Does resistin resist insulin?

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Diabetes remains one of the oldest diseases all over the world and is the major cause of morbidity and mortality in human populations. The pathology is a consequence of a lack of insulin or resistance to insulin, resulting in an increase in blood glucose levels[1]. Resistance to insulin is an important risk factor in the industrial world and is often associated with obesity. Taking the proportions of an epidemic in the West, particularly USA, obesity is a consequence of an imbalance between energy intake and energy expenditure[2]. Despite the strong clinical/pathological link between obesity and type-2 diabetes, the molecular link had remained a mystery.

Recently[3], a unique signalling molecule was identified from mouse adipocytes. This molecule, termed as resistin (resistance to insulin), was found to be over expressed in the mouse model of obesity. Circulating resistin level decreased with the administration of anti-diabetic drug, rosiglitazone. It was further shown that resistin levels increased in diet-induced and genetic forms of obesity (ob/ob and db/db). Insulin-stimulated glucose uptake was greatly reduced upon treatment of 3T3 L1 adipose cells with purified recombinant resistin. Immuno-neutralization of resistin increased insulin sensitivity, whereas insulin resistance was seen upon treatment with purified resistin. This study attempted to categorically establish the involvement of resistin in mediating insulin resistance in diet-induced obesity[3–5]. This exciting discovery, while trying to provide a molecular link between obesity and insulin resistance, generated several new questions[6]. Is there an expression-related association of a resistin with pre-disposition to obesity and/or diabetes? What is the status of resistin in naturally obese human population?

Is resistin gene expression directly related to insulin resistance?

A number of independent reports from different groups paint contrasting pictures on the association of resistin with diabetes and obesity. Using *db/db* mice model Moore *et al.*[7] showed that resistin gene expression was inhibited significantly after treatment with TZD class of anti-diabetic drug called thiazolidenidiones. While this study endorses the original observation of Steppan *et al.*[3], several other studies, however, have failed to establish such association. Way *et al.*[8] showed significantly decreased resistin expression in white adipose tissue (WAT) of several different genetic models of obesity, including the *ob/ob*, *db/db*, *tub/tub* mouse, compared to control mouse. They further showed that in response to several other classes of PPARγ agonist, the level of resistin in adipose tissue was increased in both *ob/ob* mice and Zucker diabetic fatty rats.

Using Real Time–PCR resistin gene expression was examined in fat cells, adipose tissue and muscles from 42 human subjects[9]. The subjects selected were overweight by varying degrees and showed normal insulin sensitivity, resistance or type-2 diabetes. Resistin was not expressed in the human muscle or much of the fat cells or intact biopsies. Interestingly, there was no difference in the resistin level between normal insulin resistance or type-2 diabetic individuals. In this study, the presence of splice variants of resistin was also ruled out. This study[9] concluded rather guardedly that resistin production by fat cells is not an important cause of insulin resistance at least in humans, even though some individuals may express this protein at extremely low levels. In another study which de-linked resistin from obesity and diabetes in humans[10], resistin expression in subcutaneous abdominal adipocytes was examined in 24 women volunteers. Like the previous study, the selected human volunteers exhibited a wide range of body weight and insulin resistance. This report failed to find any relationship between body weight, insulin sensitivity or other metabolic parameters on adipocyte resistin gene expression. Similarly, using RT–PCR-based assay, Savage *et al.*[11] demonstrated a lack of correlation among resistin expression, obesity, insulin resistance and PPARγ action in humans. Likewise, the reported inhibition[12] of resistin gene expression in 3T3 L1 adipocytes by insulin was further inconsistent with a role for resistin in the induction of insulin resistance.

