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ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 473–478
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Plasma glycosylphosphatidylinositol-specific phospholipase D predicts the change in insulin sensitivity in response to a low-fat but not a low-carbohydrate diet in obese women

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Received 31 July 2007; accepted 7 November 2007

Abstract

Although circulating glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD), a minor high-density lipoprotein–associated protein, is elevated in patients with insulin resistance or high triglycerides, no information is available on the effect of weight loss or changes in insulin sensitivity on circulating GPI-PLD levels. The objective of the study was to determine the effect of weight loss and changes in insulin sensitivity on plasma GPI-PLD levels. Forty-two nondiabetic obese women were included in the study, which involved a 3-month dietary intervention randomizing patients to a low-fat or a low-carbohydrate diet. The study's main outcome measures were plasma GPI-PLD levels and insulin sensitivity as estimated by the homeostasis model assessment. The very low carbohydrate diet group lost more weight after 3 months (-7.6 ± 3.2 vs -4.2 ± 3.5 kg, $P < .01$), although the decrease in insulin resistance was similar between groups. Weight loss with either diet did not alter plasma GPI-PLD levels. However, baseline GPI-PLD levels correlated with the change in insulin sensitivity in response to the low-fat diet, whereas baseline insulin sensitivity correlated with the change in insulin sensitivity in response to the low-carbohydrate diet. Plasma GPI-PLD may serve as a clinical tool to determine the effect of a low-fat diet on insulin sensitivity.

1. Introduction

Insulin resistance and type 2 diabetes mellitus are increasing worldwide, in part because of the aging of Western society as well as the prolific increase in the prevalence of obesity. Perhaps the major adverse correlate of insulin resistance is increased cardiovascular mortality. The increased risk for cardiovascular disease is due to a multitude of atherogenic changes including increased thrombosis, inflammation, hypertension, and dyslipidemia. The dyslipidemia of insulin resistance is characterized by increases in serum triglycerides and decreases in high-density lipoproteins (HDL).

We and others have described a unique, minor HDL-like particle in plasma containing apolipoproteins A-I and A-IV along with glycosylphosphatidylinositol-specific phospholipase D (GPI-PLD) [1,2]. The GPI-PLD is expressed in nearly all tissues and cells types, but the liver has the highest level of GPI-PLD expression and is the primary source of circulating GPI-PLD [3-7]. Similar to other minor, HDL-associated proteins, GPI-PLD is involved in triglyceride metabolism and associated with insulin resistance. For example, it has recently been demonstrated that circulating GPI-PLD is higher in human subjects with elevated triglycerides or insulin resistance [8]. Consistent with this

Disclosure: DLG, KDO, DAD, and BJB have nothing to declare. MAD is currently employed by and has an equity interest in Eli Lilly & Co.

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doi:10.1016/j.metabol.2007.11.007

finding, hepatic GPI-PLD messenger RNA and serum GPI-PLD levels are increased in patients with nonalcoholic fatty liver disease [9], a condition that is associated with increased serum triglycerides, fatty acid synthesis, and insulin resistance. Although a causative role for GPI-PLD in human disease has not been proven, evidence from studies of animals and cultured cells raises this possibility. Overexpressing hepatic GPI-PLD increases serum triglycerides in mice by reducing triglyceride-rich lipoprotein catabolism [9] and promotes the expression of genes involved in fatty acid synthesis in hepatoma cells [10]. Taken together, these data implicate GPI-PLD as a component of the dysregulation of lipid metabolism associated with insulin resistance.

Weight loss associated with caloric restriction is known to improve insulin sensitivity in obese people. We hypothesized that weight loss would be associated with changes in serum levels of GPI-PLD. Furthermore, we hypothesized that the macronutrient composition of the weight loss diet would influence this effect. To evaluate this hypothesis, we compared concentrations of GPI-PLD in the plasma of healthy women who participated in a randomized trial of low-carbohydrate and low-fat weight loss diets [11].

