Fasting Intact Proinsulin Is a Highly Specific Predictor of Insulin Resistance in Type 2 Diabetes

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OBJECTIVE — In later stages of type 2 diabetes, proinsulin and proinsulin-like molecules are secreted in increasing amounts with insulin. A recently introduced chemiluminescence assay is able to detect the uncleaved "intact" proinsulin and differentiate it from proinsulin-like molecules. This investigation explored the predictive value of intact proinsulin as an insulin resistance marker.

RESEARCH DESIGN AND METHODS — In total, 48 patients with type 2 diabetes (20 women and 28 men, aged 60 ± 9 years [means ± SD], diabetes duration 5.1 ± 3.8 years, BMI 31.2 ± 4.8 kg/m^2, and HbA_{1c} 6.9 ± 1.2%) were studied by means of an intravenous glucose tolerance test and determination of fasting values of intact proinsulin, insulin, resistin, adiponectin, and glucose. Insulin resistance was determined by means of minimal model analysis (MMA) (as the gold standard) and homeostatis model assessment (HOMA).

RESULTS — There was a significant correlation between intact proinsulin values and insulin resistance (MMA $P < 0.05$ and HOMA $P < 0.01$). Elevation of intact proinsulin values above the reference range (>10 pmmol/l) showed a very high specificity (MMA 100% and HOMA 92.9%) and a moderate sensitivity (MMA 48.6% and HOMA 47.1%) as marker for insulin resistance. Adiponectin values were slightly lower in the insulin resistant group, but no correlation to insulin resistance could be detected for resisitin in the cross-sectional design.

CONCLUSIONS — Elevated intact proinsulin seems to indicate an advanced stage of β-cell exhaustion and is a highly specific marker for insulin resistance. It might be used as arbitrary marker for the therapeutic decision between secretagogue, sensitizer, or insulin therapy in type 2 diabetes.

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In the past decades, insulin resistance and cardiovascular disease in type 2 diabetic patients have been linked to hyperinsulinemia as an independent risk factor (1–3). However, many studies were not able to confirm the negative impact of insulin on cardiovascular risk (4–6). One explanation for this overall confusing data may be found in the cross-reactivity between insulin and its precursor molecule, proinsulin, which was present in many assays for insulin measurement. Relatively few and contradicting data are available on the relation of proinsulin to insulin resistance, cardiovascular disease, and atherosclerosis (6–8). Prospective studies have demonstrated that the determination of the proinsulin-to-insulin ratio may be used to predict deterioration in glucose tolerance (9,10). Des31,32 proinsulin is a commonly secreted by-product of β-cell secretion in later stages of type 2 diabetes and is involved in the development of macrovascular disease, whereas des64,65 is usually not present in the circulation (11,12).

Assays that try to differentiate between intact proinsulin and the specific and unspecific proinsulin derivatives were used in cross-sectional and prospective follow-up studies to investigate the real relation of immunoreactive insulin with coronary risk and to explore and confirm the important role of intact proinsulin in the prediction and diagnosis of type 2 diabetes (13,14). Recent presentations indicate a potential value for fasting intact proinsulin to become a marker of insulin resistance, e.g., to identify patients eligible for an insulin sensitizer therapy (15), or to monitor treatment success during sensitizer treatment (16). Summarizing all scientific literature, there is a great likelihood that intact and/or total proinsulin may become an important diagnostic tool in type 2 diabetes.

The following cross-sectional study describes the relationship between intact proinsulin as measured by means of a recently introduced, very specific chemiluminescence method (17) and several other clinical and biochemical markers of insulin resistance in a well-characterized group of 48 patients with dietary and orally treated type 2 diabetes.

RESEARCH DESIGN AND METHODS

— The study was conducted at the Institute for Clinical Research and Development (Mainz, Germany) in accordance with Good Clinical Practice Guidelines and was approved by the responsible local ethics review committee. In total, 48 patients on oral anti-diabetic treatment were enrolled in the investigation (20 women and 28 men, aged 60 ± 9 years [means ± SD], diabetes duration 5.1 ± 3.8 years, BMI 31.2 ± 4.8 kg/m^2, and HbA_{1c} 6.9 ± 1.2%).

