

Abnormal Glucose Homeostasis due to Chronic Hyperresistinemia

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Resistin is an adipocyte-secreted protein that circulates at increased levels in obesity. Acute administration of resistin impairs glucose tolerance, but the effects of chronic hyperresistinemia have not been established. Here we describe the generation and characterization of transgenic mice that have high circulating levels of resistin in the setting of normal weight. Fasted blood glucose was higher in resistin-transgenic mice than in their nontransgenic littermates, and glucose tolerance was impaired in the hyperresistinemic mice. Metabolic studies in the setting of a hyperinsulinemic-euglycemic clamp protocol revealed that chronically hyperresistinemic mice have elevated glucose production. This increase in glucose production may be partly explained by increased expression of hepatic phosphoenolpyruvate carboxykinase. Thus, chronic hyperresistinemia impairs normal glucose metabolism. *Diabetes* 53:1937–1941, 2004

Type 2 diabetes is an epidemic metabolic disease that is predicted to afflict nearly one-third of people born in the U.S. in the year 2000 sometime during their lives (1). The disease is characterized by tissue resistance to the actions of insulin and is associated with obesity (2). Obese patients without diabetes commonly develop the metabolic syndrome, the hallmark of which is insulin resistance, which appears to be a major risk factor for atherosclerotic cardiovascular disease (3). Thus, the mechanisms by which obesity predisposes to insulin resistance are of tremendous interest.

Adipose tissue produces several hormones and cytokines that affect glucose homeostasis (4). Adiponectin and leptin are adipose-specific hormones that improve glucose tolerance. Although produced only in adipose tissue, adiponectin actually circulates at decreased levels in obesity

(5). Leptin levels are increased in obesity, although tissues appear to be resistant to its actions (6). Thus, abnormalities in adiponectin and leptin may both contribute to obesity-associated insulin resistance. Several other adipose-derived hormones oppose insulin action, and they circulate at increased levels in obesity, thus also possibly contributing to insulin resistance. Prominent among these are tumor necrosis factor- α and interleukin-6, two cytokines that are also produced at high levels in macrophages and were first identified as inflammatory mediators (7,8).

Resistin is also produced by adipocytes (9–11) and circulates at increased levels in obesity (9). Deletion of the resistin gene reduces the impact of obesity on glucose homeostasis (12). Conversely, acute administration of resistin impairs glucose tolerance and insulin action (9,13). Therefore, it is predicted that mice with chronic hyperresistinemia would have impaired glucose metabolism, even in the absence of obesity. This has not been systematically studied, however, and it is quite possible that compensatory mechanisms might overcome the effect of longstanding hyperresistinemia. To test this, we created transgenic mice in which circulating resistin levels are markedly and chronically elevated. These hyperresistinemic mice exhibit modest fasting hyperglycemia and glucose intolerance, associated with increased hepatic glucose production in the setting of hyperinsulinemia. These results indicate that chronic hyperresistinemia leads to impairment of glucose homeostasis.

RESEARCH DESIGN AND METHODS

Generation of hyperresistinemic mice. We opted to create hyperresistinemic mice due to ectopic expression of resistin in liver rather than adipose tissue because 1) resistin is already one of most abundant transcripts in mouse adipocytes (14) and 2) this paradigm would test the hypothesis that resistin does not need to be secreted by adipose tissue in order to have a metabolic effect. Resistin-overexpressing mice were generated using the transthyretin (TTR) minigene consisting of 3 kb of the TTR promoter region, the first and the second exons of the TTR gene, and the SV40 polyA sequence (15–17). The resistin cDNA bearing the flag epitope at the 3' end, previously shown to be biologically active (9,12), was inserted between the second exon and the polyA tail. The purified transgene DNA was injected into fertilized B6SJL F1 oocytes and implanted into pseudopregnant CD1 mice, then pups were screened by PCR of genomic DNA using primers at the 5' and 3' ends of the resistin open reading frame. Three founder mice were identified, and these were bred with C57Bl/6 females to obtain three different lines of mice to be used for experimentation. Mice were serotyped before use in experiments to ensure that they had elevated resistin levels.

