). Twenty milligrams of adipose tissue was homogenized in 300 μL of cell lysis buffer using a rotor-stator tissue homogenizer. Total RNA was purified from homogenized tissue and the RNA concentration in the sample was determined by measuring the optical density at 260 nm. All procedures involved in the use of laboratory animals in this study, including blood sampling from tail vein and sacrifice of animals by CO_2 overdosing, were approved by the Institutional Animal Care and Use Committee (IACUC) of Mount Sinai School of Medicine.

Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction

Real-time quantitative reverse-transcriptase polymerase chain reaction (RT-PCR) was used for determination of the adiponectin mRNA concentrations in the subcutaneous versus visceral adipose tissue using the Roche LightCycler-RNA amplification kit (Roche Diagnostics, Mannheim, Germany). For each reaction, aliquots (250 ng in 10 μL) of RNA isolated from adipose tissue were mixed with 4 μL of the LightCycler RT-PCR reaction mix SYBR green I, 2.4 μL of 25-mmol/L $MgCl_2$, 2 μL of the primer mix (containing 2 μmol/L primers for forward and reverse reactions), 9.2 μL of RNase free H_2O, and 0.4 μL of the LightCycler RT-PCR enzyme mix in a final volume of 20 μL in capillary reaction tubes provided by the kit. In addition, aliquots of denatured cDNA ($\sim$250 bp) corresponding to the test mRNA at a series of concentrations ranging from 0.3 ng, 1 ng, 3 ng, 10 ng, and 30 ng were treated identically as above and used as controls for standard curve construction. As a negative control, a separate reaction without RNA sample was included in each reaction. All reactions were carried out by reverse transcription (RT) at 55°C for 10 minutes, followed by PCR amplification using the program containing 45 cycles of 95°C for 30 seconds, 55°C for 10 seconds, and 72°C for 14 seconds for each cycle. At the end of reaction, the cycle threshold (Ct) values, ie, the cycle numbers at which fluorescence signals exceed background were obtained for standard and unknown samples. A standard curve was constructed by plotting the Ct values as a function of the log concentration of standard cDNA (ng). Based on the Ct values of unknown samples, their relative mRNA concentrations were obtained from the standard curve. The two adiponectin primers used in real time RT-PCR are for forward reaction 5′-ACCCAGGAGATGCTG-3′ (corresponding to rat adiponectin cDNA, 233-247 nt) and for reverse reaction, 5′-ACCTGGAGCCAGACTTGGTC-3′ (rat adiponectin cDNA, 622-641 nt). β-actin primers were purchased from Promega (Madison, WI).

Insulin Sensitivity Index

Insulin sensitivity indices (ISI) of individual animals for glycemia [ISI(gly)] and plasma FFA [ISI(ffa)] were calculated using the formula, ISI(gly) = 2/[(INSp $\times$ GLYp) + 1] and ISI(ffa) = 2/[(INSp $\times$ FFAp) + 1], respectively. Here INSp stands for fasting plasma insulin concentrations, GLYp denotes fasting blood glucose levels, and FFAp represents fasting plasma FFA concentrations, all values being converted to mmol/L (or μmmol/L), as previously described.[29] A similar homeostasis model assessment (HOMA) for calculating ISI has also been described by Matthews et al.[30] The principle of these formulas is as follows: a reduction in insulin sensitivity results in elevation of blood glucose and plasma insulin in obesity and type 2 diabetes. As a result, ISI is a function of the compound effect of elevated blood glucose and plasma insulin. Preclinical and clinical data indicate that ISI calculated on the basis of fasting blood glucose and basal plasma insulin concentrations correlates inversely with the degree of β-cell dysfunction and overall insulin resistance, as assessed by euglycemic clamp techniques.[10,29-31] In this study, this method was adopted to calculate ISI in ZDF and lean rats.

Statistics

Statistical analyses of data were performed by analysis of variance (ANOVA) using the StatView software (Abacus Concepts, Berkeley, CA). Pairwise comparisons were performed to study statistical significance between ZDF and lean rats. In addition, correlation coefficients were studied by regression analysis. Data were expressed as the mean $\pm$ SE. *P* values less than .05 are statistically significant.

