Plasma Adiponectin as a Marker of Insulin Receptor Dysfunction

Clinical utility in severe insulin resistance

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OBJECTIVE — Severe insulin resistance is associated with high morbidity. Identification of severely insulin-resistant patients who have genetic or acquired insulin receptor dysfunction may aid therapeutic decision making; however, onerous diagnostic tests allied to a low frequency of insulin receptor dysfunction often preclude formal diagnosis. Our previous observation of paradoxical hyperadiponectinemia in insulin receptoropathy provides a possible basis for a simpler and cheaper screening test.

RESEARCH DESIGN AND METHODS — Receiver operating characteristics analysis was used to determine diagnostic thresholds for insulin receptoropathy in severe insulin resistance for adiponectin and for the insulin-regulated hepatic proteins sex hormone–binding globulin (SHBG) and IGF binding protein-1 (IGFBP-1).

RESULTS — Adiponectin >7 mg/l in severe insulin resistance had a 97% positive predictive value for insulin receptoropathy and <5 mg/l a 97% negative predictive value. IGFBP-1 and SHBG had lesser, though still significant, utility.

CONCLUSIONS — Use of these markers is likely to have significant value in accelerating the diagnosis of insulin receptoropathies.

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P athology in the insulin receptor underlies at least two forms of extreme insulin resistance, namely those caused by mutations in the receptor or by autoantibodies against the receptor. While clinical features are helpful in distinguishing these syndromes from more common forms of insulin resistance, they are not definitive (1,2). Diagnostic laboratory testing for these disorders is cumbersome and, for the most part, not available from commercial resources. A simple and highly specific screening test would therefore be of special utility. We have previously reported that plasma adiponectin is elevated in these receptoropathies in contrast to other forms of severe insulin resistance (3,4), and we now formally assess the diagnostic performance of adiponectin as well as IGF binding protein-1 (IGFBP-1) and sex hormone–binding globulin (SHBG), which are both insulin responsive (5,6).

RESEARCH DESIGN AND METHODS

— All patients had severe insulin resistance, defined as fasting insulin 150 pmol/l or peak insulin on oral glucose tolerance test 1,500 pmol/l in nondiabetic patients. Complete insulin deficiency was defined as an insulin requirement 3 units $\cdot$ kg^{-1} $\cdot$ day^{-1}. Those with partial β-cell decompensation and clinical features including acanthosis nigricans were included at the investigators' discretion. All patients gave informed consent with approval of the local research ethics committee in Cambridge, U.K., or of the institutional review board of the National Institute of Diabetes and Digestive Kidney Diseases. Patients aged <1 year were excluded. Genetic insulin receptoropathy was defined by homozygous $n = 5$), compound heterozygous ($n = 6$), or heterozygous ($n = 9$) mutations in the insulin receptor gene. All patients had the insulin receptor gene sequenced. Type B insulin resistance was diagnosed on the basis of severe insulin resistance with anti-insulin receptor antibodies, determined by immunoprecipitation of insulin receptor using patient serum followed by immunoblotting (1,7). Anti-insulin receptor antibody assays were undertaken in patients with features suggestive of type B insulin resistance such as rapid onset of severe insulin resistance in adulthood and associated autoimmune disease (1).

Other patients had severe insulin resistance that was either idiopathic ($n = 62$) or due to mutations in the genes encoding lamin A/C ($n = 15$), Akt2 ($n = 2$), or AGPAT2 ($n = 2$). Blood was drawn in the fasting state and plasma stored at $-20°C$. Insulin and adiponectin dissociation-enhanced lanthanide fluorescence immunoassays (DELFIA) have previously been described (4,8). IGFBP-1 was determined using an immunoradiometric assay (Diagnostic Systems Laboratories) and SHBG using an IMMULITE 1,000 solid phase chemiluminescent enzyme immunometric assay (Siemens Medical Solutions Diagnostics).