Immunohistochemistry. Liver tissue from wild-type and transgenic mice was fixed in neutral buffered formalin and used to prepare sections that were

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G6-Pase, glucose 6-phosphatase; TTR, transthyretin.
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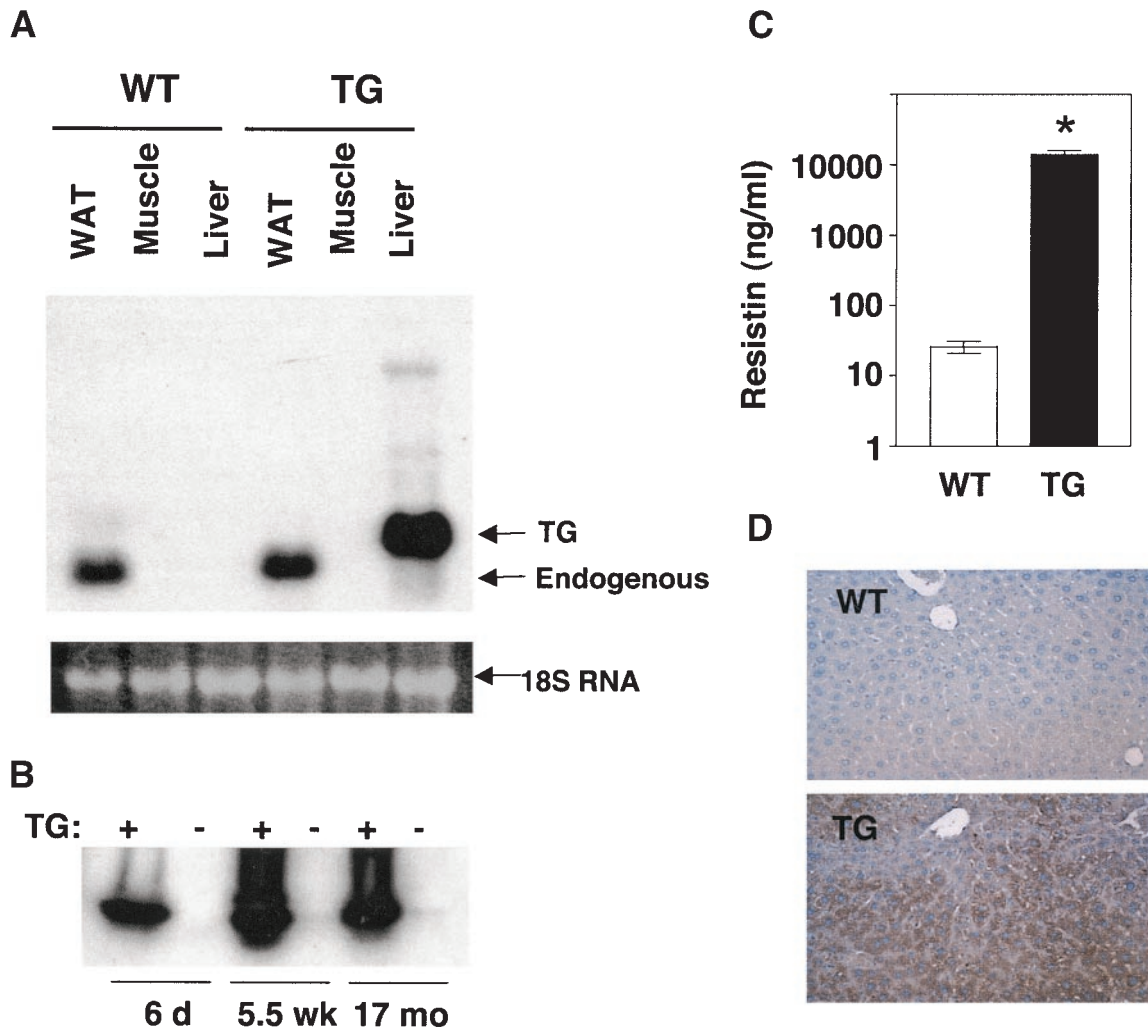