RESULTS

Adiponectin Expression in ZDF Versus Lean Rats

In this study, 13 male ZDF and 5 age-matched male lean littermates were used to characterize adipose tissue depot-specific expression of adiponectin in relation to insulin resistance. ZDF rats at the age of 5 months became severely obese with an average body weight of 392 $\pm$ 27 g, in comparison to their lean littermates (body weight, 212 $\pm$ 6 g) (Fig 1A). In addition, ZDF rats exhibited hyperglycemia (Fig1B), hyperinsulinemia (Fig 1C), and hyperlipidemia (Fig 1D and E), accompanied by elevated plasma TNF-α levels (Fig 1F).

To study the expression level of adiponectin in ZDF versus lean rats, plasma adiponectin levels were determined under both nonfasting and fasting conditions. As shown in Fig 2, ZDF rats exhibited significantly reduced plasma adiponectin concentrations in comparison to their lean littermates (13.3 $\pm$ 1.7 *v* 8.1 $\pm$ 0.4 μg/mL, $P < .005$). However, in response to a 24-hour fast, a significant difference in plasma adiponectin expression was revealed between ZDF rats and their lean littermates. Plasma adiponectin concentrations were greatly reduced in lean animals after 24 hours of fasting (9.2 $\pm$ 0.6 *v* 13.3 $\pm$ 1.7 μg/mL under nonfasting conditions, $P < .005$). In contrast, no significant changes in plasma adiponectin concentrations were detected in ZDF rats before (8.1 $\pm$ 0.5 μg/mL) and after 24 hours fasting (8.9 $\pm$ 1.2 μg/mL).

Depot-Specific Expression of Adiponectin in ZDF Rats

To study the relative contribution of adiponectin production in visceral versus subcutaneous fat to the overall plasma adiponectin, the relative abundance of adiponectin mRNA in these two adipose tissues was determined by real-time quantitative RT-PCR using β-actin mRNA as an internal control. As shown in Fig 3, adiponectin was differentially expressed in subcutaneous and visceral fat. In lean animals, visceral fat contributes more to the overall plasma adiponectin, as evidenced by a 2-fold higher expression level of adiponectin in visceral adipocytes than in subcutaneous fat cells. In contrast, this depot-specific expression of adiponectin is reversed in ZDF rats. Here the relative abundance of adiponectin mRNA in subcutaneous fat is relatively higher than that in visceral fat, but overall the expression levels of adiponectin in both subcutaneous and visceral adipose tissues were markedly reduced in ZDF rats, which correlated with their plasma adiponectin levels (Fig 2).