The patients arrived in the morning of the study day after an overnight fast, and

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Abbreviations: HOMA, homeostatis model assessment; MMA, minimal model analysis.

Table 1—*Patient characteristics for insulin-resistant and insulin-sensitive patients according to MMA and HOMA analysis*

	Insulin resistant	Insulin sensitive	*P*
MMA			
n	35	13	
Age (years)	59 ± 9	60 ± 6	NS
Disease duration (months)	54 ± 42	65 ± 46	NS
Body weight (kg)	94 ± 19	85 ± 13	NS, 0.118
BMI (kg/m^2)	31.8 ± 5.2	29.5 ± 3.3	NS, 0.145
Hypertension (number, %)	28 (80%)	10 (77%)	NS
HbA_{1c} (%)	7.1 ± 1.2	6.2 ± 0.6	<0.05
Fasting glucose (mg/dl)	119 ± 33	102 ± 22	NS, 0.093
Fasting insulin (μU/ml)	13.1 ± 6.6	9.4 ± 3.9	NS, 0.063
Fasting intact proinsulin (pmol/l)	19.9 ± 11.7	6.0 ± 2.6	<0.01
Proinsulin/insulin ratio (pmol/mU)	1.21 ± 0.70	0.71 ± 0.36	<0.05
Fasting resistin (ng/l)	0.07 ± 0.12	0.11 ± 0.18	NS
Fasting adiponectin (μ/ml)	7.05 ± 4.43	9.70 ± 5.66	NS, 0.095
Fibrinogen (mg/dl)	2.9 ± 0.9	2.6 ± 0.6	NS
HOMA analysis			
n	34	14	
Age (years)	58 ± 9	62 ± 6	NS, 0.92
Disease duration (months)	54 ± 34	63 ± 59	NS
Body weight (kg)	96 ± 17	81 ± 14	<0.01
BMI (kg/m^2)	32.3 ± 4.9	28.5 ± 3.5	<0.05
Hypertension (%)	27 (79%)	11 (79%)	NS
HbA_{1c} (%)	7.2 ± 1.2	6.2 ± 0.8	<0.05
Fasting glucose (mg/dl)	126 ± 29	86 ± 13	<0.001
Fasting insulin (μU/ml)	14.6 ± 5.5	6.0 ± 1.7	<0.001
Fasting intact proinsulin (pmol/l)	15.4 ± 11.6	5.3 ± 2.1	<0.005
Proinsulin/insulin ratio (pmol/mU)	1.58 ± 0.66	0.66 ± 0.36	<0.001
Fasting resistin (ng/l)	0.08 ± 0.15	0.08 ± 0.11	NS
Fasting adiponectin (μg/ml)	6.95 ± 4.33	9.77 ± 5.68	NS, 0.068
Fibrinogen (mg/dl)	2.8 ± 0.9	2.7 ± 0.6	NS

Data are means ± SD unless otherwise indicated.

blood samples were taken for the determination of insulin, intact proinsulin, glucose, resisitin, adiponectin, and lipids. Thereafter, a standardized modified frequently sampled intravenous glucose tolerance test was performed for the determination of insulin resistance by means of the minimal model analysis (MMA) according to Bergman et al. (18). On the investigational day, all antidiabetic medications were discontinued in the morning. Before starting the procedure, capillary blood glucose had to be <180 mg/dl. Glucose (300 mg/kg body wt, glucose 40%) was injected in a bolus over a maximum time period of 2 min at baseline. Twenty minutes later, insulin (0.07 units/kg body wt) (Huminsulin, Eli Lilly) was injected in a bolus as previously described (19). Frequent blood samples for the determination of blood glucose and serum insulin were taken from −15 to 180 min. Calculation of insulin sensitivity was performed using the MMA according to Bergmann by means of the SAAM 1.2 software package (SAAM Institute, Seattle, WA). Precision parameters estimate was expressed as fractional SD (the ratio between the SD of the estimate and the estimated value expressed as a percentage). Estimation of S_i with fractional SD >25% were excluded from further evaluation. Insulin resistance was defined as values $<1.5 \times 10^{-4} \cdot min^{-1} \cdot \mu U^{-1} \cdot ml^{-1}$ (20).