FIG. 1. Generation of a transgenic (TG) mouse with chronic hyperresistinemia. **A:** Expression of resistin mRNA in white adipose tissue (WAT) and liver of resistin-transgenic mice and their wild-type (WT) littermates. **B:** Serum resistin levels are markedly elevated from birth to adulthood in resistin-transgenic mice. Serum (1 μ l) from representative mice was subjected to immunoblotting. **C:** Quantitation of resistin circulating levels by radioimmunoassay. * $P = 0.0016$. ($n = 3$ each for WT and TG). **D:** Resistin expression and normal architecture of transgenic livers. Liver sections from adult WT and TG mice were stained for peroxidase and counterstained with hematoxylin. Resistin staining is brown.

stained for resistin using guinea pig anti-mouse resistin antibody (Linco) according to an indirect peroxidase-conjugated streptavidin procedure. Sections were counterstained with hematoxylin.

Mouse phenotyping. Serum parameters were measured as previously described (18) at 0900 to 1200 in the ad libitum-fed state and after overnight fasting (from 1700 to 0900 the next morning). Serum levels of resistin were measured by immunoblotting as well as radioimmunoassay (Linco) as previously described (12). Liver enzymes were measured using commercially available kits (Stanbio). Glucose tolerance testing, Northern blot analysis, and body fat composition by dual-energy X-ray absorptiometry were performed as previously described (18).

Euglycemic-hyperinsulinemic clamp studies. Mice were studied under hyperinsulinemic-euglycemic conditions as previously described (12).

Statistical analyses. Statistical comparisons were performed using Student's *t* test.

RESULTS

A transgenic mouse model of hyperresistinemia. We used the TTR promoter to overexpress resistin with a COOH-terminal Flag epitope that retains biological activity (9,12–14). Resistin transgenic mice expressed abundant resistin mRNA in the liver, which is the main site of TTR expression (Fig. 1A). Consistent with the expression of the TTR promoter in liver (15–17), a marked elevation of

serum resistin levels was sustained from birth to adulthood, demonstrated semiquantitatively by immunoblot (Fig. 1B) and quantified in the adult by radioimmunoassay (Fig. 1C). Resistin transgenic livers contained abundant resistin within hepatocytes, but no morphologic or structural abnormalities were observed (Fig. 1D). In addition, serum levels of liver enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were similar in the wild-type and resistin-transgenic mice, indicative of normal liver function (Table 1).

Hyperresistinemic transgenic mice exhibit increased fasting glucose. Body weights and body fat composition assessed by dual-energy X-ray absorptiometry were similar in the hyperresistinemic transgenic mice and their wild-type littermates (Table 1). Blood glucose levels in mice fed ad libitum were also similar in resistin-transgenic and wild-type mice (Fig. 2A). However, following an overnight fast, plasma glucose levels were significantly elevated in the hyperresistinemic mice (Fig. 2B). Similar results were obtained in progeny from three independent founders (Fig. 3).