Further contributing to this controversy, was the report describing decreased resistin expression in mice with different sensitivities to a high fat diet[13]. The expression of resistin in isolated adipocytes was shown to be as abundant as that encoding fatty acid synthase, a functionally important protein in adipose tissue. However, a comparison of adipose resistin expression in mice which were differently sensitive to diet-induced obesity revealed that the obese phenotype was related to decreased resistin expression in adipocytes, and high fat feeding *per se* did not directly modulate resistin expression. Juane *et al.*[14] reported suppressed expression of adipocyte resistin gene in an insulin-resistant rat model; free fatty acids levels were elevated and this was found to suppress resistin expression.

Does single nucleotide polymorphism analysis support resistin as a link between diabetes and obesity?

Single nucleotide polymorphism (SNP) analyses of resistin gene were investigated to find an association, if any, with type-2 diabetes or obesity at the population level. In one study[15], an association of 5′ flanking variants of resistin with obesity in individuals from Qubec, Canada was reported. In another report using PCR direct sequencing of 24 Japanese type-2 diabetic patients, 3 SNPs in the introns, but none in the coding regions were identified[16]. No association of these SNPs or haplotypes defined by these SNPs in linkage disequilibrium with type-2 diabetes was evident. In yet another study, SNPs within the coding sequence of the three exons of resistin gene together with its 5′ regulatory region and 3′ untranslated region was analysed in 58 type-2 diabetic, 59 obese

and 60 normal human subjects. A single sequence variant was detected in the 3′ untranslated region of exon 3. Interestingly however, no evidence of association of this novel SNP with type-2 diabetes and/or obesity in Italian subjects could be found. This study[17] therefore concluded that genetic defects of the resistin gene are unlikely to play a role in the etiology of these common disorders in their population.

Is resistin truly the molecule that is responsible for insulin resistance? Search for other adipocytokines

Although body fat is generally associated in a negative way in the context of human health, the adipocytes produce and secrete, in addition to resistin, several other bioactive molecules or hormones known as adipokines or adipocytokines. Association of dysregulated production of adipocytokines like TNF-α, leptin and plasminogen-activator inhibitor type-1 (PA1-1) with pathophysiology of obesity-related insulin resistance and thrombosis has been reported[18–21]. Another adipocytokine, the adiponectin/ACRP30 produced exclusively in human, monkey and mice adipose tissue, is abundantly present in plasma. However, adipose mRNA and plasma levels decrease in obesity and type-2 diabetes. Involvement of adiponectin in insulin sensitivity has been reported earlier[22]. Further evidence on the insulin-sensitizing ability of adiponectin comes from the observation that treatment of mice with adiponectin/ACRP30 improves diabetes in mice[23–25]. In a recent study[26], adiponectin knockout mice were used to investigate the biological function of this important adipokine. Mice that were genetically deficient for adiponectin/ACRP30 exhibited delayed clearance of free fatty acid in plasma, low levels of fatty acid transport proteins-1 (FAT P-1) mRNA in muscle, high levels of TNF-α mRNA in adipose tissue and high plasma TNF-α concentration. These knockout mice showed a weight gain comparable to that of control mice when placed on a high fat diet. However, in contrast to controls, they developed moderate to severe insulin resistance, a characteristic feature of type-2 diabetes. Such knockout mice showed increased response to mechanical injury of the femoral artery, thereby producing a model mimicking human atherosclerosis. These studies clearly highlight the importance of adiponectin/ACRP30 in diet-induced insulin resistance. While pharmacological intervention aimed at increasing plasma adiponectin levels compared to those of plasma TNF-α should be useful in the treatment of diabetes and atherosclerosis, adiponectin appears to have established its candidature as a critical molecular link between obesity, diabetes and atherosclerosis.

The murine resistin gene sequence is identical to a number of the recently reported *fizz* family of secreted cysteine-rich proteins. It therefore, appears that the use of the term resistin for the human counterpart of *fizz 3* may not be appropriate until more studies are carried out to explain the conflicting results about association of resistin with obesity and type-2 diabetes. It would not be unreasonable to predict that some other molecule(s) may emerge as important player(s) in this otherwise complicated and complex disorder. The coming years will expectedly see the emanation of new candidate molecules in this competitive and challenging field of research.

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