Homeostatis model assessment (HOMA) score calculation was applied as a second measure for insulin resistance analysis as previously described (21). The estimate of insulin resistance by HOMA score was calculated with the following formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5. As described by Hedblad et al. (22), patients with HOMA score values exceeding the 75th percentile (i.e., 2.0) were considered to have insulin resistance.

Glucose levels were determined using a standard laboratory reference method (glucose oxidase method) (Super GL RLT, Möhnesee, Germany). Lipid values (triglycerides, total cholesterol, and HDL cholesterol) were determined using standard dry chemistry methods.

Insulin and proinsulin were determined by means of specific chemiluminescence tests (MLT insulin [intra- and interassay coefficient of variation {CV} 3.8 and 2.3%, reference range <30 μU/ml] and MLT Intact Proinsulin [intra- and interassay CV 5.2 and 8.6%, reference range <10 pmol/l]; Sciema Diagnostics, Mainz, Germany). The tests were carried out as previously described (17).

Resistin concentrations were determined by means of a clinical research

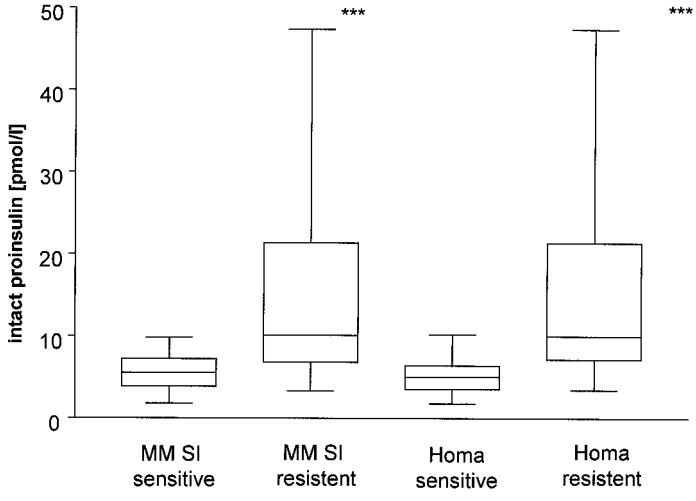

Figure 1—*Analysis of fasting intact proinsulin values in relation to the insulin sensitivity status as assessed by MMA and HOMA analysis.* ***P < *0.001*

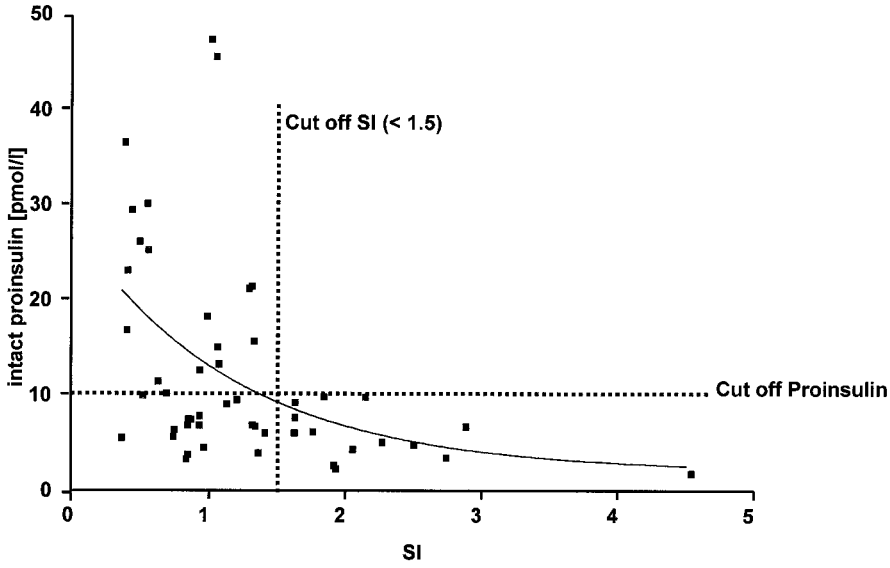

Figure 2—*Correlation between fasting intact proinsulin values and the insulin sensitivity index according to the MMA.*

enzyme-linked immunosorbent assay provided by Immundiagnostik, Bensheim, Germany (intra- and interassay CV 4.2 and 16.2%, reference range not defined).

