Metabolism
Clinical and Experimental

VOL 53, NO 4 APRIL 2004

PRELIMINARY REPORT

Resistin Serum Levels in Type 1 Diabetes Pre- and Post-Islet Transplantation

Anath Shalev, Noelle B. Patterson, Boaz Hirshberg, Kristina I. Rother, and David M. Harlan

Resistin is a recently described secretory protein produced in adipocytes that is thought to be involved in insulin resistance, diabetes, and inflammation. While resistin can be detected in mouse and human serum, very little is known about the regulation of serum resistin levels, especially in humans. To test whether resistin levels are affected by type 1 diabetes mellitus (T1DM), we measured serum resistin levels in samples from 5 healthy volunteers and 6 patients with T1DM pre- and 3 months post-islet transplantation using a human resistin enzyme immunoassay (EIA). Interestingly, serum resistin levels were significantly higher in T1DM patients before transplantation compared to normal controls, but decreased to normal levels after islet transplantation. Thus, our results suggest that human resistin may be involved in the pathophysiology of T1DM and thereby reveal a heretofore unappreciated aspect of human resistin biology.
© *2004 Elsevier Inc. All rights reserved.*

RESISTIN, also called FIZZ3 (found in inflammatory zone 3)[1] or adipocyte-secreted factor (ADSF),[2] is a secretory protein produced by adipocytes.[3] Resistin was first identified in a screen for proteins that were downregulated by the insulin-sensitizing antidiabetic drug rosiglitazone and it was shown to cause insulin resistance.[3] Although still controversial,[4] evidence suggests that resistin plays a role in glucose homeostasis both in mice and humans.[5-7] Resistin is also thought to represent a novel cytokine[4] and to be involved in inflammation.[1] Resistin is measurable in mouse and human serum,[3,8-10] but data regarding human serum resistin levels and its metabolic regulation are very limited. In this study we therefore investigated whether human serum resistin levels are affected by type 1 diabetes mellitus (T1DM) associated with impaired glucose homeostasis, as well as autoimmunity/inflammation, or by islet transplantation, a treatment used increasingly for selected individuals with T1DM.[11]

MATERIALS AND METHODS

Patients were enrolled in the islet transplantation protocol approved by the National Institute of Diabetes and Digestive and Kidney Disease (NIDDK) Institutional Review Board of the National Institutes of Health and provided full informed consent.[12] Since all 6 islet recipients were Caucasian females, 5 healthy Caucasian female volunteers were used as controls. The mean body mass index (BMI) was 24.9 ± 3.6 for controls and 22.3 ± 2.7 for T1DM patients. Average diabetes duration was 33 ± 13.2 years, average daily insulin requirements were 0.5 ± 0.1 U/kg, and mean hemoglobin A_{1C} (HbA_{1C}) was 8.1% ± 1.1%. A final mass of 4,000 to 13,500 islet equivalents (IEQs)/kg body weight was infused into the portal vein and recipients were treated with tacrolimus, sirolimus, and daclizumab as described previously.[11,12] Fasting serum samples were obtained from healthy volunteers and from T1DM patients before and approximately 3 months after islet transplantation and frozen for later analysis.

Serum resistin levels were measured using a resistin (51-108) enzyme immunoassay (EIA) kit (Phoenix Pharmaceutical Inc, Phoenix, AZ) according to the manufacturer's instructions. This kit has been validated by the supplier, demonstrating a high specificity with no cross-reactivity with other peptides such as insulin, leptin, ghrelin, orexin, or neuropeptide Y. The test was further found to be sensitive with a minimum detectable concentration of 5 ng/well. Intra-assay error was less than 5% and interassay error less than 14%. Standard curves were plotted on a lin/log scale and R_2 values ~0.95 were obtained. Positive controls included 5 ng/mL resistin peptide (51-108), resistin standard in EIA buffer, and normal serum spiked with 2 ng/mL peptide (51-108). Bovine serum albumin 1 μg/mL served as a negative control.

From the Transplantation and Autoimmunity Branch, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Department of Health and Human Services, Bethesda, MD.

Submitted August 25, 2003; accepted November 4, 2003.

Current affiliation for A.S.: Endocrinology, Diabetes and Metabolism Section, Department of Medicine, University of Wisconsin, Madison, WI.

Address reprint requests to Anath Shalev, MD, H4/526 Clinical Science Center, 600 Highland Ave, Madison, WI 53792.

© 2004 Elsevier Inc. All rights reserved.