RESULTS — Adiponectin was assayed in 20 patients with insulin receptor mutations, 14 patients with type B insulin resistance, and 81 patients with other forms of severe insulin resistance. Mean

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A

Adiponectin, mg/l	PPV	NPV
>5	81%	97%
>6	86%	94%
>7	97%	94%

B

IGFBP-1, µg/l	PPV	NPV
>20	81%	83%
>30	81%	75%

C

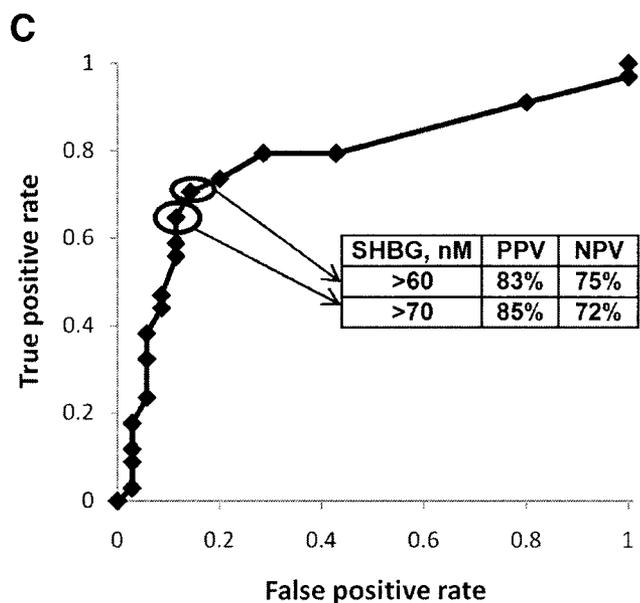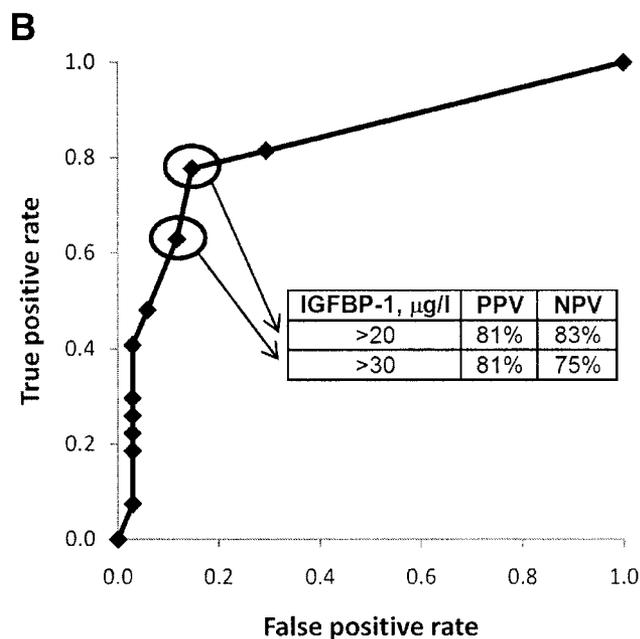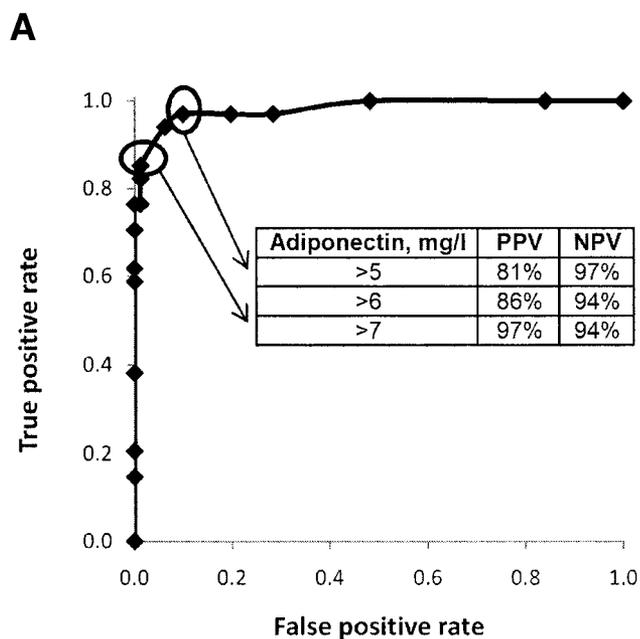| SHBG, nM	PPV	NPV
>60	83%	75%
>70	85%	72%

