

Paradoxical Elevation of High-Molecular Weight Adiponectin in Acquired Extreme Insulin Resistance Due to Insulin Receptor Antibodies

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Total plasma adiponectin and high-molecular weight (HMW) polymeric adiponectin are strongly positively correlated with insulin sensitivity. However, we have recently reported paradoxical hyperadiponectinemia in patients with severe insulin resistance due to genetically defective insulin receptors. This implies either that the insulin receptor has a critical physiological role in controlling adiponectin production and/or clearance or that constitutive insulin receptor dysfunction influences adiponectin levels through developmental effects. The aim of the current study was to distinguish between these possibilities using a human model of reversible antibody-mediated insulin receptor dysfunction and to refine the previous observations by determining adiponectin complex distribution. Cross-sectional and longitudinal determination of fasting plasma adiponectin and adiponectin complex distribution was undertaken in patients with extreme insulin resistance due to insulin receptor mutations, anti-insulin receptor antibodies (type B insulin resistance), or an undefined cause. Despite extreme insulin resistance, patients with type B insulin resistance (all women; mean age 42 years [range 12–54]) had dramatically elevated total plasma adiponectin compared with the general population (mean 43.0 mg/l [range 31.3–54.2] vs. 8.9 mg/l [1.5–28.5 for BMI <25 kg/m²]), which was accounted for largely by HMW polymers. Hyperadiponectinemia resolved in parallel with reduction of insulin receptor antibodies and clinical resolution of insulin resistance. Although the well-established inverse relationship between plasma insulin and adiponectin levels may, in part, reflect positive effects of adiponectin on insulin sensitivity, these data suggest that the magnitude of the effect of insulin action on adiponectin levels may have been underestimated. *Diabetes* 56:1712–1717, 2007

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FPLC, fast-protein liquid chromatography; HMW, high molecular weight.

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The ability of white adipose tissue to elaborate molecules with endocrine actions of relevance to fuel metabolism is well established (1). Most abundant of these adipose tissue-derived factors is adiponectin, a multimeric protein with homology to complement factor 1q (1). Adiponectin has excited considerable interest as a marker of insulin resistance because of the strong correlation between its plasma levels and insulin sensitivity (1) and because low plasma adiponectin is predictive of future type 2 diabetes (2). Furthermore, based on the elevation of adiponectin seen upon treatment with thiazolidinediones (3), the insulin-sensitizing effect of either infusion or transgenic overexpression of adiponectin in insulin-resistant rodents (4–6), and the significant association between genetic variants in the adiponectin gene and type 2 diabetes risk in human populations (1), it has been suggested that defects in adiponectin production and/or action may be an etiological factor in a significant proportion of human insulin resistance. Correcting suppressed adiponectin in insulin resistance has thus become an attractive therapeutic strategy.

However, we have recently reported paradoxical hyperadiponectinemia in patients with insulin receptor loss-of-function mutations and have suggested that this arises either from abnormal adipose tissue development or from loss of insulin action in mature adipose tissue (7). In this study, we sought to discriminate between these possibilities by studying a group of patients with acquired loss of insulin receptor function and extreme insulin resistance due to insulin receptor blocking antibodies (type B insulin resistance).

The proportion of adiponectin accounted for by high-molecular weight (HMW) adiponectin multimers or the absolute concentration of HMW multimers correlates better with insulin sensitivity in normal and type 2 diabetic populations than total plasma adiponectin (1), and human mutations in the adiponectin gene that are associated with type 2 diabetes produce mutant species that show impaired multimerization (8). This led us also to refine the previous findings by determining adiponectin complex distribution in patients with either congenital or acquired insulin receptor dysfunction or idiopathic severe insulin resistance.

TABLE 1
Characteristics of the patients with type B insulin resistance studied

| Patient | Sex | Age (years) | Ethnic origin | Associated autoimmune disease | Duration of severe insulin resistance before diagnosis of type B insulin resistance |
|---------|--------|-------------|------------------|-------------------------------|---|
| 1 | Female | 52 | Peruvian | MCTD | 6 months |
| 2 | Female | 12 | African American | SLE | 3 months |
| 3 | Female | 50 | African American | None | 16 months |
| 4 | Female | 54 | African American | None | 6 months (10 years of type 2 diabetes) |
| 5 | Female | 44 | African American | SLE | 4 years |
| 6 | Female | 37 | Indian | SLE | 7 months |
| 7 | Female | 20 | African American | MCTD | 6 months |

MCTD, mixed connective tissue disease; SLE, systemic lupus erythematosus.