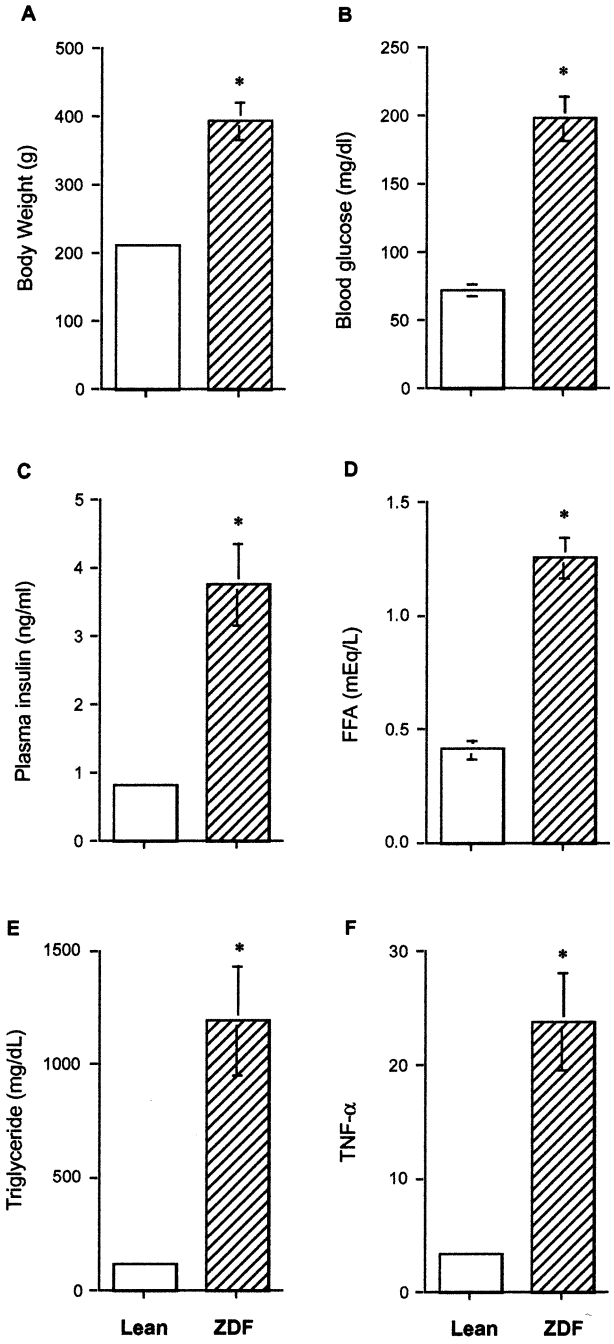

Fig 1. Body weight and blood chemistries. Lean (n = 5) and ZDF (n = 13) rats at age 5 months were measured for (A) body weight, (B) fasting blood glucose levels, (C) basal plasma levels of insulin, (D) FFA, (E) triglycerides, and (F) TNF-α. *P < .001 *v* lean animals.

Relationship Between Adiponectin Expression and Insulin Resistance in ZDF Rats

To study the relationship between adiponectin production and insulin resistance in ZDF and lean rats, we correlated plasma adiponectin concentrations with fasting blood glucose and basal plasma insulin levels. Negative correlations were produced between plasma adiponectin concentrations and the degree of hyperglycemia (Fig 4A) and hyperinsulinemia (Fig 4B). Likewise, plasma adiponectin concentrations were also negatively correlated with plasma levels of FFA (Fig 4C), triglycerides (Fig 4D), TNF-α (Fig 4E), and the percentage of visceral fat mass in ZDF and lean rats (Fig 4F).

To determine the degree of insulin resistance in ZDF and lean rats, we calculated ISI(gly) and ISI(ffa) based on fasting blood glucose and basal plasma insulin (or FFA) levels as described earlier. In these calculations, the mean ISI in lean animals was set to 1 and ISIs in individual ZDF and lean rats were calculated accordingly. Since ISI is an inverse function of insulin resistance, it should decrease with increasing insulin resistance. Indeed, ISI(gly) and ISI(ffa) were 0.21 ± 0.04 and 0.18 ± 0.03 in ZDF rats, compared to the corresponding values of 1.0 ± 0.04 and 1.0 ± 0.03 in lean animals. When correlated with plasma adiponectin, both ISI(gly) for glycemic control and ISI(ffa) for FFA metabolism increased with increasing plasma adiponectin concentrations (Fig 4G and H).

DISCUSSION

Adiponectin is an adipocyte-derived protein that is abundantly expressed and secreted from adipocytes. Evidence is