Adiponectin measurements were performed by means of the Human Adiponectin Radioimmunoassay (Linco, St. Charles, MO). As given by the manufacturer, this assay has intra- and interassay CVs of 1.8–6.2% and 6.9–9.3%, respectively (reference range not defined).

Statistical analysis was performed using descriptive statistics and appropriate parametrical and nonparametrical tests. A Student's *t* test and Mann-Whitney's *U* test were used to compare the means of the variables measured. All tests were two-sided. Regression analyses were performed by means of the nonparametric method of Passing and Bablok (23). Results with P values <0.05 were considered statistically significant. All calculations were made with the SPSS version 9.0 statistical package.

RESULTS — MMA resulted in 35 insulin-resistant and 13 insulin-sensitive patients, including 49% (17 of 35) and 0 (0 of 13) of patients with elevated intact proinsulin levels, respectively ($P < 0.05$). HOMA analysis of the same group revealed 34 resistant (16 with elevated intact proinsulin, 47%) and 14 sensitive patients (1 with elevated proinsulin, 7%, $P < 0.01$). HOMA and MMA proved to be significantly correlated in the contingency table analysis ($P < 0.001$). The demographic data and the laboratory results of the respective groups are given in Table 1. There were significant differences between insulin-sensitive and insulin-resistant patients for both methods for a variety of clinical and laboratory parameters, including HbA_{1c}, fasting intact proinsulin (Fig. 1), and the proinsulin-to-insulin ratios. It could be demonstrated that the elevation of intact proinsulin values above the reference range showed a very high specificity (MMA 100% and HOMA 92.9%) and a moderate sensitivity (MMA 48.6% and HOMA 47.1%) as a marker for insulin resistance. The hyperbolic correlation between intact proinsulin values and the MMA value and the linear correlation between the HOMA score values and the intact proinsulin concentrations are given in Figs. 2 and 3.

The clinical characteristics of the patients with elevated or normal intact proinsulin levels in consideration of their insulin resistance status as assessed by MMA is given in Table 2. Although patients with elevated intact proinsulin values were significantly different from the combined other two groups with regard to body weight, BMI, HbA_{1c}, fasting glucose, fasting insulin, fasting intact proinsulin, and proinsulin-to-insulin ratio, there were no significant differences between the insulin-resistant or insulin-sensitive patients with normal fasting morning intact proinsulin concentrations. When comparing the patients with elevated intact proinsulin levels individually to the other two groups of resistant and sensitive patients with normal proinsulin levels, the differences from the insulin-sensitive group were much more pronounced than the differences from the insulin-resistant group (Fig. 3 and Table 2).

No significant differences between the groups were observed with regard to

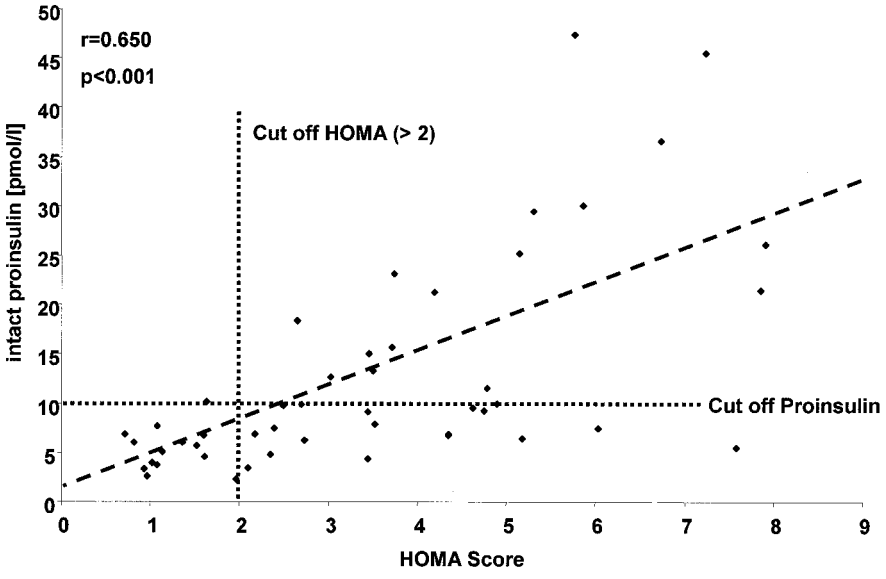

Figure 3—*Correlation between fasting intact proinsulin values and the S_i according to the HOMA analysis.*

