Insulin Resistance and Obesity: Resistin, A Hormone Secreted by Adipose Tissue
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A newly discovered hormone named adipocyte-secreted factor, or resistin, is secreted by adipocytes in mice. Expression of resistin is low during food deprivation and in diabetes, and increased greatly during refeeding and insulin treatment. It is found in serum in mice and humans, and is greatly increased in obesity. Resistin inhibits adipocyte differentiation and may function as a feedback regulator of adipogenesis. Administration of resistin to mice resulted in increased glucose production and blood glucose levels. Therefore, resistin also functions as a regulator of glucose homeostasis and a physiologic antagonist to hepatic insulin action.

Key words: adipocyte-secreted factor, resistin, adipocytes, peroxisome proliferator-activated receptor γ.

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expression vector of the gene was transfected into COS cells, and the cells secreted resistin into the medium.

Resistin-mRNA (R-mRNA) was not detectable in 3T3-L1 pre-adipocytes. Expression was induced by causing the pre-adipocytes to differentiate into adipocytes by means of glucocorticoid treatment. Resistin was expressed, along with such markers of adipocytes as peroxisome proliferator-activated receptor γ (PPARγ). Primary pre-adipocytes lacked resistin, but showed a large increase in expression in the later stages of differentiation, an induction confined to adipose tissue.

The expression of R-mRNA was regulated nutritionally as follows. Resistin from adipose tissue of food-deprived mice was quite low and increased 25-fold upon feeding a carbohydrate-rich, fat-free, lipogenic diet within 16 hours; in contrast, fatty acid synthase mRNA increased 34-fold. Insulin injection into diabetic mice produced an almost identical effect: R-mRNA increased 23-fold in 30 minutes.

The authors then incubated differentiating 3T3-L1 pre-adipocytes in a medium containing resistin, which was obtained from COS cells transfected with the epitope-tagged vector of the resistin gene (R-HA). Whereas differentiation of control cells incubated in medium lacking resistin induced PPARγ and other indicators of adipocyte differentiation abundantly, the 3T3-L1 cells in the resistin-containing medium exhibited a dramatic (80%) decline in PPARγ induction. Therefore, resistin acted by inhibiting adipocyte differentiation.

The discovery of resistin by Steppan et al.5, made simultaneously with Kim et al.,4 was based on a somewhat different approach. Steppan et al.5 carried out their investigation on a class of antidiabetic drugs called thiazolidinediones (TZDs), which are known to function by increasing insulin sensitivity of target tissues through binding with high affinity to the nuclear receptor PPARγ. This receptor, when combined with TZDs, activates a gene that causes increased insulin sensitivity. Another effect of PPARγ activation is the stimulation of adipocyte differentiation. The conclusions of Steppan et al.,5 then, were based on the following facts: (a) TZDs lower insulin resistance and/or increase insulin sensitivity, (b) TZDs activate PPARγ, and (c) PPARγ expression accompanies or is required for pre-adipocyte differentiation into adipocytes. The investigators asked the question: could adipocyte differentiation be accompanied by the induction of insulin resistance? Or, quite generally, is there an adipocyte signal that mediates insulin resistance?5

Steppan et al.5 screened for genes induced during differentiation of pre-adipocytes and repressed by TZDs in mature adipocytes. They found that a cDNA was expressed during differentiation, coding for a protein they named “resistin” (found to be identical to ADSF4), which was sequenced. R-mRNA was induced in cultured pre-adipocytes (3T3-L1) when made to differentiate, along with PPARγ-mRNA. Resistin was secreted into the culture media by the differentiated adipocytes. In mature adipocytes, TZDs inhibited resistin gene expression and secretion.

As found by Kim et al.,4 resistin was expressed solely in WAT. By immunohistochemistry, Steppan et al.5 showed that resistin occurred abundantly in adipocyte cytoplasm. The human homolog of resistin was localized to chromosome 19.

Immunoreactive resistin was detected in normal rat and mouse serum. As also determined by Kim et al., it declined upon fasting and reappeared upon refeeding. TZD treatment caused virtual disappearance of serum resistin.