0026-0495/04/5304-0015$30.00/0
doi:10.1016/j.metabol.2003.11.014

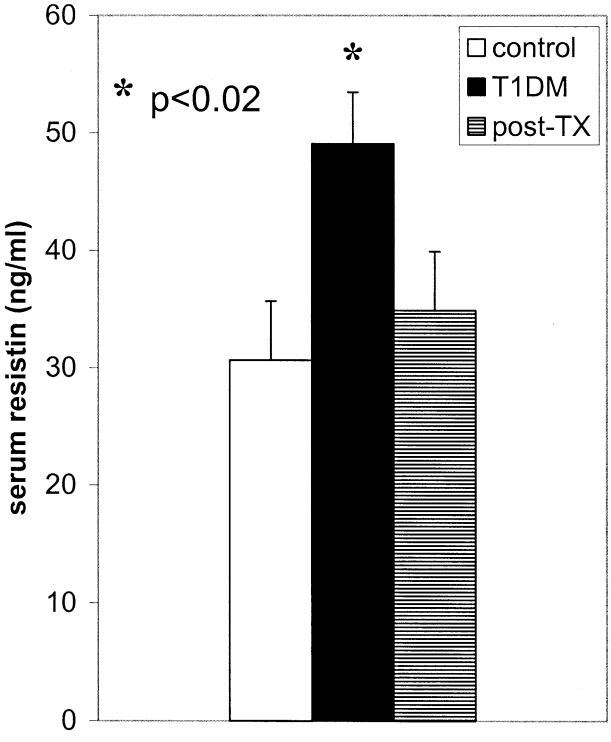

Fig 1. Human serum resistin levels. Bars represent average serum resistin concentration (ng/mL) ± SEM as measured by human resistin EIA in 5 healthy controls (□), 6 patients with T1DM (■), or the same 6 patients 3 months after islet transplantation (▤). All measurements were done in duplicates as described in the Methods. The *P* value was calculated with a 2-sided Student *t* test. Serum resistin levels were significantly higher in patients with T1DM compared to healthy controls or to post-transplant values. No significant difference was observed between post-transplant values and normal controls.

RESULTS AND DISCUSSION

The average serum resistin level in healthy control subjects was 30.7 ng/mL (Fig 1). Interestingly, based on several baseline measurements, serum resistin levels were significantly elevated in patients with T1DM before islet transplantation compared to healthy controls (Fig 1). Potential explanations for these findings include insulin resistance associated with long-term insulin therapy of T1DM, suboptimal metabolic control pre-islet transplantation, and the autoimmune/inflammatory process presumed to underlie T1DM. However, we did not find any correlation with patient age, weight, BMI, insulin requirement, or HbA_{1C} (data not shown).

Even more surprisingly, we found that islet transplantation normalized serum resistin levels (Fig 1). Three patients became and remained insulin-independent,[12] but again no trend between transplant outcome and resistin levels was detected (data not shown). The 3 insulin-independent transplant recipients were assessed for insulin sensitivity as described previously,[13,14] and demonstrated a mean fasting glucose to insulin (G/I) ratio of 10.6 ± 5.6 and an insulin sensitivity index (Si) (calculated using the frequently sampled intravenous glucose tolerance test and the minimal model) of 7.7 ± 3.4. These values, both within the normal range, suggest that no major insulin resistance was present in these patients, at least in this early post-transplant phase. While we did see a reduction in mean body weight from 60.8 ± 6.5 kg pretransplant to 58.3 ± 6.5 kg post-transplant, no correlation was found between the amount of weight loss and the reduction in resistin levels (data not shown). This makes loss of body fat mass as an explanation unlikely and suggests that islet transplantation and/or the immunosuppression may have improved the pretransplant conditions.

In summary, we found that resistin levels are elevated in T1DM and are normalized by islet transplantation. Our results raise the possibility that resistin may be involved in the pathophysiology of T1DM and provide new insight into human resistin biology.

REFERENCES

1. Holcomb IN, Kabakoff RC, Chan B, et al: FIZZ1, a novel cysteine-rich secreted protein associated with pulmonary inflammation, defines a new gene family. EMBO J 19:4046-4055, 2000

2. Kim KH, Lee K, Moon YS, et al: A cysteine-rich adipose tissue-specific secretory factor inhibits adipocyte differentiation. J Biol Chem 276:11252-11256, 2001

3. Steppan CM, Bailey ST, Bhat S, et al: The hormone resistin links obesity to diabetes. Nature 409:307-312, 2001

4. Hotamisligil GS: The irresistible biology of resistin. J Clin Invest 111:173-174, 2003

5. Rajala MW, Obici S, Scherer PE, et al: Adipose-derived resistin and gut-derived resistin-like molecule-beta selectively impair insulin action on glucose production. J Clin Invest 111:225-230, 2003

6. McTernan CL, McTernan PG, Harte AL, et al: Resistin, central obesity, and type 2 diabetes. Lancet 359:46-47, 2002

7. Smith SR, Bai F, Charbonneau C, et al: A promoter genotype and oxidative stress potentially link resistin to human insulin resistance. Diabetes 52:1611-1618, 2003

8. Fehmann HC, Heyn J: Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. Horm Metab Res 34:671-673, 2002

9. Kielstein JT, Becker B, Graf S, et al: Increased resistin blood levels are not associated with insulin resistance in patients with renal disease. Am J Kidney Dis 42:62-66, 2003

10. Yannakoulia M, Yiannakouris N, Bluher S, et al: Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. J Clin Endocrinol Metab 88:1730-1736, 2003

11. Ryan EA, Lakey JR, Paty BW, et al: Successful islet transplantation: Continued insulin reserve provides long-term glycemic control. Diabetes 51:2148-2157, 2002

12. Hirshberg B, Rother KI, Digon BJ, et al: Benefits and risks of solitary islet transplantation for type 1 diabetes mellitus using steroid sparing immunosuppression: The NIH experience. Diabetes Care 26: 3288-3295, 2003

13. Legro RS, Finegood D, Dunaif A: A fasting glucose to insulin ratio is a useful measure of insulin sensitivity in women with polycystic ovary syndrome. J Clin Endocrinol Metab 83:2694-2698, 1998

14. Bergman RN: Lilly Lecture 1989. Toward physiological understanding of glucose tolerance. Minimal-model approach. Diabetes 38: 1512-1527, 1989