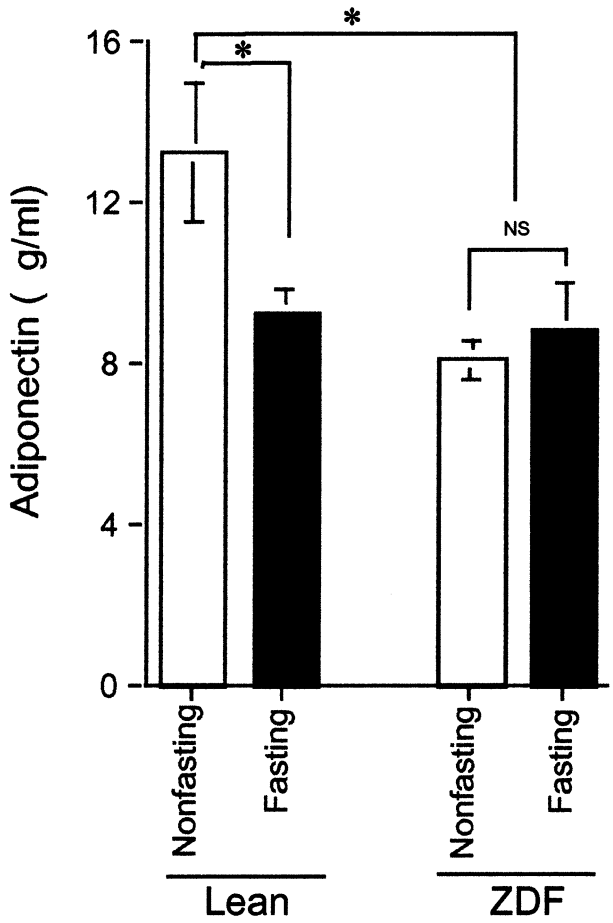

Fig 2. Plasma adiponectin levels in ZDF and lean rats under nonfasting conditions and after 24 hours fasting. *P < .005. NS, not significant.

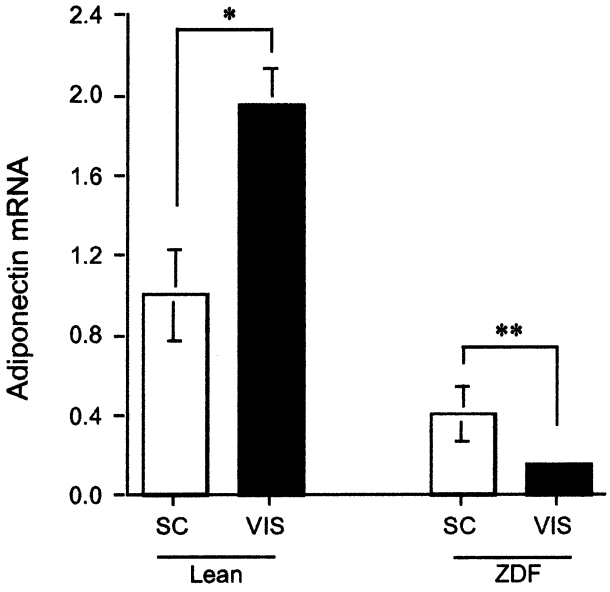

Fig 3. Adiponectin expression in subcutaneous (SC) and visceral (VIS) fat in ZDF *v* lean rats. Adiponectin mRNA expressed in visceral and subcutaneous fat was quantified by real-time RT-PCR using β-actin mRNA as internal standards. After normalizing to the amount of β-actin mRNA, the relative abundances of adiponectin mRNA in visceral and subcutaneous tissues were calculated for ZDF and lean animals. *$P < .001$. **$P < .05$. Data in a given tissue between ZDF and lean rats were also significant ($P < .005$).

accumulating that adiponectin expression correlates closely with whole-body insulin sensitivity.[2,13,16,17,19,23,24] This close correlation between plasma adiponectin levels and insulin sensitivity has led to the idea that adiponectin functions as an insulin-sensitizing adipocytokine in controlling fuel metabolism and energy balance.[2,5] In support of this idea, our present studies provide additional data showing that adiponectin expression was significantly reduced in ZDF rats, in comparison to their age-matched lean littermates. Furthermore, we showed that plasma adiponectin levels correlated positively with insulin sensitivity and negatively with the degree of hyperglycemia, hyperinsulinemia, hyperlipidemia, and the percentage of visceral fat pad mass. Thus, reduced adiponectin production is associated with increased insulin resistance, accompanied by impaired glucose and lipid metabolism in ZDF rats.

In addition, we showed that plasma adiponectin concentrations correlated inversely with plasma TNF-α in ZDF rats. Increased TNF-α production in adipose tissue is known to be a causative factor for the pathogenesis of insulin resistance.[1] Interestingly, TNF-α shares a great degree of structural similarity with adiponectin, although no significant homology exists in amino acid sequences between these 2 proteins.[32] Based on these observations, it is postulated that TNF-α and adiponectin act in an antagonist manner in modulating insulin action. There is evidence that TNF-α administration causes increased lipid synthesis and accumulation in the liver, resulting in hepatic insulin resistance.[33-35] Conversely, adiponectin injection enhances insulin action in the liver and increases FFA oxidation in the skeletal muscle, contributing significantly to improved whole-body insulin sensitivity in type 2 diabetic mice.[24] More recently, it was shown that adiponectin and TNF-α mutually suppress each other's expression in adipocytes in a dose-dependent manner.[36] Together these results suggest that decreased plasma adiponectin, coupled with increased TNF-α production, is an aggravating factor for the pathogenesis of insulin resistance in obesity-related type 2 diabetes.