RESEARCH DESIGN AND METHODS

Subjects with severe insulin resistance were recruited with informed consent in line with procedures approved either by the local research ethics committee in Cambridge, U.K., or by the institutional review board of the National Institute of Diabetes, Digestive, and Kidney Diseases, Bethesda, Maryland. Type B insulin resistance was diagnosed on the basis of clinical and biochemical evidence of severe hyperinsulinemia with detectable insulin receptor binding antibodies. Anti-insulin receptor antibody titers were determined by immunoprecipitation of insulin receptor preparations using patients' sera followed by Western blotting, using one of two closely related protocols (9,10).

Venous blood was drawn in the fasting state, and plasma was immediately extracted and stored at -20°C . Leptin and adiponectin assays have been described previously (7). Adiponectin complex distribution was determined by separating 20 μl of human serum over a Superdex 200 10/300 GL column (GE Healthcare Bio-Sciences) using an AKTA fast-protein liquid chromatography (FPLC) system (GE Healthcare Bio-Sciences). The column was equilibrated in PBS, pH 7.4, and 0.215-ml fractions were collected. Samples (40 μl) were collected over the entire elution of adiponectin and incubated with 10 μl 5 \times Laemmli sample buffer before electrophoresis on a Criterion precast 26-well gel (Bio-Rad). Immunoblotting using 1:500 polyclonal anti-adiponectin (NH_2 -terminal) antibody followed by incubation with IR-Dye 800-coupled goat anti-rabbit secondary antibody (Rockland) was undertaken. The fluorescence signal at 30 kDa was quantified using the LI-COR Odyssey infrared imaging system in conjunction with Odyssey v1.2 software (LI-COR Biotechnology, Lincoln, NE). Samples were from six normal control subjects (three men and three women), four patients with insulin receptor mutations (one man: 16 years old, insulin receptor P193L homozygote; and three women: 14 years, insulin receptor P193L homozygote; 41 years, insulin receptor F382V homozygote; and 28 years, insulin receptor K460E/Q672X compound heterozygote; metabolic parameters for all described previously [7]), initial samples from patients 1, 2, and 4 with type B insulin resistance (Tables 1 and 2), and three women patients with idiopathic severe insulin resistance (mean age 34.3 years, mean fasting blood glucose 197 mg/dl, mean fasting insulin 279 pmol/l, and mean total adiponectin 2.8 mg/l).

Normative adiponectin and leptin data were derived from the MRC Ely Study cohort, representative of an ethnically homogeneous European population in Eastern England (11). Those with diabetes on the basis of fasting blood glucose or of oral glucose tolerance testing were excluded from analysis. Complete data (fasting insulin, leptin, and adiponectin) were available for 872 nondiabetic participants (357 men and 515 women) and were used to generate sex- and BMI-specific reference ranges for adiponectin. Samples from patients with type 1 diabetes were obtained as part of the Oxford Regional Prospective Study of type 1 diabetes (12).

RESULTS

The characteristics of patients with type B insulin resistance were in keeping with the cumulative published experience of type B insulin resistance (9) (Table 1). Despite marked fasting hyperinsulinemia at presentation with extremely high requirements for exogenous insulin and oral insulin-sensitizing agents, all patients had total plasma adiponectin well above the sex- and BMI-specific reference range, with low or normal leptin. Plasma adiponectin values of all patients at the height of

their insulin resistance were significantly above the highest plasma adiponectin recorded in the 872 nondiabetic control subjects (28.5 mg/l). Clinical improvement with resolution of insulin resistance and reduction of anti-insulin receptor antibody titer was associated with decreases of plasma adiponectin to within the normal range, increases in BMI, improved glycemia and insulinemia, and reduction or cessation of treatment for diabetes. Patient 2 showed an increase in adiponectin over 3 months of therapy, but although the insulin receptor antibody titer waned over this period, glycemic control deteriorated despite unchanged insulin and metformin doses, suggesting worsening insulin resistance. Data from patient 1, who received no immunomodulatory therapy and in whom insulin resistance and anti-insulin receptor antibodies abated spontaneously over 3 years, demonstrate the marked and reciprocal responses of leptin and adiponectin to insulin sensitization (Fig. 1A). Importantly, only patient 5 had evidence of renal disease (systemic lupus erythematosus nephritis), and her measured creatinine clearance of 76 ml/min was only modestly depressed. No correlation was seen in other patients between changes in creatinine clearance and plasma adiponectin (Table 2).