TABLE 1

Serum parameters measured in the ad libitum-fed state in transgenic hyperresistinemic mice and their wild-type littermate controls at 8–11 weeks of age

	Wild-type	Hyperresistinemic
Body weight (g)	24 ± 0.5	24.1 ± 0.6
Glucose (mg/dl)	180.1 ± 9.2	185.1 ± 7.6
Free fatty acid (mEq/l)	0.66 ± 0.03	0.67 ± 0.03
Triglycerides (mg/dl)	190 ± 21	188 ± 17
Insulin (ng/ml)	0.95 ± 0.17	0.89 ± 0.19
Leptin (ng/ml)	2.86 ± 0.22	2.55 ± 0.30
AST (units/l)	12.9 ± 0.8	13.4 ± 1
ALT (units/l)	9.0 ± 0.6	9.4 ± 0.7
Adiponectin (μg/ml)	5.7 ± 0.4	5.9 ± 0.5
Body fat (%)	11.9 ± 0.7	11.3 ± 0.7

Data are means ± SE. For serum measurements, $n = 10$ for wild-type and $n = 11$ for transgenic. For body fat determination, $n = 9$ (wild-type) and $n = 11$ (transgenic). ALT, alanine aminotransferase; AST, aspartate aminotransferase.

Glucose tolerance and glucose production are abnormal in hyperresistinemic transgenic mice. The abnormal glucose homeostasis in resistin-transgenic mice was further evaluated by dynamic testing. Glucose tolerance was impaired relative to wild-type littermates (Fig. 3).

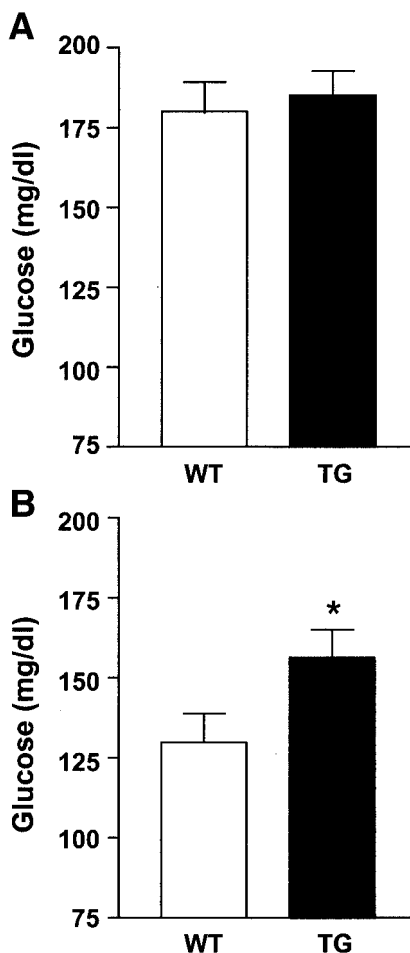


FIG. 2. Increased fasting glucose levels in chronically hyperresistinemic mice. **A:** Serum glucose in ad libitum-fed mice 9–11 weeks of age. **B:** Serum glucose in fasted mice 8–10 weeks of age. $n = 10$ males for wild-type (WT) and $n = 11$ males for transgenic (TG). $*P < 0.05$.

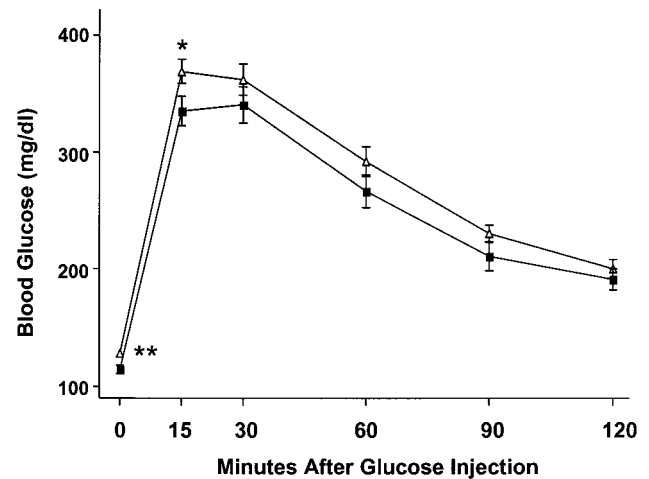


FIG. 3. Impaired glucose tolerance of hyperresistinemic mice. Mice used in this experiment were male, 14–22 weeks of age. $n = 20$ –34 for wild-type (■) and $n = 20$ –29 for transgenic (△). $**P = 0.004$, $*P = 0.038$.