Intra-abdominal adiposity is causative for the development

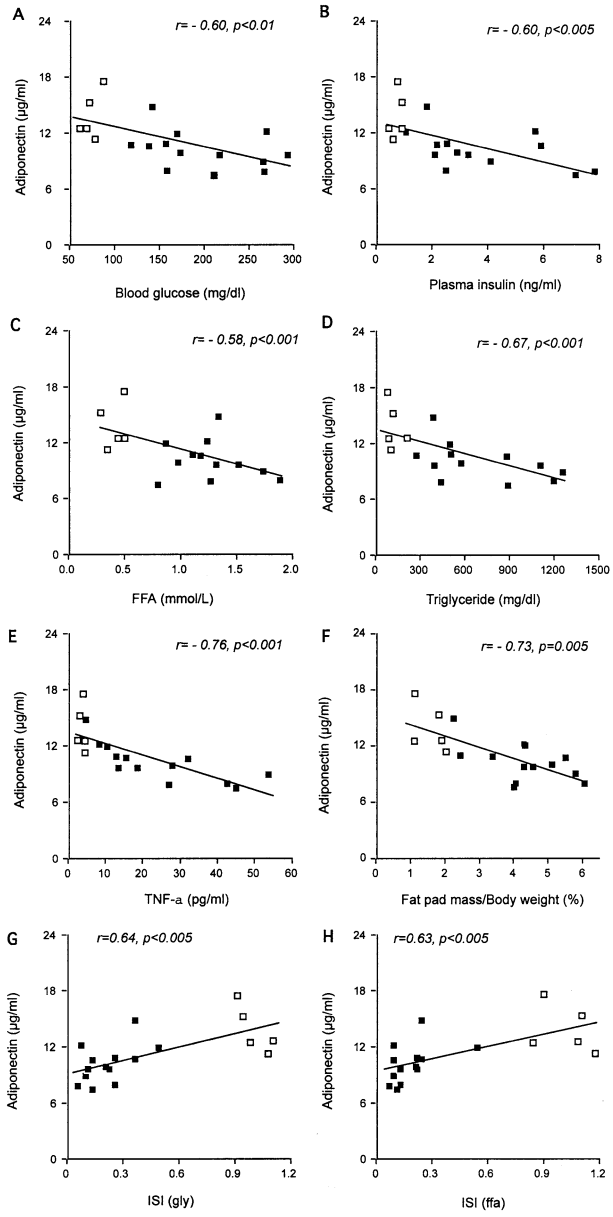

Fig 4. Correlation between plasma adiponectin levels with insulin sensitivity indices. Plasma adiponectin levels were correlated with (A) blood glucose levels, (B) plasma levels of insulin, (C) FFA, (D) triglycerides, (E) TNF-α, (F) the percentage of intra-abdominal fat pad mass, as well as insulin sensitivity indices (G) ISI(gly) and (H) ISI(ffa) in ZDF rats and their lean littermates. (■) ZDF rats; (□) lean controls.

