Association between Serum Concentrations of Persistent Organic Pollutants and γ Glutamyltransferase: Results from the National Health and Examination Survey 1999–2002

We recently reported a relationship between serum concentrations of persistent organic pollutants (POPs) and diabetes mellitus in the general population *(1)*. Furthermore, serum γ-glutamyltransferase (GGT) activity, even within its usual reference interval, strongly predicted type 2 diabetes in prospective studies through unclear mechanisms *(2)*. Because exposure to high concentrations of POPs in occupational or accidental settings increased serum GGT *(3)*, serum GGT may predict type 2 diabetes related to exposure to POPs. Thus, in the general population, low concentrations of POPs would be related to normal concentrations of serum GGT, and adjustment for POPs would attenuate the relationship of GGT and diabetes.

This hypothesis was tested in the National Health and Nutrition Examination Survey (NHANES) 1999–2002 public-use dataset in 2016 persons ≥20 years of age. Serum concentrations of POPs and GGT activity were measured by high-resolution gas chromatography/high-resolution mass spectrometry and by a Hitachi 737 analyzer, respectively. We selected the 6 POPs for which at least 80% of study participants had concentrations greater than the limit of detection, as listed in Table 1.

For each POP, persons with serum concentrations less than the limit of detection were assigned to the refer-

Table 1. Adjusted geometric means of serum GGT by category of 6 persistent organic pollutants.

Analyte	Not detectable	Detectable					P_{trend}
		<25th	25th–<50th	50th–<75th	75th–90th	≥90th	
PCB 153							
Concentration (ng/g of lipid)		14.3	36.7	60.2	93.6	164	
No. of subjects	413	397	404	401	237	164	
Model 1[a]	22.2	22.4	23.3	24.0	22.6	24.5	0.34
Model 2[b]	22.4	22.9	22.9	24.3	22.4	24.3	0.32
1,2,3,4,6,7,8-hpcdd							
Concentration (pg/g of lipid)		20.7	37.8	60.8	97.5	170	
No. of subjects	263	436	439	439	262	177	
Model 1[a]	23.3	21.3	23.6	23.1	23.8	25.3	0.15
Model 2[b]	22.2	21.8	23.6	22.9	23.6	26.0	0.02
1,2,3,4,6,7,8,9-ocdd							
Concentration (pg/g of lipid)		194	323	514	805	1485	
No. of subjects	390	401	410	408	241	166	
Model 1[a]	23.3	22.4	22.2	23.3	23.3	26.0	0.35
Model 2[b]	22.4	22.2	22.6	24.0	23.6	25.5	0.06
Oxychlordane							
Concentration (ng/g of lipid)		8.5	15.4	25.1	39.1	65.5	
No. of subjects	359	404	422	416	249	166	
Model 1[a]	20.3	22.0	22.9	25.3	24.5	26.0	<0.01
Model 2[b]	20.9	22.4	22.0	25.0	24.0	26.6	<0.01
p, p'-DDE							
Concentration (ng/g of lipid)		112	292	717	1560	3700	
No. of subjects	0	502	506	502	304	202	
Model 1[a]		21.5	22.2	22.6	26.0	26.3	<0.01
Model 2[b]		22.4	22.4	22.4	25.0	25.8	<0.01
Trans-nonachlor							
Concentration (ng/g of lipid)		11.0	21.7	35.7	60.6	114	
No. of subjects	203	451	455	451	274	182	
Model 1[a]	19.1	21.3	23.1	24.3	26.6	25.0	<0.01
Model 2[b]	20.5	21.5	22.6	23.6	26.3	26.0	<0.01
Sum of 5 POPs[c]							
Sum of category ranking		0–5	6–9	10–14	15–19	20–25	
No. of subjects		463	505	527	275	246	
Model 1[a]		20.1	22.2	24.0	24.8	25.5	<0.01
Model 2[b]		20.5	22.2	23.6	24.8	26.0	<0.01