Next, Steppan et al.5 determined resistin in serum of mice made obese by feeding a high-fat diet. At the time—after 4 weeks—when the mice became obese and insulin resistant (both serum insulin and serum glucose levels nearly doubled), their serum resistin rose dramatically, as was also found in genetically obese mice (ob/ob and db/db mice). When serum resistin was neutralized by intraperitoneal injection of anti-resistin-IgG into mice with diet-induced obesity, their elevated blood glucose declined to normal levels and insulin sensitivity was significantly increased. This experiment showed that the increased circulating resistin was the cause of insulin resistance in diet-induced obesity or, in other words, the cause of type 2 diabetes.

To determine whether injected recombinant resistin would have the same effect as the endogenous protein, Steppan et al.5 transfected resistin-cDNA tagged with a carboxy-terminal epitope (“Flag”) into 293T cells, and isolated and purified Flag-tagged resistin on a Flag-agarose column. Upon injection of resistin-Flag (R-Flag), serum resistin levels increased within 30 to 60 minutes, blood glucose levels rose by 28%, and insulin levels rose (though not significantly).

Steppan et al.5 tested the hypothesis that resistin functions in an autocrine or paracrine manner. Adipocytes in culture secreted resistin into the medium. When the cells were exposed to anti-R-IgG, the insulin-stimulated uptake of [3H]2-deoxyglucose was increased 42%. Conversely, treatment of the insulin-stimulated adipocytes with R-Flag caused a 37% decline in glucose uptake.

To summarize the results of the studies described above, resistin is a hormone secreted by WAT, occurs in serum, and is induced during adipogenesis. Its expression is reduced by the antidiabetic TZD drugs. Its level is increased in diet-induced and genetic obesity, and therefore it is causally connected to obesity-associated insulin resistance. Steppan et al.5 suggested that resistin “is a unique hormone whose effects on glucose metabolism
are antagonistic to those of insulin.” Indeed, Rajala et al.9 showed that acute increases in circulating resistin increased glucose production, possibly by activation of the glucose-6-phosphatase gene.

To explain the function of resistin on a molecular basis, Steppan et al.5 proposed the following hypothesis: Because its expression is reduced by TZD drugs, resistin is the downstream target of PPARγ action, and the insulin-sensitizing effect of PPARγ may be due to inhibition of synthesis and secretion of resistin. This hypothesis, however, is controversial. Way et al.10 found that TZDs, acting as PPARγ agonists, caused an increased expression of resistin in obese, insulin-resistant mice.

The long-term in vivo function of resistin could best be ascertained by a knockout of its gene. This was achieved by Banerjee et al.,11 who generated resistin-deficient (R−/−) mice by replacing the coding exon of the resistin gene with the reporter gene LacZ, which then appeared exclusively in WAT. The R−/− mice expressed neither R-mRNA nor resistin protein in WAT, and resistin was not found in R−/− serum. Blood glucose levels in the R−/− mice, when fed a high-fat diet, were 20% to 30% lower than in wild-type (WT) controls. Intraperitoneal injection of recombinant resistin restored blood glucose to normal levels.

Glucose tolerance was similar in the R−/− and WT mice when given a chow diet. The mutants had a better glucose tolerance when fed a high-fat diet (Figure 1). By means of a euglycemic glucose clamp and continuous infusion of insulin, producing hyperinsulinemia, Banerjee et al.11 determined that the R−/− mice so treated had greater insulin tolerance. They found reduced glucose production compared with WT controls. Gluconeogenesis is normally down-regulated by activation of adeno-

sine monophosphate-activated protein kinase (AMPK).12 This activation process was lacking in the R−/− mice; therefore, their glucose production was reduced and restored upon resistin injection (Figure 2). The resistin-knockout experiments, therefore, suggest that one of the in vivo functions of R is “to act on the liver to inhibit AMPK and thereby increase glucose production.”12

An ingenious method to produce a transgenic mouse with a dominant negative form of resistin was developed by Kim et al.13 The idea is based on the fact that resistin occurs both as a monomer and a homodimer. Further, it can form heterodimers with other members of the family (FIZZ or RELM).