Fasted insulin levels and insulin tolerance testing were similar in the two groups (Table 1) (data not shown). As the insulin tolerance test is relatively acute, we assessed whole-body glucose homeostasis in hyperinsulinemic ($3.6 \text{ mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)-euglycemic glucose clamp studies. Resistin-transgenic mice required a lower rate of glucose infusion than their wild-type littermates in order to maintain euglycemia, although this difference did not reach statistical significance ($P = 0.11$). Glucose disposal was nearly equivalent in the two groups (Fig. 4B). Remarkably, however, glucose production in the setting of hyperinsulinemia was increased 2.8-fold in the hyperresistinemic mice (Fig. 4C).

Increased hepatic PEPCK gene expression in hyperresistinemic transgenic mice. The increased glucose production of hyperresistinemic transgenic mice subjected to hyperinsulinemic-euglycemic clamping suggested that hepatic gluconeogenesis was inappropriately high under these conditions (19). Consistent with this, hepatic PEPCK gene expression was significantly higher in resistin-transgenic than in wild-type littermates under hyperinsulinemic conditions (Fig. 5A). In addition, glucose 6-phosphatase (G6-Pase) gene expression was ~30% higher in the resistin-transgenic mice, although this difference was not statistically significant (Fig. 5B). Together these results suggest that increases in hepatic gluconeogenic gene expression may underlie the increase in glucose production observed in our resistin-transgenic mice.

DISCUSSION

We have created and characterized a novel model of chronic hyperresistinemia. These results demonstrate that resistin can function as a circulating hormone to regulate glucose homeostasis and that chronic elevation of serum resistin levels leads to fasting hyperglycemia, glucose intolerance, and increased hepatic glucose output.

The parameters of whole-body glucose metabolism in our mice with chronic hyperresistinemia were similar to those described in the setting of acutely elevated resistin levels in the rat (13). In both cases the main abnormality was failure to normally suppress hepatic glucose output in

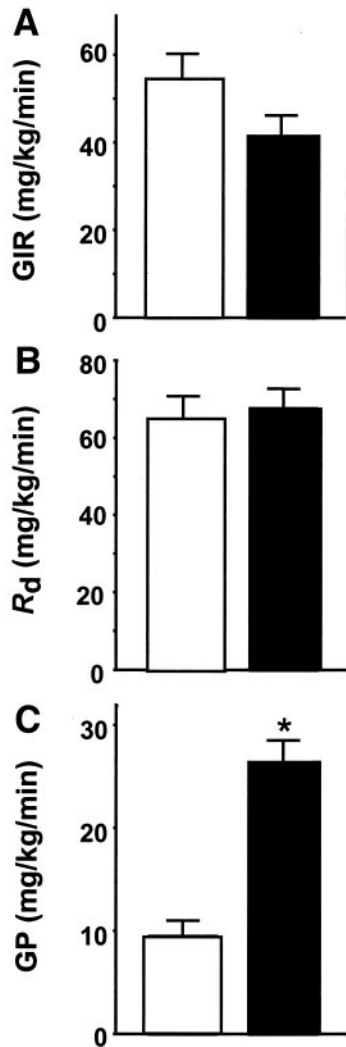


FIG. 4. Hyperinsulinemic-euglycemic clamp studies. **A:** Glucose infusion rate (GIR). **B:** Whole-body glucose disposal (R_d). **C:** Glucose production (GP). Eight male wild-type (□) and eight male transgenic (■) mice were studied. * $P < 0.0001$.

the setting of hyperinsulinemia. Hepatic expression of the transgene did not alter liver morphology or synthetic function, suggesting that although the liver was the main source of resistin in the transgenic mice, the metabolic changes resulted from increases in levels of circulating resistin. It should be noted that resistin levels were quite high in our mouse model. However, Rajala et al. (13) did not observe a significant difference in the induction of hepatic insulin resistance between 2- to 5-fold versus 10- to 15-fold increases in plasma resistin levels in the rat.