[a] Model 1: adjusted for age, sex, race and ethnicity, poverty income ratio, body mass index, cigarette smoking, alcohol consumption, and exercise.
[b] Model 2: additional adjustment for serum alanine aminotransferase.
[c] The sum of category ranking of 1,2,3,4,6,7,8-hpcdd, 1,2,3,4,6,7,8,9-ocdd, oxychlordane, p, p'-DDE, and trans-nonachlor; PCB153 was omitted

ence group, and those with detectable POPs concentrations were divided into categories with cut-points at the 25th, 50th, 75th, and 90th percentiles. To yield a cumulative measure of exposure to POPs, we summed the category ranking of the 5 POPs (SUMPOP5) that were positively associated with serum GGT, thus making a score from 0 to 25, and divided across the 25th, 50th, 75th, and 90th percentiles (omitting PCB153; 0 for nondetectable, 1 for detectable below the 25th percentile, and so on, up to 5 for above the 90th percentile). Adjustment for confounders was performed by general linear models; adjusting variables are provided in Table 1. The association between serum GGT and prevalence of diabetes *(1)* was also examined after adjusting for POPs. All statistical analyses were performed with SAS 9.1 (SAS Institute) and SUDAAN 9.0 (Research Triangle Institute).

Serum concentrations of 5 of the POPs were positively associated with serum GGT. The associations were strengthened by additional adjustment for serum alanine aminotransferase (ALT), which was inversely associated with POPs. Adjusted geometric means of serum GGT by categories of SUMPOP5 were 20.5, 22.2, 23.6, 24.8, and 26.1 (P for trend <0.01). In these data, serum GGT was positively associated with the prevalence of diabetes, with the adjusted odds ratios of 1.0, 2.2, 2.1, 2.8, and 3.1 (P for trend <0.01), but further adjustment for POPs did not change the strength of this association.

POPs are known hepatotoxins *(4)*. In humans, hepatic abnormalities, including abnormally increased serum GGT and ALT, have been observed after exposure to high concentrations of POPs *(3)*. In this study, however, exposure to low concentrations of POPs was positively associated with serum GGT activities within the routine reference interval despite an inverse association with serum ALT, contradicting the interpretation of hepatotoxicity as the mechanism linking POPs to GGT. Similar to the current findings, patterns of association of serum antioxidant concentrations and C-reactive protein with serum ALT differed from those with GGT, both within their routine reference intervals *(5, 6)*. Similarly, serum GGT within its routine reference interval was positively associated with low concentrations of blood lead or urinary cadmium in general population *(7)*. As cellular GGT catalyzes the first step in the degradation of extracellular glutathione *(8)*, all these findings suggest that serum GGT within its routine reference interval may be a biomarker reflecting the extent of exposure to environmental xenobiotics, especially biotransformed through conjugation of glutathione. Of further interest, the secular trend of serum GGT showed a strong increase among Koreans independent of changes in health behaviors including obesity *(9)*; Korea is experiencing increasing exposure to various environmental pollutants, including POPs.

Although serum GGT was positively associated with the prevalence of diabetes, this association was not explained by POPs. Despite the generally positive associations between POPs and serum GGT, the associations of specific POPs with diabetes and serum GGT were inconsistent. For example, PCB153 was strongly associated with diabetes, but not with serum GGT. Thus, serum GGT may predict diabetes as a general marker of exposure to various environmental xenobiotics, including diabetes-related POPs and those unrelated to diabetes, such as lead or cadmium.

We recently proposed serum GGT within its routine reference interval as a marker of oxidative stress *(8)*. Serum GGT may also be a marker of the combined exposure to various environmental xenobiotics. This further hypothesis does not contradict our assertion that serum GGT is a marker of oxidative stress because exposure to xenobiotics directly induces oxidative stress *(10)*. Our hypothesis does add another dimension to the interpretation of serum GGT within its routine reference interval.