2. Materials and methods

2.1. Participants

We previously reported the results of a randomized trial comparing low-fat and very low carbohydrate diets in 42 obese women [11]. The mean age was 43.73 ± 7.72 years, the mean body mass index (BMI) was 33.63 ± 1.86 kg/m^2, and the mean percentage of body fat was 41.36% ± 3.22%. Participants were randomized either to a reduced-calorie, low-fat diet (mean self-reported macronutrient content after 3 months was 28% fat, 18% protein, and 54% carbohydrate) or to an ad libitum low-carbohydrate diet (mean reported macronutrient content after 3 months was 57% fat, 28% protein, and 15% carbohydrate). Both dietary groups reported a reduced caloric intake by approximately 450 calories after 3 months. The very low carbohydrate diet group lost more weight after 3 months (−7.6 ± 3.2 vs −4.2 ± 3.5 kg, $P < .01$) [11]. The magnitude of improvement in insulin resistance did not differ between the dietary groups (Table 1) [12]. Baseline and 3-month frozen plasma samples (obtained in the fasting state) were assayed for GPI-PLD. All participants gave written, informed consent for participating in the study. The Institutional Review Board of the University of Cincinnati approved this study.

Table 1
Effect of dietary intervention on serum GPI-PLD levels

	Low fat			Low carbohydrate		
	Baseline	3 mo	Δ	Baseline	3 mo	Δ
GPI-PLD (μg/mL)	189 ± 50	187 ± 55	−1.4 ± 25.7	186 ± 37	193 ± 49	7.0 ± 30.2
HOMA-IR	5.3 ± 2.2	4.1 ± 2.8 *		4.5 ± 2.1	2.9 ± 1.9*	

Serum GPI-PLD was determined as described in Materials and methods before and after dietary intervention. There was no statistically significant effect of either diet on GPI-PLD as determined by paired *t* test. The HOMA-IR was determined as described in Materials and methods and reported elsewhere [12]. The HOMA-IR is shown to aid the reader.

* $P < .03$ vs baseline.

2.2. Biochemical assays

Plasma GPI-PLD mass was determined by enzyme-linked immunosorbent assay as described previously [8]. Glycosylphosphatidylinositol-specific phospholipase D mass is stable in the frozen state for at least 3 years (M Deeg, unpublished observation). Glycosylphosphatidylinositol-specific phospholipase D activity was not measured because serum triglycerides will alter GPI-PLD activity in vitro [8]. Other chemistries (glucose, insulin, cholesterol, triglycerides, low-density lipoprotein [LDL], HDL, and leptin) were determined as previously described [11]. Serum amyloid A (SAA), C-reactive protein (CRP), and interleukin-6 were measured as markers of systemic inflammation and were determined as reported elsewhere for this cohort [11]. Insulin resistance was calculated using the homeostasis model assessment (HOMA-IR) as previously described [13].

2.3. Statistical analyses

Continuous variables were compared by paired *t* test, unpaired *t* test, or 1-way analysis of variance as appropriate. Pearson correlation coefficients were calculated to examine the relationship between variables. Multivariate analyses were done by best subset regression, and all baseline variables were included in the analysis. Results are expressed as mean ± SD, and $P < .05$ was considered statistically significant. Statistical analyses were performed with SigmaStat (v3.1; SyStat Software, San Jose, CA).

3. Results

3.1. Effect of weight loss on plasma GPI-PLD levels

At baseline, plasma GPI-PLD levels did not differ between the 2 groups (Table 1). The baseline level of plasma GPI-PLD in this cohort was nearly twice that of a cohort with nonalcoholic fatty liver disease [10] but similar to that of a cohort with type 2 diabetes mellitus and low HDL [14]. Baseline plasma GPI-PLD levels did not correlate with baseline values for BMI ($P = .637$), systolic blood pressure ($P = .34$), diastolic blood pressure ($P = .90$), percentage of body fat ($P = .53$), percentage of lean body mass ($P = .38$), or percentage of bone mineral content ($P = .78$). There was no correlation of baseline GPI-PLD levels with any dietary parameters, including total intake of calories ($P = .30$), carbohydrates ($P = .54$), protein ($P = .38$), fat ($P = .29$), saturated fat ($P = .31$), monounsaturated fat ($P = .67$), or polyunsaturated fat ($P = .68$).

