PRELIMINARY REPORT

Insulin Resistance Is Associated With Increased Serum Levels of Glycosylphosphatidylinositol-Specific Phospholipase D

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The dyslipidemia of the metabolic syndrome is associated with alterations in triglyceride and high-density lipoprotein (HDL) metabolism. We examined the serum levels of glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD), a minor HDL-associated protein, in a cohort with a wide range of insulin sensitivity. The mean serum GPI-PLD mass from 109 subjects was 58.9 ± 18.4 μg/mL (mean ± SD). GPI-PLD levels directly correlated with cholesterol, apolipoprotein AI, triglycerides, insulin, and homeostasis model assessment (HOMA) but not C-reactive protein. These results suggest that increased serum GPI-PLD is associated with the insulin resistance.
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THE DYSLIPIDEMIA of the metabolic syndrome is associated with alterations in triglyceride and high-density lipoprotein (HDL) metabolism. We recently described a minor HDL particle that contains glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) along with apolipoproteins AI and AIV.[1] Since GPI-PLD is associated with HDL, we hypothesized that serum GPI-PLD would also be altered in patients with insulin resistance. In this study, we examined serum GPI-PLD levels in a cohort with a wide range of insulin sensitivity.

MATERIALS AND METHODS

Fasting venous blood samples were obtained from subjects undergoing bariatric surgical procedures (n = 39), outpatient adipose tissue biopsy (n = 26), or venipuncture (n = 44). Blood samples were taken prior to surgery, and the serum separated and frozen at −70°C. The institutional review boards of Indiana University-Purdue University at Indianapolis, and St. Vincent's Hospital, Carmel, IN, approved all protocols. All subjects provided informed consent.

Analyte assays were performed by the Indiana University Analyte Core using commercial kits: total cholesterol, total triglycerides, apolipoprotein AI, glucose, HDL-cholesterol (all from Roche Diagnostics, Indianapolis, IN), high-sensitivity C-reactive protein (hsCRP; Diagnostic Systems Laboratory, Webster, TX), and insulin (Linco Research, St Charles, MO).

Standards for the enzyme-linked immunosorbent assay (ELISA) were generated by quantitating GPI-PLD in 2 human serum samples using Western blotting with purified GPI-PLD[2] as a reference. Human serum was diluted 10,000-fold with phosphate-buffered saline (PBS) and 50 μL was allowed to bind to enzyme immunoassay (EIA) plates (Costar, Cambridge, MA) overnight at 4°C. Plates were then washed 3 times in 100 μL of PBS/0.1% bovine serum albumin (BSA) (wt/vol; buffer A) and blocked by adding 50 μL of buffer A and incubating for 30 minutes at 37°C. Anti-GPI-PLD^{771} (50 μL of 5 μg/mL in buffer A) was added and incubated for 60 minutes at 37°C.[3] Plates were washed and incubated with donkey anti-rabbit antibody horseradish peroxidase (Pierce, Rockford, IL; 50 μL of a 260 ng/mL solution in buffer A) for 60 minutes at 37°C. Plates were washed with PBS and 50 μL of 1-step ultra TMB ELISA (Pierce) was added and incubated for 2 minutes at 30°C. The color reaction was stopped by adding 50 μL of 2N H_2SO_4 and color read at 450 nm. The sensitivity of the assay was 10 ng/mL with a working range of 20 to 100 ng/mL. The intra- and inter-assay variations were less than 5% and less than 15%, respectively.

Data are expressed as the mean ± SD. Since many of the measured variables were not normally distributed, Spearman rank correlation coefficient was used to determine correlations between the measured variables and GPI-PLD rather than have a mix of transformed and untransformed variables. A *t* test was used to compare GPI-PLD levels in males and females since GPI-PLD itself was distributed normally. $P < .05$ was considered statistically significant.

RESULTS AND DISCUSSION

We examined serum GPI-PLD mass from 109 individuals with a wide range in body mass index (BMI) (Table 1). The mean serum GPI-PLD was 58.9 ± 18.4 μg/mL. Serum GPI-PLD mass was higher in women compared to men (59.9 ± 16.1 *v* 51.5 ± 20.4 μg/mL, respectively, $P = .036$). GPI-PLD mass did not correlate with BMI or age.