Figure 1—*Receiver operating characteristics analysis of adiponectin (A), IGFBP-1 (B), and SHBG (C) as diagnostic discriminators of insulin receptor dysfunction in patients with severe insulin resistance. Included in the adiponectin analysis were 20 patients with insulin receptor mutations (17 female, 3 male; mean age 18.1 years [range 4–46]), 14 patients with antibody-mediated insulin receptor dysfunction (all female; mean age 47.5 years [12–81]), and 81 patients with severe insulin resistance but no insulin receptor mutation or antibodies (66 female, 15 male; mean age 27.4 years [1.8–66]). Available subsets of these groups were used for IGFBP-1 (13, 14, and 34 patients, respectively) and SHBG (20, 14, and 35 patients, respectively) analyses.*

adiponectin levels were 19.7, 36.1, and 2.4 mg/l, respectively (online appendix 1, available at http://dx.doi.org/10.2337/dc07-2194). Receiver operating characteristics analysis was then used; i.e., true-positive and false-positive rates of diagnosis of insulin receptoropathy were determined across the adiponectin range. The receiver operating characteristics curve reveals that adiponectin performs outstandingly as a diagnostic test for insulin receptoropathy (Fig. 1*A*). An adiponectin level <7 mg/l in severe insulin resistance has a 97% positive predictive value (PPV) for insulin receptoropathy, whereas a level <5 mg/l has a 97% nega-

tive predictive value (NPV) (Fig. *A*). The only patient with insulin receptoropathy and adiponectin <5 mg/l had morbid obesity and the heterozygous Cys225Ser insulin receptor mutation. This probably represents receptor haploinsufficiency producing severe insulin resistance only in conjunction with obesity and suggests a gray area where adiponectin is a less accurate screening test. Adiponectin <8 mg/l had a 100% NPV for type B insulin resistance when genetic insulin receptoropathies were excluded.

Similar analyses were undertaken for IGFBP-1 and SHBG (Figs. 1*B* and *C*, respectively), suggesting diagnostic thresh-

olds of 20 µg/l for IGFBP-1 (PPV for level <20, 81%; NPV for level <20, 83%) and 60 nmol/l for SHBG (PPV for level 60, 83%; NPV for level <60, 75%). Although these analytes are thus of value in discriminating insulin receptoropathy, neither performs as well as adiponectin.

Diagnosis of insulin receptoropathy has several benefits: It allows consideration of immunomodulatory therapy in type B insulin resistance and identification of severe autosomal recessive genetic defects, enabling future antenatal testing. Futhermore, confirming milder autosomal dominant defects permits family screening, often leading to identification

of hitherto undiagnosed, severely insulin-resistant diabetes in relatives.

There are important caveats, however. The suggested thresholds must only be applied in the context of severe insulin resistance, as ~50% of healthy control subjects have adiponectin levels above these values. Moreover, because adiponectin levels are high in healthy infants and the youngest patient with receptoropathy in this report was aged 4 years, the thresholds should not be used to screen for insulin receptoropathy in younger children without further study. Finally, lack of a universal adiponectin reference standard means that there are significant between-assay differences in calibration. Comparison of the autoDELFIA assay used here with a widely used radioimmunoassay (Linco) suggests that values in this report should be multiplied by 1.7 if applied to adiponectin measured using the Linco radioimmunoassay (online appendix 2) and similar comparisons should be undertaken for each different adiponectin assay.

CONCLUSIONS — Assay of plasma adiponectin in patients with severe insulin resistance affords a simple and inexpensive means of discriminating among patients with loss of insulin receptor function, permitting directed genetic or antibody testing. This should facilitate rapid definitive diagnosis in such patients. Plasma IGFBP-1 and SHBG have utility as alternative, although less accurate, diagnostic discriminators.

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