Peak adiponectin levels corresponded not only to extreme insulin resistance but also to poor glycemic control, as evidenced by high A1C values (Table 2). However, in 10 female subjects with type 1 diabetes (mean age 17.0 years [range 13.1–20.6]) and similar A1C levels (mean 11.3% [9.2–13.2]), we found adiponectin levels to be much lower than in type B insulin-resistant patients, with no overlap between groups (mean 9.4 mg/l [range 3.5–13.7] vs. 43.0 mg/l [31.3–54.2]), suggesting that hyperglycemia and relative insulin deficiency per se do not explain the observation. Although this control group was younger than most of the type B patients studied, published experience suggests that in adult type 1 diabetic patients, too, the observed increase in plasma adiponectin is modest compared with healthy control subjects and does not approach the magnitude of the difference in the type B patients (13).

To determine whether insulin receptor dysfunction leads to changes in the distribution of higher order multimers of adiponectin, FPLC was undertaken of plasma from normal volunteers and from patients with insulin receptor mutations, type B insulin resistance, or idiopathic severe insulin resistance. The high total adiponectin in genetic and antibody-mediated insulin receptor dysfunction was accounted for largely by HMW adiponectin (Fig. 2), in marked contrast to patients with idiopathic severe insulin resistance, who had a preponderance of lower-

TABLE 2
Treatment, clinical, and biochemical parameters of type B insulin-resistant patients during therapy

| Patient | Time from diagnosis (months) | BMI (kg/m ²) | Fasting blood glucose (mg/dl) | Fasting plasma insulin (pmol/l) | A1C (%) | Leptin (μg/l) | Adiponectin (mg/l)* | Diabetes treatment | Immunosuppression | Anti-insulin receptor antibody titer | Serum creatinine (mg/dl)/CrCL (ml/min)† |
|---------|------------------------------|--------------------------|-------------------------------|---------------------------------|---------|---------------|---------------------|--|--|--------------------------------------|---|
| 1 | 0 | 20.4 | 244 | 11952 | 10.2 | <0.1 | 51.1 | Metformin, 4,000 IU insulin/day | Nil | ++ | 0.4/149 |
| | 6 | 21.8 | 66 | 17682 | 8.6 | 1.2 | 21.9 | 2,350 IU insulin/day | Nil | ++ | 0.3/152 |
| | 11 | 23.4 | 78 | 11681 | 8.7 | 2.3 | 16.3 | 1,250 IU insulin/day | Nil | + | 0.4/157 |
| | 17 | 23.8 | 55 | 14015 | 8 | 9.8 | 15.6 | 1,250 IU insulin/day | Nil | - | 0.4/143 |
| | 35 | 30.1 | 81 | 82 | 5.7 | 55.7 | 6.2 | Nil | Nil | - | 0.6/113 |
| 2 | 0 | 19.1 | 85 | 490 | 5.1 | 35.6 | 42 | Rosiglitazone, metformin, 450 IU insulin/day | 60 mg prednisone/day | +++ | 0.5/152 |
| | 3 | 21.8 | 70 | 212 | 7.6 | 18.6 | 60 | Rosiglitazone, metformin, 450 IU insulin/day | 5 mg prednisone/day Plasmapheresis, rituximab, and cyclophosphamide 20 mg prednisone/day | + | 0.5/134 |
| 3 | 0 | 23 | 378 | 6876 | 8.7 | 6 | 31.3 | 1,000 IU insulin/day | Nil | ++ | 0.5/NA |
| | 10 | 24.9 | 69 | 249 | 7.3 | 48.4 | 21.8 | Nil | Nil | ++ | 0.7/166 |
| 4 | 0 | 31.1 | 237 | 17029 | 12.1 | <0.1 | 54.2 | 1,400 IU insulin/day | Nil | ++ | 0.6/NA |
| | 0 | 25.6 | 108 | 2979 | 8.4 | 8.5 | 36.2 | 1,200 IU insulin/day | Nil | ++ | 1.4/76 |
| 6 | 0 | 16.8 | 63 | 12,500 | 12.1 | 0.4 | 32.0 | 2,800 IU insulin/day | Nil | +++ | 0.3/NA |
| | 0 | 20.7 | 321 | 42852 | 11.9 | 1.7 | 54.4 | 19,000 IU insulin/day | Nil | +++ | 0.6/97 |