We recently reported that mice lacking resistin have reduced glucose production associated with decreased expression of genes encoding the gluconeogenic enzymes PEPCK and G6-Pase expression (12). Conversely, here we have found that mice with chronic hyperresistinemia have increased glucose production along with increased hepatic expression of PEPCK and G6-Pase. The gluconeogenic phenotype of resistin null mice correlated with activation of hepatic AMP kinase (AMPK). In the hyperresistinemic transgenic mice, we were unable to document significant changes in hepatic activation of AMPK or other gluconeogenic factors such as phospho-Akt or peroxisome proliferator-

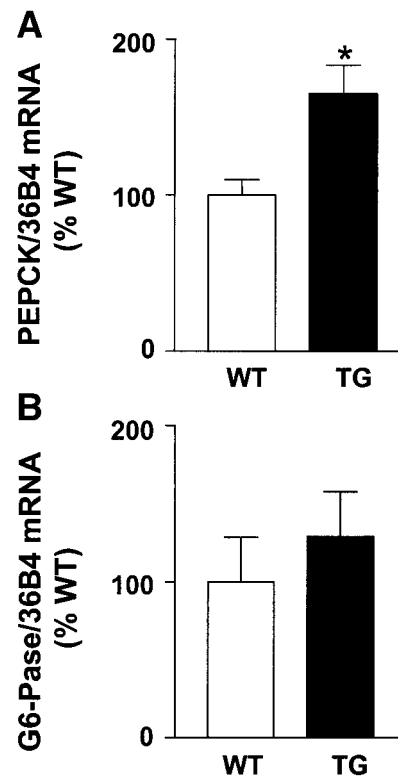


FIG. 5. Hepatic expression of gluconeogenic genes. **A:** PEPCK, normalized to 36B4 expression. **B:** G6-Pase, normalized to 36B4 expression. $n = 9$ wild-type (WT) and $n = 8$ transgenic (TG) mice. * $P < 0.005$.

ator-activated receptor γ coactivator (PGC-1) (data not shown). This may be due to subtle differences requiring larger numbers of mice to achieve significance, consistent with the much more robust changes in G6-Pase gene expression in the resistin null mice. In addition, although effects on the liver are the most pronounced in this model, effects of resistin on glucose disposal in skeletal muscle and adipose tissue have been described and may be relevant in other settings (9,20), and adipose-derived resistin may function in a paracrine manner. Nevertheless, together with earlier studies demonstrating that antibody neutralization of resistin lowers serum glucose levels in the setting of hyperresistinemia (9) and that acute systemic administration of resistin impairs glucose homeostasis (13), our results strongly suggest that resistin functions as an endocrine hormone to modulate glucose homeostasis. This has important implications for humans, where circulating resistin may be primarily derived from macrophages rather than adipocytes (21,22).

Obesity- and nutrient-related defects in glucose homeostasis are multifactorial, which is why we studied hyperresistinemic transgenic mice of normal weight that were fed a normal laboratory diet. Thus, the abnormalities in glucose metabolism observed in these mice were due to the hyperresistinemia per se, rather than other factors associated with obesity. The resistin-transgenic mice had high levels of resistin nearly from the time of birth, and it is likely that counterregulatory mechanisms and/or desensitization to the actions of resistin contribute to the modest phenotype. Nevertheless, the fasting hyperglycemia, impaired glucose tolerance, and altered whole-body glucose metabolism in the resistin-transgenic mice suggest

that counterregulation and/or desensitization are insufficient to completely overcome the detrimental effects of prolonged hyperresistemia. Compensation may be even less of a factor when signaling by counterregulatory factors such as glucocorticoids and adiponectin are also altered, as occurs in obesity.

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