Table 2
Multivariate analyses of baseline GPI-PLD

	β	P	R^2
Model 1			
SAA	−6.053	.003	0.315
Model 2			
SAA	−5.350	.005	0.445
Leptin	−2.204	.029	

In univariate analyses, baseline plasma GPI-PLD correlated with CRP ($r = -0.321$, $P = .04$) and SAA ($r = -0.365$, $P = .019$) but not with glucose ($P = .162$), insulin ($P = .97$), HOMA-IR ($P = .87$), leptin ($P = .099$), total cholesterol ($P = .65$), triglycerides ($P = .15$), HDL ($P = .107$), LDL ($P = .44$), or interleukin-6 ($P = .91$). In a multivariable analysis, baseline GPI-PLD levels were predicted by a model including leptin and SAA (model 2, Table 2).

After 3 months, the weight loss was greater in the very low carbohydrate group compared with the low-fat group; but the improvement in insulin resistance was similar between dietary groups (Table 1). Plasma GPI-PLD levels were not affected by either diet (Table 1). After weight loss, plasma GPI-PLD did not correlate with any of the dietary variables (total kilocalories, macronutrient content, quantity or type of fat). In the total cohort, the change in GPI-PLD did not correlate with the change in any other variable examined. However, the change in GPI-PLD at 3 months did correlate with the change in SAA in participants on the low-fat diet ($r = 0.505$, $P = .0326$), but not in those on the low-carbohydrate diet ($r = -0.0167$, $P = .941$).

3.2. Effect of diet on insulin resistance

At baseline, both cohorts had mild insulin resistance as estimated by HOMA-IR (Table 1). Previous analyses showed that both diets improved insulin resistance as demonstrated by a reduction in HOMA-IR, with most patients having normal insulin sensitivity after 3 months [12]. The change in HOMA correlated with the change in SAA but not with changes in CRP or weight [12]. Because GPI-PLD has been shown in other studies to correlate with insulin resistance [8,10], we examined the effect of macronutrient composition on the relationship between plasma GPI-PLD and insulin resistance. Unlike previous cohorts [8,10], baseline plasma GPI-PLD did not correlate with baseline HOMA-IR ($P = .87$). The change in HOMA-IR did not correlate with the changes in either BMI or dietary fat for the total cohort or each diet group. The change in HOMA-IR did not correlate with any of the changes in serum chemistries, although it did approach statistical significance for HDL (-0.301, $P = .0553$). However, the change in HOMA-IR was predicted by 2 baseline variables: GPI-PLD