Table 2—*Results for patients with different intact proinsulin secretion status*

	Elevated intact proinsulin (>10 pmol/l)	Normal intact proinsulin (<10 pmol/l)	Insulin resistant + normal intact proinsulin	Insulin sensitive + normal intact proinsulin
n	17	31	18	13
Age (years)	58 ± 10	60 ± 8	60 ± 9	60 ± 6
Disease duration (months)	60 ± 45	63 ± 46	65 ± 54	61 ± 34
Body weight (kg)	99 ± 18	88 ± 17*	90 ± 19	85 ± 13*
BMI (kg/m^2)	33.2 ± 5.2	30.1 ± 4.3*	30.5 ± 4.9	29.5 ± 3.3*
HbA_{1c} (%)	7.5 ± 1.3	6.5 ± 1.0†	6.8 ± 1.1	6.2 ± 0.6†
Fasting glucose (mg/dl)	127 ± 32	107 ± 29*	112 ± 33	102 ± 22*
Fasting insulin (μU/ml)	15.8 ± 5.6	10.1 ± 5.6†	10.6 ± 6.6*	9.4 ± 3.9†
Fasting intact proinsulin (pmol/l)	23.7 ± 11.3	6.3 ± 2.2‡	6.6 ± 1.9‡	6.0 ± 2.6‡
Proinsulin/insulin ratio (pmol/mU)	1.58 ± 0.64	0.79 ± 0.50‡	0.86 ± 0.58†	0.71 ± 0.35‡
Resistin (ng/l)	0.07 ± 0.13	0.08 ± 0.15	0.07 ± 0.12	0.11 ± 0.18
Adiponectin (μg/ml)	6.46 ± 3.94	8.49 ± 5.24	9.70 ± 5.66	7.61 ± 4.86
Triglycerides (mg/dl)	197 ± 123	161 ± 83	151 ± 59	176 ± 110
Total cholesterol (mg/dl)	208 ± 37	201 ± 48	194 ± 39	212 ± 59
HDL cholesterol (mg/dl)	44 ± 7	47 ± 10	47 ± 9	47 ± 10
Fibrinogen (mg/dl)	3.0 ± 1.0	2.7 ± 0.7	2.7 ± 0.8	2.6 ± 0.7
Hip/waist circumference ratio	1.03 ± 0.07	1.03 ± 0.07	1.04 ± 0.07	1.02 ± 0.06
Hypertension	14 (82%)	24 (77%)	14 (78%)	10 (77%)
Complications				
Macrovascular	4 (24%)	6 (19%)	5 (28%)	1 (8%)
Microvascular	6 (35%)	6 (19%)	3 (17%)	3 (23%)
Therapy				
No drug	4 (24%)	6 (19%)	4 (22%)	2 (15%)
Sulfonylurea	12 (71%)	11 (35%)	6 (33%)	5 (38%)
Metformine	8 (47%)	17 (55%)	9 (50%)	8 (62%)

Data are means ± SD or *n* (%). *$P < 0.05$, †$P < 0.001$, and ‡$P < 0.001$ vs. elevated proinsulin group.

the resistin or lipid values. There was a tendency toward lower adiponectin concentration in the insulin resistant patients.

CONCLUSIONS — Type 2 diabetes is characterized by impaired insulin sensitivity and deficient β-cells, both occuring with different degrees of severity. Understanding of the metabolic situation by the physician has, however, a major impact on the proper selection of an effective therapy. The two reference methods for measurement of insulin resistance, the hyperinsulinemic-euglycemic clamp test and the frequently sampled intravenous glucose test, cannot be used in daily practice (24). Therefore, simple clinical scores and biochemical markers are under investigation in order to provide the clinical diagnosis of insulin resistance and secretion disorder with sufficient specificity and sensitivity to be suitable in general practice.

HOMA is accepted with some limitations as a standard method for the determination of insulin resistance in epidemiological studies (37). Like the use of insulin alone, the determination of insulin resistance by HOMA is limited to the early stage of type 2 diabetes because β-cell secretion in later stages is also comprised of intact proinsulin and split products. Thus, insulin levels and HOMA score can be low or normal even in progressed stages of insulin resistance.