of hyperinsulinemia and insulin resistance in type 2 diabetes. Despite increased fat mass, plasma adiponectin levels are significantly reduced in obesity with type 2 diabetes, and yet the molecular mechanism underlying this paradoxical decrease in plasma adiponectin levels with increased adiposity in relation to insulin resistance is unknown. In this study, we showed that adiponectin is differentially expressed in visceral versus subcutaneous adipose tissues. In lean animals, adiponectin expression is significantly higher in visceral fat than in subcutaneous adipose tissue, suggesting that visceral fat is a predominant contributor to plasma adiponectin. However, this depot-specific expression of adiponectin is greatly impaired in ZDF rats. Although adiponectin expression levels in both tissues were significantly reduced, contributing to the reduced plasma adiponectin concentrations in ZDF rats, the amplitude of reduction was different between visceral and subcutaneous tissues. In comparison to a 50% reduction of adiponectin expression in subcutaneous fat, adiponectin expression in visceral adipose tissue was suppressed to a basal level in ZDF rats. This pronounced defect in adiponectin production in visceral fat, as opposed to that in subcutaneous adipose tissue, is a major contributing factor for the greatly reduced plasma adiponectin concentrations, which underlies the greater importance of visceral adiposity in the pathogenesis of insulin resistance associated with obesity and type 2 diabetes. Consistent with this interpretation is the observation that adiponectin production in intra-abdominal adipocytes, but not in other adipose tissue, was significantly increased in *db/db* mice in response to insulin-sensitizing drug therapy.[21] Furthermore, a more recent study by Milan et al[37] showed that body weight reduction, caused by food restriction, significantly improved insulin sensitivity and also restored adiponectin production in visceral adipose tissue to normal in ZDF rats.

In conclusion, we showed in a rat model of obesity and type 2 diabetes that plasma adiponectin is correlated positively with insulin sensitivity and negatively with plasma TNF-α levels and the percentage of intra-abdominal fat mass. Furthermore, we present evidence that adiponectin is differentially expressed in visceral vs. subcutaneous adipose tissue. These results are consistent with the idea that visceral fat plays an important role in modulating whole-body insulin sensitivity by controlling the expression and secretion of different metabolic adipocytokines.[1,38,39] Impaired adiponectin secretion in visceral fat is a significant contributing factor for the pathogenesis of insulin resistance in ZDF rats.

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REFERENCES

1. Greenberg AS, McDaniel ML: Identifying the links between obesity, insulin resistance and β-cell function: Potential role of adipocyte-derived cytokines in the pathogenesis of type 2 diabetes. Eur J Clin Invest 32:24-34, 2002

2. Tsao TS, Lodish HF, Fruebis J: ACRP30, a new hormone controlling fat and glucose metabolism. Eur J Pharmacol 440:213-221, 2002

3. Steppan CM, Lazar MA: Resistin and obesity-associated insulin resistance. Trends Endocrinol Metab 13:18-23, 2002

4. Havel PJ: Control of energy homeostasis and insulin action by adipocyte hormones: Leptin, acylation stimulating protein, and adiponectin. Curr Opin Lipidol 13:51-59, 2002

5. Berg AH, Combs TP, Scherer PE: ACRP30/adiponectin: An adipokine regulating glucose and lipid metabolism. Trends Endocrinol Metab 13:84-89, 2002

6. Caro JF, Sinha MK, Kolansky JW, et al: Leptin: The tale of an obesity gene. Diabetes 45:1455-1462, 1996

7. Peraldi P, Spiegelman B: TNF-α and insulin resistance: Summary and future prospects. Mol Cell Biochem 182:169-175, 1998

8. Nadler ST, Stoehr JP, Schueler KL, et al: The expression of adipogenic genes is decreased in obesity and diabetes mellitus. Proc Natl Acad Sci USA 97:11371-11376, 2000

9. Arita Y, Kihara S, Ouchi N, et al: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun 257:79-83, 1999

10. Hara K, Boutin P, Mori Y, et al: Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. Diabetes 51:536-540, 2002

11. Comuzzie AG, Funahashi T, Sonnenberg G, et al: The genetic basis of plasma variation in adiponectin, a global endophenotype for obesity and the metabolic syndrome. J Clin Endocrinol Metab 86:4321-4325, 2001

12. Hotta K, Funahashi T, Bodkin NL, 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 50:1126, 2001

13. Weyer C, Funahashi T, Tanaka S, et al: Hypoadiponectinemia in obesity and type 2 diabetes: Close association with insulin resistance and hyperinsulinemia. J Clin Endocrinol Metab 86:1930-1935, 2001

14. Stefan N, Vozarova B, Funahashi T, et al: Plasma adiponectin concentration is associated with skeletal muscle insulin receptor tyrosine phosphorylation, and low plasma concentration precedes a decrease in whole-body insulin sensitivity in humans. Diabetes 51:1884-1888, 2002