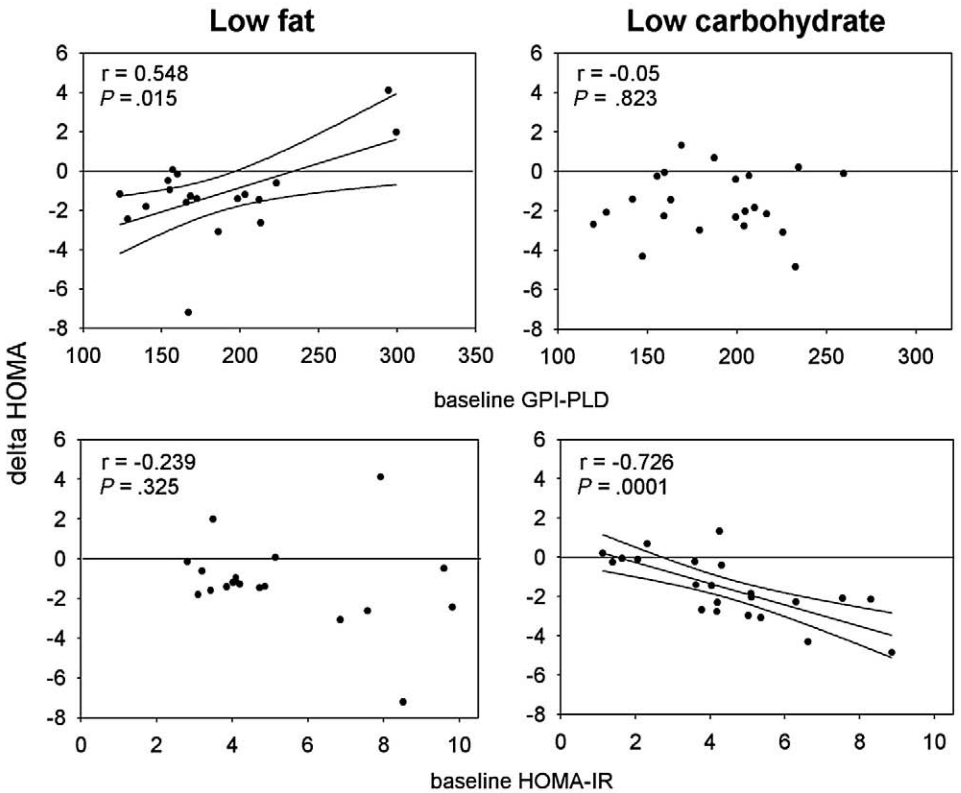

Fig. 1. Correlation between baseline GPI-PLD or HOMA-IR with changes in insulin resistance. The correlation between baseline GPI-PLD or HOMA-IR with the changes in HOMA-IR was determined as described in Materials and methods. For each comparison, the regression line is shown (solid line) along with its 95% confidence intervals (dotted line). An increase in HOMA-IR is consistent with a worsening of insulin resistance.

($r = -0.348$, $P = .0259$) and baseline HOMA ($r = -0.419$, $P = .00646$).

When these associations were examined within each dietary cohort, GPI-PLD predicted the change in insulin resistance in subjects randomized to the low-fat diet ($r = 0.548$, $P = .0151$) but not in response to those randomized to the very low carbohydrate diet ($r = 0.05$, $P = .823$) (Fig. 1). After adjusting for weight loss, baseline GPI-PLD was still an independent predictor of the change in insulin sensitivity. In contrast, baseline HOMA-IR predicted the change in HOMA-IR on the low-carbohydrate diet ($r = -0.726$, $P = .000132$) but not the low-fat diet ($r = -0.239$, $P = .325$) (Fig. 1).

4. Discussion

Weight loss is associated with improvements in various metabolic parameters including reductions in lipids, inflammatory markers, and insulin resistance. There are 2 primary findings in this study: (1) Weight loss did not produce a change in plasma GPI-PLD despite improvements in many parameters including a reduction in insulin resistance. This suggests that, in the simplest model, plasma GPI-PLD levels are not regulated by insulin resistance per se, although this conclusion is limited by the relatively small improvement in insulin sensitivity; the only variable associated with the change in GPI-PLD was the change in SAA. (2) Improvements in HOMA-IR in response to the low-fat diet correlated with baseline GPI-PLD. There are 2 broad means of affecting plasma GPI-PLD levels: regulation of GPI-PLD expression and synthesis in the liver and alterations in metabolism of the HDL-like particle containing GPI-PLD in the plasma compartment. A few regulators of GPI-PLD expression in the liver have been identified including insulin [15]. Hepatic GPI-PLD expression is increased in mouse models of type 1 and type 2 diabetes mellitus [15,16]. Plasma GPI-PLD levels also may be affected by metabolism of the GPI-PLD–containing particle within the plasma compartment [9,14]. Interestingly, low-fat and low-carbohydrate diets have different effects on minor HDL particles in humans [17]. Hence, it is conceivable that the different diets do affect either GPI-PLD expression or plasma compartment metabolism but the effects oppose each other, resulting in no or little change in plasma levels. More detailed studies are needed to examine this hypothesis.

Another interesting observation of this study is the correlation of GPI-PLD with SAA: both baseline levels and changes in response to the diets. Like GPI-PLD, SAA is carried primarily on HDL in the plasma [18]; and its levels are raised in the setting of obesity [12,19-21] and insulin resistance [19,22]. Although both GPI-PLD and SAA are produced primarily by the liver [15,18], both are also produced in macrophages [23,24]. The contribution of macrophages to circulating GPI-PLD and SAA is unknown. Thus, GPI-PLD and SAA share many similarities and may be physically or physiologically linked via HDL metabolism. For these reasons, it is perhaps not surprising that GPI-PLD and SAA levels should correlate strongly in humans.