The presented study was performed to investigate the suitability of fasting intact proinsulin as measured by a recently introduced chemiluminescence assay to assess β-cell function and to predict insulin resistance. Significant differences were seen and had to be expected with HOMA for the glucose and insulin values due to the calculation procedure of the HOMA score. Proinsulin and insulin secretion are closely related in later stages of type 2 diabetes, which is confirmed by the regression analysis for the intact proinsulin levels and the insulin secretion–dependent HOMA score values. A hyperbolic correlation was seen between the MMA values and the fasting intact proinsulin levels, with cutoff values of >10 pmol/l for intact proinsulin and <1.5 for the MMA.

Proinsulin concentrations are related to atherosclerosis and cardiovascular disease (6–9,25,26). A therapeutic trial for the treatment of type 2 diabetes with externally administered biosynthetic proinsulin was stopped prematurely because of an increase in the incidence of cardiovascular events in the proinsulin-treated study population (27). In our cross-sectional study, 24% of the patients with elevated intact proinsulin levels and 19% of the patients with normal intact proinsulin levels suffered from macrovascular disease.

Our findings suggest that even after a short disease duration, patients with type 2 diabetes may belong to any of the following three groups: *1*) patients with subclinical insulin resistance (possibly representing an earlier pathophysiological stage), *2*) patients with pronounced clinical insulin resistance without significant deterioration of β-cell secretion

(possibly an intermediate stage), and 3) patients with pronounced clinical insulin resistance with significant deterioration of β-cell secretion (possibly a later stage) as indicated by an increase in fasting intact proinsulin secretion. It is remarkable that patients with impairment of β-cell function were not different from the others with regard to their disease duration or other clinical features. This may be explained by the well-known delay of diabetes diagnostics. Already 20–30% of patients at the time of their first diagnosis of diabetes are in a progressed stage of type 2 diabetes with micro- and macrovascular complications (28).

Such a potential classification might have substantial therapeutic consequences. It is common practice to treat patients initially with diet and exercise and continue with oral treatment (most commonly with secretagogues or metformin), before the introduction of sensitizer and/or insulin treatment. Although diet and metformin therapy have both been reported to decrease insulin resistance and proinsulin levels in patients with type 2 diabetes (29,30), blood proinsulin levels showed no change or were increased in patients treated with sufonylurea (31). In our cross-sectional study population, 71% of the insulin-resistant patients with elevated proinsulin concentration were treated with sulfonylurea alone or in combination with metformin, whereas only 33% of the insulin-resistant patients with normal intact proinsulin had this kind of therapy. However, prospective studies will be a more appropriate measure to investigate the influence of sulfonylurea therapy on β-cell deterioration as measured by the fasting intact proinsulin chemiluminescence. It has already been demonstrated with a less specific proinsulin assay in Japanese patients that a 12-week treatment with pioglitazone alone or in combination with a sulfonylurea leads to a decrease in fasting proinsulin levels, whereas treatment with sulfonylurea alone did not decrease proinsulin in the third comparator group (16). Considering our findings, it can be speculated (with all limitations of our study design and power) that even after a short disease duration half of the insulin-resistant patients would most likely not benefit from a secretagogue treatment, but would better be treated with a sensitizer (i.e., exercise plus diet, metformin, glitazones, or combinations thereof) or insulin therapy to protect β-cell function.

In contrast to intact proinsulin, there was only a tendency to decreased adiponectin levels in the insulin-resistant group. This finding is in line with other recent investigations that have also shown lower adiponectin plasma levels in subjects with insulin resistance (32). As reported by other groups, there was also no difference in resistin values between sensitive and resistant patients (33). The clinical implications of resistin measurements in humans are still a matter of debate, but our findings indicate that resistin may not be a powerful predictive marker for insulin resistance in a cross-sectional study design.

Further larger cross-sectional and prospective studies are required to confirm our findings and hypotheses that fasting intact proinsulin, as measured by means of the new chemiluminescence assay, may become an interesting diagnostic tool for both selection of an appropriate therapy and evaluation of treatment success in patients with type 2 diabetes. In conclusion, however, elevated proinsulin is a highly specific marker for clinically relevant insulin resistance.

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