Reference ranges for leptin and adiponectin are the 5th–95th percentiles from sex- and BMI-matched nondiabetic controls. *Normal ranges for adiponectin from a control population of 515 healthy control women are 4.4–17.7 mg/l for BMI <25 kg/m², 3.5–15.5 mg/l for BMI 25–30 kg/m², and 2.6–14.9 mg/l for BMI 30–35 kg/m². †CrCL, creatinine clearance, determined from 24-h urine collection.

molecular weight oligomers (Fig. 2). Furthermore, comparison of complex distribution before and after clinical resolution of type B insulin resistance in one patient revealed a marked shift in profile toward lower-molecular weight species with restitution of insulin receptor function (Fig. 1B). Representative elution profiles of adiponectin are also shown (Fig. 2B–F), demonstrating the ability of the technique used to detect trimers, hexamers, and HMW species.

DISCUSSION

Plasma adiponectin consistently correlates positively with insulin sensitivity in normal human populations (1), and we have previously established that this relationship also holds in almost all patients with severe insulin resistance, in whom adiponectin is extremely low (7). Striking exceptions have proved to be patients with loss-of-function mutations in the insulin receptor, in whom plasma adiponectin is not only an order of magnitude higher than seen in other states of severe insulin resistance but is also significantly higher than in the normal population (7). We suggested that this discordance between high plasma adiponectin and extreme insulin resistance may be accounted for either by

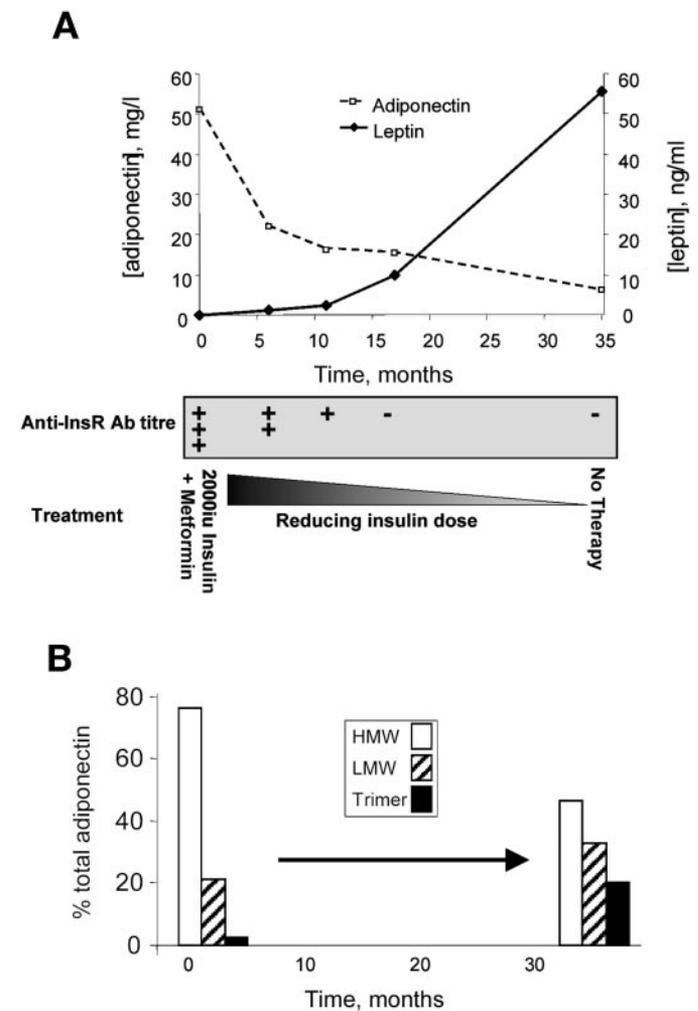


FIG. 1. Longitudinal course of patient 1 during spontaneous resolution of type B insulin resistance. Total plasma adiponectin (A) and adiponectin complex (B) distribution determined before and after resolution of insulin resistance.