Interestingly, baseline plasma GPI-PLD predicted the change in insulin sensitivity in response to the low-fat diet; and baseline HOMA predicted the change in insulin sensitivity in response to the very low carbohydrate diet. The latter observation has been observed in other studies [25]. Few biomarkers have been identified that predict the change in insulin sensitivity. These include adiponectin [26] and tissue fat content, particularly skeletal and hepatic fat content [27-29].

What are the differences between losing weight with a low-fat vs a low-carbohydrate diet? Both result in reductions in (1) weight loss and (2) insulin resistance. In the short-term (<3 months) studies, the very low carbohydrate diet generally results in greater weight loss and improvements in insulin resistance, although we did not see the latter effect in this cohort. Another difference is the effect on hepatic fat content.

Hepatic fat content closely correlates to hepatic and whole-body insulin sensitivity [27]. Hypocaloric, low-fat diets result in the reduction in hepatic fat, whereas low-carbohydrate diets have no effect or increase hepatic fat content [28,30,31]. We have also noted that serum GPI-PLD and liver GPI-PLD messenger RNA are increased in patients with fatty liver [10]. Hence, it is possible that circulating GPI-PLD may be a marker for hepatosteatosis. However, the fact that we did not see plasma GPI-PLD decrease on the low-fat diet may reflect that (1) hepatic fat did not change significantly in this cohort or (2) plasma GPI-PLD levels are regulated in a complicated fashion as discussed above. Further work is needed to test this hypothesis directly.

Another obvious difference between the diets is the absolute and relative types of fat in the diet. The total and type of fat in the diet influence the effect on insulin sensitivity. In eucaloric but not hypocaloric diets, high-fat diets induce hepatic steatosis and worsen insulin sensitivity [32]. Diets high in saturated fat worsen insulin resistance but only when total fat is less than 37% of total calories [33]. Hence, there is a complicated interaction between dietary fat and insulin sensitivity. Although we did not see a relationship between GPI-PLD and total fat or type fat in this study, we have noted in animal studies that dietary fat influences hepatic GPI-PLD expression and serum levels (M Deeg, unpublished observation). One of the limitations of our study is that dietary intake was only assessed by self-report. Other studies have documented that people under-report dietary intake and that obese persons under-report more than nonobese [34-36].

Based on its association with insulin resistance, one would have expected GPI-PLD level to fall with weight loss in the very low carbohydrate diet group; but it did not. One possible explanation for this is that the high cholesterol content of the very low carbohydrate diet induced

inflammation that up-regulated GPI-PLD expression, thereby counteracting the GPI-PLD–lowering effect of improved insulin resistance. In addition to the liver, GPI-PLD also can be produced by macrophages [37]. One recent study has demonstrated that addition of cholesterol to a diabetogenic diet dramatically increases abdominal adipose tissue macrophage content in LDL-receptor null mice [38], although the addition of dietary cholesterol does not result in weight gain greater than that seen with a diabetogenic diet alone. Thus, at least in that animal model, dietary cholesterol induced abdominal inflammation that was dissociated from the degree of weight gain.

This study has limitations. The HOMA is an indirect marker of insulin resistance and may also reflect changes in insulin secretion. The length of the study (3 months) may also result in numerous compensatory changes that were not measured and therefore not included in our analyses.

In summary, plasma GPI-PLD was not altered by weight loss regardless of diet type but did predict the change in insulin sensitivity in response to a low-fat but not low-carbohydrate diet. Hence, measuring GPI-PLD may serve as a clinical tool to determine the effect of a low-fat diet on insulin sensitivity. Additional studies are needed to confirm this hypothesis.

Acknowledgment

This work was supported by a Veterans Affairs Merit Award (MAD); the American Heart Association (BJB); University of Cincinnati Obesity Research Center (BJB); University of Cincinnati Research Council (BJB); Children's Hospital Medical Center Clinical Research Center (supported in part by US Public Health Service grant M01-RR-08084 from the General Clinical Research Center Program, National Center for Research Resources, National Institutes of Health); and National Institutes of Health grants DK-54263, DK-56863, and DK57900 (DAD). We thank Dr Kieren Mather for insightful comments.

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