Kim et al.13 produced a resistin protein to which was fused a bulky protein. This was done by engineering the resistin gene attached to the gene of the human immunoglobulin-γ-constant region (the heavy-chain Fc region of IgGγ, R-hFc). The R-hFc gene so made was cloned into a vector with an adipocyte fatty acid-binding protein (aP2) promoter/enhancer, with which the R-hFc transgenic mice were generated.

The hypothesis developed by Kim et al.13 was that the R-hFc protein would dimerize with endogenous resistin and other members of this gene family, and such a dimer, consisting of the bulky R-hFc molecule attached to the smaller resistin molecule, would inhibit the normal functioning of resistin.

To test the first hypothesis, R-hFc and resistin tagged with hemagglutinin (for ease of detection on Western blots, resistin-hemagglutinin [R-hFc]) was transfected separately or together into COS7 cells. The conditioned medium showed homodimers of R-hFc and R-HA when analyzed by Western blots. Also, R-hFc co-immunoprecipitated with R-HA, thus proving the hypothesis that the heavy R-hFc had dimerized with resistin.

To test the second hypothesis, that the R-hFc-R-HA heterodimer would have a dominant negative effect on the function of resistin, Kim et al.13 investigated the action of R-hFc on adipocyte differentiation. Their previous studies4 had shown that resistin inhibited the differentiation of pre-adipocytes into adipocytes. Conditioned media from COS7 cells transfected with R-hFc or R-HA or both were incubated with 3T3-L1 pre-adipocytes. Treatment with R-HA media, as expected, showed inhibition of lipid accumulation and inhibition of the induction of molecular markers of differentiation to adipocytes, such as PPARγ and aP2, compared with control media. On the other hand, 3T3-L1 cells treated with media containing R-hFc or R-hFc plus R-HA differentiated into adipocytes exactly like cells treated with control media. Kim et al.13 concluded that the bulky molecule of R-hFc, by combining with R-HA, prevented the inhibitory action of resistin on adipocyte differentiation.

Having proved that, in vitro, R-hFc had a dominant
negative action on resistin function, Kim et al. next engineered mice expressing R-hFc in their adipose tissue. These mice, in effect, would be the functional equivalent of resistin knockout mice. Both low (L) and high (5-fold; H) expression level lines were generated. R-hFc was expressed only in WAT, brown adipose tissue, and serum. In the latter, the level of R-hFc was much higher than resistin in WT serum, the R-hFc apparently being a more stable protein than resistin.

To arrive at an insight into the physiologic function of resistin, long-term in vivo experiments were carried out with the resistin-suppressing R-hFc transgenic mice. These mice, expressing R-hFc in their adipose tissue, were fed either a chow or a high-fat diet for 10 weeks. The H-line R-hFc transgenic mice had a 20% greater body weight and their fat pads weighed 56% to 68% more than the WT controls when fed the chow or the high-fat diets, even though their food intakes were identical. Adipose cell size was enlarged. Molecular markers of adipocyte differentiation, such as PPARγ, showed significant increases, as well as late markers of differentiation (stearoyl CoA desaturase, fatty acid-binding protein). Increased fat pad weights correlated, both in L- and H-line mice, with level of expression of R-hFc. In short, in chow-fed and in high-fat diet-fed transgenic mice, the expression of the R-hFc gene resulted in increased adipocyte differentiation, no doubt because the normal function of resistin in inhibiting adipocyte differentiation was abolished.

Resistin was found to impair insulin action and glucose tolerance. Therefore, Kim et al. next studied the effect of in vivo suppression of resistin in the R-hFc transgenic mice on glucose and on insulin tolerance. Chow-fed R-hFc transgenic mice, in a glucose tolerance test, showed lower blood glucose levels and in insulin tolerance tests became hypoglycemic compared with WT mice. To discover a clue to the connection between obesity and insulin resistance, a high-fat diet was fed to R-hFc transgenic and WT mice. Whereas the WT mice exhibited the expected insulin resistance characteristic of obesity, showing a poor glucose tolerance, the R-hFc mice tolerated the glucose challenge well. Upon insulin injection, the obese WT mice were resistant, whereas the R-hFc transgenic mice responded normally to the hormone (Figure 3). Therefore, the R-hFc transgenic mice resisted obesity-mediated glucose intolerance and insulin resistance. These results firmly establish the role of resistin in the diet-induced impairment of glucose tolerance and insulin action (Figure 4).