15. Lindsay RS, Funahashi T, Hanson RL, et al: Adiponectin and development of type 2 diabetes in the Pima Indian population. Lancet 360:57-58, 2002

16. Maeda N, Shimomura I, Kishida K, et al: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 8:731-737, 2002

17. Yang WS, Lee WJ, Funahashi T, et al: Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J Clin Endocrinol Metab 86:3815-3819, 2001

18. Moore GB, Chapman H, Holder JC, et al: Differential regulation of adipocytokine mRNAs by rosiglitazone in db/db mice. Biochem Biophys Res Commun 286:735-741, 2001

19. Maeda N, Takahashi M, Funahashi T, et al: PPARgamma ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein. Diabetes 50:2094-2099, 2001

20. Yang WS, Jeng CY, Wu TJ, et al: Synthetic peroxisome proliferator-activated receptor-gamma agonist, rosiglitazone, increases plasma levels of adiponectin in type 2 diabetic patients. Diabetes Care 25:376-380, 2002

21. Combs TP, Wagner JA, Berger J, et al: Induction of adipocyte complement-related protein of 30 kilodaltons by PPARgamma agonists: A potential mechanism of insulin sensitization. Endocrinology 143:998-1007, 2002

22. Berg AH, Combs TP, Du X, et al: The adipocyte-secreted

protein Acrp30 enhances hepatic insulin action. Nat Med 7:947-953, 2001

23. Fruebis J, Tsao TS, Javorschi S, et al: Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. Proc Natl Acad Sci USA 98:2005-2010, 2001

24. Yamauchi T, Kamon J, Waki H, et al: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 7:941-946, 2001

25. Ostman J, Arner P, Engfeldt P, et al: Regional differences in the control of lipolysis in human adipose tissue. Metabolism 28:1198-1205, 1979

26. Engfeldt P, Arner P: Lipolysis in human adipocytes, effects of cell size, age and of regional differences. Horm Metab Res 19(suppl): 26-29, 1988

27. Randle PJ: Regulatory interactions between lipids and carbohydrates: The glucose fatty acid cycle after 35 years. Diabetes Metab Rev 14:263-283, 1998

28. Ravussiin E, Smith SR: Increased fat intakes, impaired fat oxidation, failure of fat cell proliferation results in ectopic fat storage, insulin resistance, and type 2 diabetes. Ann NY Acad Sci 967:363-378, 2002

29. Belfiore F, Iannello S, Volpicelli G: Insulin sensitivity indices calculated from basal and OGTT-induced insulin, glucose, and FFA levels. Mol Genet Metab 63:134-141, 1998

30. Matthews DR, Hosker JP, Rudenski AS, et al: Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28:412-419, 1985

31. Belfiore F, Iannello S: Insulin resistance in obesity: Metabolic mechanisms and measurement methods. Mol Genet Metab 65:121-128, 1998

32. 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

33. Feingold KR, Serio MK, Adi S, et al: Tumor necrosis factor stimulate hepatic lipid synthesis and secretion. Endocrinology 124: 2336-2342, 1989

34. Nachiappan V, Curtiss D, Corkey BE, et al: Cytokine inhibit fatty acid oxidation in isolated rat hepatocytes: Synergy among TNF-α, IL-6, and IL-1. Shock 1:123-129, 1994

35. Lang CH, Dobrescu C, Bagby GJ: Tumor necrosis factor impairs insulin action on peripheral glucose disposal and hepatic glucose output. Endocrinology 130:43-52, 1992

36. Fasshauer M, Klein J, Neumann S, et al: Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. Biochem Biophys Res Commun 290:1084-1089, 2002

37. Milan G, Granzotto M, Scarda A, et al: Resistin and adiponectin expression in visceral fat of obese rats: Effect of weight loss. Obesity Res 10:1197-1199, 2002

38. Vidal H: Quantification of lipid-related mRNAs by reverse transcription-competitive polymerase chain reaction in human white adipose tissue biopsies. Methods Mol Biol 155:83-88, 2001

39. Vidal H: Gene expression in visceral and subcutaneous adipose tissues. Ann Med 33:547-555, 2001