Serum GPI-PLD mass was directly proportional to total cholesterol ($r = 0.30$, $P = 0.002$), triglyceride ($r = 0.21$, $P = .032$), and apolipoprotein AI ($r = 0.22$, $P = 0.022$) consistent with previous reports[4,5]; however, serum GPI-PLD did not correlate with HDL cholesterol ($r = 0.14$, $P = 0.143$) or glucose ($r = 0.09$, $P = 0.370$). Assuming a 1:1 stoichometry in the complex with apolipoprotein AI,[1] GPI-PLD is associated with approximately 1% of the total apolipoprotein AI in serum.

Serum GPI-PLD mass correlated with insulin ($r = 0.27$, $P = .005$) and the homeostasis model assessment (HOMA; $r = 0.23$, $P = .016$), ie, serum GPI-PLD increased with insulin resistance. Similarly, serum GPI-PLD mass is increased in mice fed a high-fructose diet (manuscript in preparation), a model of insulin resistance. These results demonstrate that insulin resistance is associated with increases in serum GPI-PLD. Using adenovirus-mediated gene transfer in mice, we have shown that overexpression of hepatic GPI-PLD with the resulting increase in serum GPI-PLD mass is associated with increased fasting triglycerides and accumulation of triglyceride-rich lipoprotein remnants during a fat tolerance test (manu-

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Table 1. Population Description

Variable	Mean ± SD	Median	Range
Gender	26 males		
Age	40 ± 11	39	22-64
BMI	38.0 ± 14.9	40.5	17.3-74.0
GPI-PLD (μg/mL)	58.9 ± 18.4	59.0	18.3-106.7
Cholesterol (mg/dL)	197 ± 45	197	98-321
Triglycerides (mg/dL)	157 ± 108	129	30-602
Apolipoprotein AI (mg/dL)	136 ± 28	132	67-220
HDL-cholesterol (mg/dL)	44 ± 14	40	23-89
Glucose (mg/mL)	99 ± 7	90	60-261
Insulin (ng/ml)	0.69 ± 0.59	0.48	0.10-3.39
HOMA*	4.63 ± 5.10	2.74	0.62-29.45
hsCRP (mg/L)	18.8 ± 36.3	8.38	0.019-344.6

*HOMA was calculated as previously described.[12] A HOMA $>$ 3.5 is consistent with insulin resistance.[13]

script in preparation). These results along with our observation here suggest that GPI-PLD may play a direct role in altered triglyceride metabolism in insulin resistance and diabetes.

Metabolic syndrome is also associated with an inflammatory state. The degree of inflammation may also influence serum GPI-PLD mass. In previous studies in humans with different pathological conditions, serum GPI-PLD activity has been either increased or decreased.[4-6] In this study, C-reactive protein correlated with insulin levels ($r = 0.23$, $P = .016$), but did not correlate with serum GPI-PLD ($r = -0.16$, $P = .113$). This suggests that low levels of inflammation do not influence serum GPI-PLD mass. Serum GPI-PLD mass may also vary secondary to changes in the catabolism of GPI-PLD and/or the rate of synthesis and catabolism of apolipoprotein AI and/or HDL. Although GPI-PLD is primarily associated with lipoproteins that contain only apolipoprotein AI (LpAI),[1] it is unlikely that the changes in GPI-PLD mass are due to changes in LpAI mass since LpAI does not differ between diabetics and nondiabetics.[7,8]

Serum levels of GPI-PLD may be regulated by a number of different hormones or metabolites. In mice, serum GPI-PLD mass correlates with liver steady-state levels of GPI-PLD mRNA,[9,10] consistent with liver as the principal source of serum GPI-PLD and that the rate of hepatic synthesis as the principle component influencing serum levels. Insulin, glucose, and oxidative stress regulate GPI-PLD mRNA in various cell types.[3,9,11] The factor(s) that regulate hepatic GPI-PLD expression in insulin resistance is unknown at this time.

In summary, serum GPI-PLD levels are associated with increased insulin resistance in humans. Preliminary experiments suggest that GPI-PLD plays an active role in lipoprotein metabolism and may contribute to the dyslipidemia of insulin resistant patients. Hence, it is conceivable that serum GPI-PLD may be a "risk factor" for atherosclerosis and a potential therapeutic target.

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