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Reliability of the Thrombin-Generation Assay in Frozen-Thawed Platelet-Rich Plasma

To the Editor:

The recent introduction of a general coagulation function test, namely the thrombin-generation assay (TGA), has enabled efficient assessment of the global functioning of the hemostatic system. By using a fluorogenic substrate, the TGA produces thrombin-generation curves in a fully automated manner that may be useful and sensitive enough to screen for either hypercoagulable states or hemorrhagic diatheses. In the recent report, Hézard et al. *(1)*, concluded that the TGA can be reliably used to screen patients needing further specific thrombophilia testing. Specifically, a thrombin generation lag time ≤1.5 min indicates the need for factor V Leiden genotyping, whereas a peak thrombin concentration >433 nmol/L indicates the need for factor II G20210A genotyping. As reported in that study, the experiments were performed on thawed, previously frozen, platelet-rich plasma (PRP), and little indication is provided on either the collection procedure or the storage conditions of these samples, both of which are essential requisites to enable reliable TGA results *(2, 3)*.

Previous exhaustive evaluations of TGA demonstrated that although the integral amount of thrombin generated in time, expressed by the endogenous thrombin potential (ETP), appears substantially unmodified in frozen-thawed PRP, thrombin generation is accelerated and the maximum amount of generated thrombin is increased, apparently as a result of cold-induced platelet activation, membrane damage, and procoagulant phospholipid exposure *(2, 3)*. Accordingly, in frozen-thawed PRP, the lag time decreases substantially, up to one third, compared with nonfrozen specimens *(2)*. The freezing also affects the maximum concentration of thrombin (c_{max}), which is substantially higher in frozen-thawed than in fresh PRP. Thus, it seems likely that assessing thrombin generation in frozen-thawed PRP would introduce a substantial bias in several measurements, especially lag time and peak concentration. Consequently, the ETP would appear to be the single variable that can be assessed in PRP, regardless of the storage conditions *(2)*. However, this is further disputed by Chantarangkul et al. *(4)*, who demonstrated that when the phospholipids are omitted, such as in the experimental conditions of Hézard et al. *(1)*, there is a linear relationship between the ETP value and the number of residual platelets in thawed specimens. Platelets are not an ideal surrogate for exogenous phospholipids, as the fatty acid composition of membrane phospholipids in platelets might be heterogeneous, depending basically on dietary lipid modifications *(5)*. Additionally, the interindividual variability of several TGA indicators measured in PRP is considerably higher, especially in the presence of very low concentrations (≤3 pmol/L) of tissue factor *(2)*. Potential artifacts in thawed specimens, such as platelet debris or the presence of procoagulant material, are detrimental to assay reliability *(2)*. Although ideally TGA should be evaluated on whole blood or PRP, we recommended that frozen plasma is suitable, provided that it is filtered before testing to eliminate the unwanted effect of residual platelets *(6)*. Earlier data showed an increased sensitivity to activated protein C (APC) in frozen-thawed PRP compared with fresh PRP *(3)*. To minimize the influence of using frozen-thawed PRP preparations, Regnault et al. *(3)* suggested that 6.7 nmol/L exogenous APC be added, instead of 25 nmol/L, the latter being the experimental conditions of Hézard et al. *(1)*.

Because the freezing-thawing effects on ETP, lag time, and c_{max} cannot be anticipated and depend on heterogeneous interindividual functional characteristics of platelets *(7)*, the use of thawed PRP is likely to introduce an unpredictable bias, influencing result comparability and transferability within the same study protocol, especially during assessment of the APC-induced thrombin potential inhibition *(2)*. Additionally, although the use of frozen PRP may be justified to screen for the presence of lupus anticoagulants *(3)*, there are no clear reasons to use PRP to screen for inherited coagulation disorders that do not directly involve platelet pathophysiology, such as factor V Leiden and the factor II G20210A sequence variant *(8)*.

In conclusion, we acknowledge that, on the whole, the TGA might be potentially useful for the laboratory assessment of a large spectrum of clotting abnormalities. Nevertheless, as with other areas of coagulation testing, we suggest that rigorous preanalytic and experimental conditions for the TGA ought to be fulfilled and standardized *(2–4, 6)* to provide reliable information on clinically meaningful hypercoagulable states.

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