How does suppression of resistin in the R-hFc transgenic mice affect plasma triglycerides and free fatty acids? Kim et al. found that, when given a high-fat diet, levels of plasma triglycerides and free fatty acids were lower in the R-hFc transgenic mice compared with WT mice, despite their adiposity. The authors state: “...the adipose tissues from obese R-hFc transgenic mice still have the ability to maintain normal tissue lipid homeostasis.” This effect was no doubt caused by the increased formation and secretion of leptin and adi-
Adipose tissue leptin and adiponectin were four times higher in the R-hFc than in the WT mice, with similar increases in plasma levels.

In conclusion, recent research\(^1\) has determined that resistin is a hormone from adipose tissue that regulates adipocyte differentiation through a negative feedback mechanism to limit adipose tissue formation in response to increased energy intake. It also functions as a regulator of glucose homeostasis and a physiologic antagonist to hepatic insulin action.

What is the relevance of these findings to human obesity and insulin resistance? Resistin is present in human serum\(^1\) and is correlated to the extent of obesity.\(^5\) Resistin expression in humans is proportional to insulin resistance,\(^6\) though its serum level does not appear to be correlated with insulin resistance.\(^7\) Surprisingly, resistin is not found in human adipocytes,\(^8\) but has its source in circulating macrophages\(^9\) and may have a role in the inflammatory response.\(^10\) Banerjee et al.\(^11\) speculate that “human and mouse resistin may have similar metabolic functions despite their divergent sites of production.”

Central Obesity and Elevated Liver Enzymes
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Nonalcoholic fatty liver disease is commonly associated with obesity, a growing epidemic worldwide. A new large, population-based investigation has shown a statistically significant association between central adiposity and elevated liver enzymes. This finding adds to the growing research specifically linking central adiposity, and more specifically, visceral adiposity, with adverse health effects.

Key words: obesity, liver enzymes, fatty liver, nonalcoholic fatty liver disease, adipose tissue, central adiposity, visceral adiposity

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Nonalcoholic fatty liver disease (NAFLD), currently defined as fat accumulation in the liver exceeding 5% to 10% by weight,1 is estimated to affect 10% to 24% of the general population.2 The term refers to a wide range of liver damage, from steatosis to steatohepatitis, advanced fibrosis, and cirrhosis. Requiring alcohol consumption to be less than 14 units/week or 20 g/day,1 NAFLD is still the most common etiology of abnormal liver test results among adults in the United States.2 NAFLD is a syndrome with a multifactorial etiology, with which obesity is most commonly associated.3 Obesity is a growing worldwide epidemic with links to numerous medical conditions such as diabetes, cardiovascular disease, ischemic stroke, hypertension, obstructive sleep apnea, gout, osteoarthritis, and a higher incidence of some cancers. Steatosis is found in more than two-thirds of the obese population, and in more than 90% of the morbidly obese.2 Steatohepatitis affects 3% of the lean population, 19% of the obese population, and almost half of the morbidly obese population.2 A recent study by Stranges et al.4 evaluated the relationship between central fat accumulation, body mass index (BMI, kg/m2), and the liver enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and γ-glutamyltransferase (GGT). It is the first large, population-based investigation to do so, and the findings seem to support a role of central adiposity independent of overall adiposity in predicting increased liver enzymes and potential liver damage.

The study by Stranges et al.4 was based on data obtained from a sample of residents of Erie and Niagara Counties in New York State from September 1995 to May 2001; 6837 people were identified and 4065 agreed to participate. Exclusion criteria were: a self-reported history of chronic or acute hepatitis, cirrhosis, or noncirrhotic liver disease; coronary artery disease; missing anthropometric measurements; missing data on education, smoking, and drinking habits; and missing blood determination of liver enzymes. Of the total population,