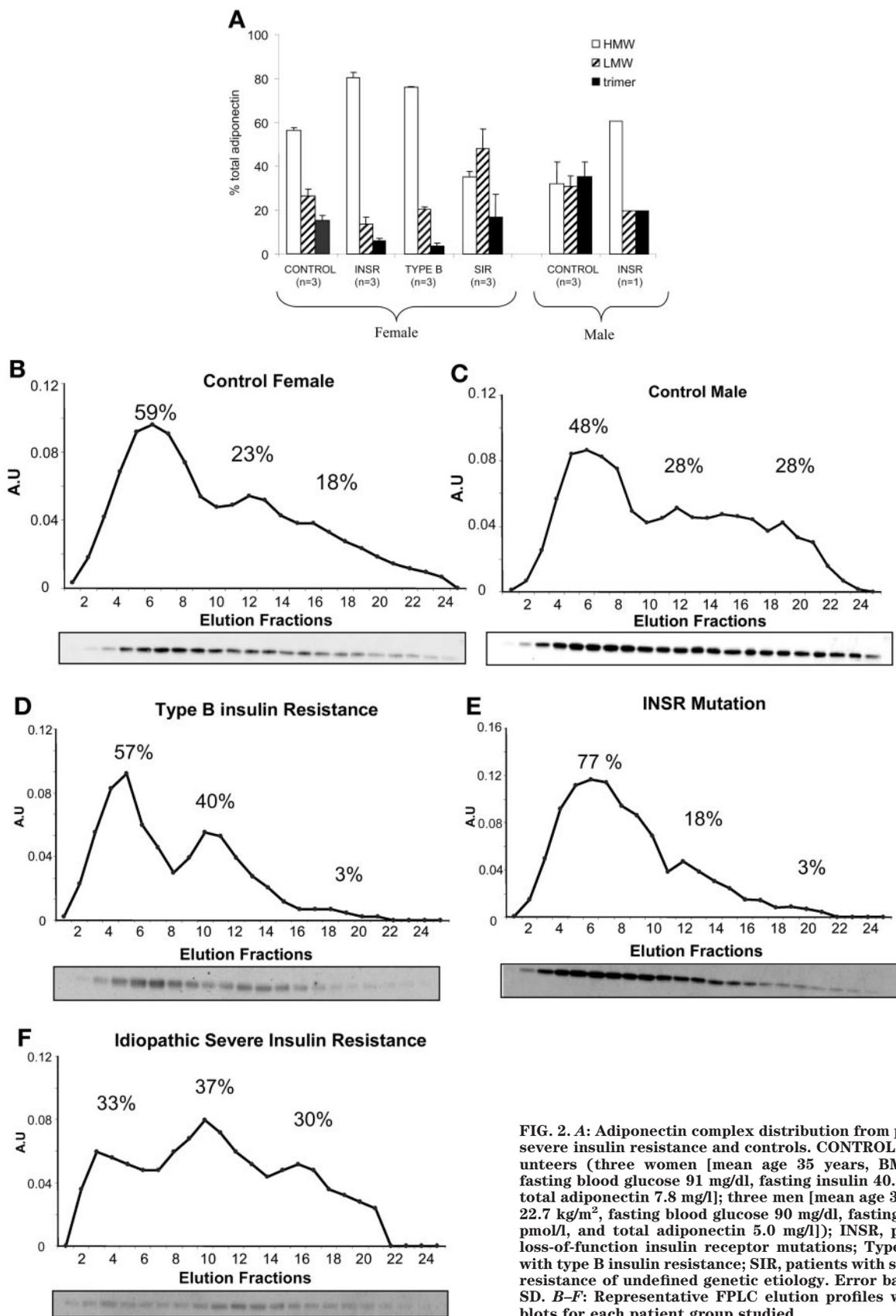


FIG. 2. A: Adiponectin complex distribution from patients with severe insulin resistance and controls. CONTROL, healthy volunteers (three women [mean age 35 years, BMI 20 kg/m², fasting blood glucose 91 mg/dl, fasting insulin 40.3 pmol/l, and total adiponectin 7.8 mg/l]; three men [mean age 35 years, BMI 22.7 kg/m², fasting blood glucose 90 mg/dl, fasting insulin 40.3 pmol/l, and total adiponectin 5.0 mg/l]); INSR, patients with loss-of-function insulin receptor mutations; Type B, patients with type B insulin resistance; SIR, patients with severe insulin resistance of undefined genetic etiology. Error bars represent SD. **B–F:** Representative FPLC elution profiles with Western blots for each patient group studied.

direct effects in adipocytes of the loss of insulin receptor function or by the effects on adipose tissue development of severely impaired insulin receptor function in utero and beyond (7). Using type B insulin resistance as

a model of acquired and reversible insulin receptor dysfunction in adult life, we now have established that dramatic hyperadiponectinemia with loss of insulin receptor function is not dependent on receptor dysfunc-

tion during development and, moreover, is reversible on restitution of receptor function. Our finding that the hyperadiponectinemia in states of insulin receptor dysfunction is accounted for by a large preponderance of HMW multimers further accentuates the dissociation between plasma adiponectin and insulin sensitivity because HMW adiponectin has been shown to correlate better with insulin sensitivity than total plasma adiponectin (1). In contrast, the patients studied with idiopathic severe insulin resistance showed the same unexplained leftward shift in complex distribution that is seen in highly prevalent but milder insulin resistance.

The robust association of hypoadiponectinemia with the earliest detectable stages of insulin resistance has raised the possibility that hypoadiponectinemia may play a causal role in prevalent forms of insulin resistance. However, the causal link in humans has yet to be established, and hyperadiponectinemia in states of extreme insulin resistance due to insulin receptor dysfunction demonstrates that they may be entirely dissociated in some settings. Nevertheless, this observation is not at odds with the notion of an etiological role for hypoadiponectinemia in other, more common, forms of insulin resistance: Hypersecretion of adiponectin by adipocytes with a very proximal defect in insulin action could be regarded as an extreme compensatory response aimed at systemic insulin sensitization.

The mechanistic basis for this radical dissociation between insulin sensitivity and circulating adiponectin is unclear. It may, in principle, be accounted for by increased adiponectin secretion from adipocytes, by reduced clearance of circulating adiponectin, or by a combination of these. Several lines of evidence suggest that an effect on secretion is dominant: the 60% increase in plasma adiponectin reported in mice with adipose-specific deletion of the insulin receptor provides evidence that there is an adipocyte-specific role of the insulin receptor in determining plasma adiponectin levels *in vivo* (14), whereas the shift in complex distribution toward HMW forms reported here, allied to previous demonstration that higher order adiponectin multimers do not interconvert in the circulation *in vivo* (15), is also suggestive of a change in adipocyte secretory activity.

Hypersecretion of adiponectin from adipocytes with reduced or absent insulin receptor function could be attributable to 1) loss of direct suppression of adiponectin synthesis and secretion at a transcriptional or posttranscriptional level by insulin receptor activation or 2) to a more indirect effect mediated by changes in cellular metabolic or redox status in the absence or reduction of functional insulin receptor. A direct suppressive effect of insulin on adipocyte synthesis and secretion of adiponectin is generally not supported by studies *in vitro* and *ex vivo* to date (16–19); however, chronic insulin action in the context of a more physiological hormonal, nutritional, and cellular milieu may elicit a different response. A longitudinal study of the development of insulin resistance in rhesus monkeys found no change in adipose tissue adiponectin mRNA despite low plasma adiponectin, suggesting that posttranscriptional events may predominate (17).

Hypersecretion of adiponectin could instead be related to the unrestrained catabolic mode of adipocytes with hypofunctional insulin receptors, consistent with the modestly elevated adiponectin seen in poorly controlled type 1 diabetes (13). However, the most physiological insulinopenic catabolic state is fasting, and neither medium-

term fasting nor anorexia nervosa have consistently been shown to produce elevations in adiponectin (20,21). A further possibility is that lack of insulin receptor function leads to elevated adiponectin through a reduction in intracellular reactive oxygen species, which have been shown to suppress adiponectin expression *in vitro* and *ex vivo* (22,23) and to be modulated by insulin receptor activity (24).

This report attests to the utility of using human disease models such as type 1 diabetes (pure insulin deficiency) and insulin receptor mutations or type B insulin resistance (isolated loss of insulin receptor function with severe hyperinsulinemia) to make novel observations of relevance to the biology of human insulin action *in vivo*.

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