

# Can persistent organic pollutants explain the association between serum $\gamma$ -glutamyltransferase and type 2 diabetes?

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**Abstract** The results of several epidemiological studies of serum  $\gamma$ -glutamyltransferase (GGT) led us to hypothesise that associations of GGT within its normal range with type 2 diabetes may reflect detrimental effects of xenobiotics found in the environment, such as persistent organic pollutants (POPs). Epidemiological observations showed that serum GGT activity within its normal range strongly predicted future type 2 diabetes; the predictability of diabetes from obesity was low with GGT at the low end of the normal range; and GGT showed a positive association with known markers of oxidative stress or inflammation. Experimental findings on cellular GGT suggest that serum GGT levels within the normal range may reflect oxidative stress related

to the re-synthesis of intracellular glutathione; however, this interpretation is not completely satisfying because, in its role of regenerating intracellular glutathione, GGT activity should be antioxidative. Alternatively, serum GGT activity may reflect amounts of glutathione conjugates formed during the metabolism of xenobiotics. Accordingly, we postulate a two-part hypothesis: that the association of serum GGT with type 2 diabetes reflects exposure to POPs, as these substances, which have a very long half-life, may influence diabetes risk by residing in adipose tissue as endocrine disruptors; and that POPs or similar substances may interact with obesity to cause type 2 diabetes. Supporting this hypothesis, cross-sectional investigation of background exposure to POPs in the National Health and Nutrition Examination Survey showed relationships similar to those observed for GGT, including a powerful association with prevalent diabetes and no association between obesity and diabetes for very low POP concentrations. Our hypothesis can be tested in both prospective studies and toxicological studies.

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## Abbreviations

CARDIA	Coronary Artery Risk Development in Young Adults
FinMONICA	Finland Multinational Monitoring of Trends and Determinants in Cardiovascular Disease
GGT	$\gamma$ -glutamyltransferase
GSH	glutathione
NHANES	National Health and Nutrition Examination Survey
OC pesticides	organochlorine pesticides

POPs            persistent organic pollutants  
ROS             reactive oxygen species

### $\gamma$ -Glutamyltransferase and diabetes mellitus

Our hypothesis and observations regarding persistent organic pollutants (POPs) and type 2 diabetes arose from interpretations of several studies of serum  $\gamma$ -glutamyltransferase (GGT). Serum GGT activity within the normal range predicted type 2 diabetes in Korean men [1]. Furthermore, the association between serum GGT and type 2 diabetes was stronger among the obese, and obesity did not predict the risk of type 2 diabetes among participants with GGT levels at the low end of the normal range [1]. A similar pattern of associations was observed in datasets from the Coronary Artery Risk Development in Young Adults (CARDIA) study [2], the Finland Multinational Monitoring of Trends and Determinants in Cardiovascular Disease (FinMONICA) study [3], the National Health and Nutrition Examination Survey (NHANES) [4], and in German women, but not men [5], leading us to search for new interpretations of serum GGT.

Cellular GGT, found on the plasma membrane in most tissues [6], metabolises extracellular glutathione (GSH), the main intracellular antioxidant, allowing precursor amino acids to be reutilised for intracellular GSH re-synthesis [7]—a process known as the ‘ $\gamma$ -glutamyl cycle’ (Fig. 1, left of

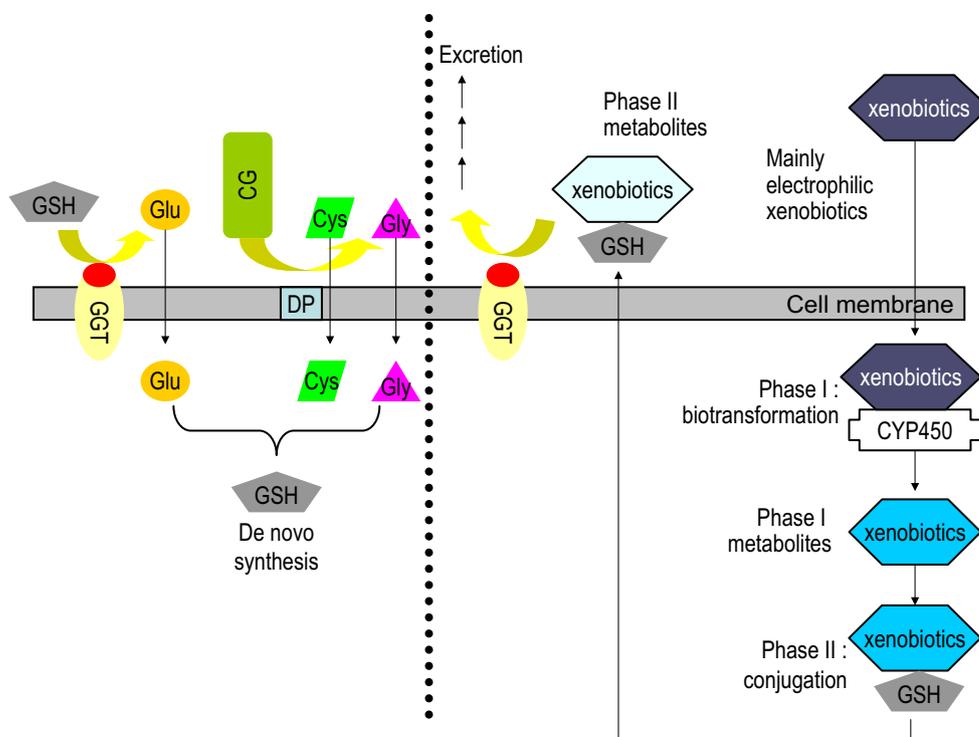
the dotted line) [8]. Cellular GGT may also be involved in the generation of reactive oxygen species (ROS) in the presence of transition metals [9].

This information on cellular GGT led us to hypothesise that serum GGT levels within the normal range reflect oxidative stress [10]. Supporting this hypothesis, serum GGT is inversely associated with serum antioxidants [11] and positively associated with  $F_2$ -isoprostanes and C-reactive protein [2].

However, this interpretation of serum GGT presented a dilemma. In terms of the  $\gamma$ -glutamyl cycle, an increase in GGT should facilitate regeneration of intracellular GSH, eventually leading to increased levels, even though the initial increase may be a compensatory response to the depletion of GSH as a result of oxidative stress. Thus, an increase in GGT should theoretically lead to a decrease in oxidative stress and consequent pathological changes. However, prospective cohort studies have consistently reported that a slight increase in serum GGT activity precedes an increase in type 2 diabetes risk.

Some researchers interpreted that serum GGT predicts type 2 diabetes because it acts as a pro-oxidant or a marker of hepatic steatosis [12–14]. These mechanisms may partly explain some of the associations between GGT and type 2 diabetes, especially those observed at relatively high levels of serum GGT activity. However, it is important that even a slight increase in serum GGT activity at the low end of the normal range, where hepatic steatosis is unlikely, increased the risk of type 2 diabetes [1–3].

**Fig. 1** The section to the right of the dotted line shows the role of GGT in the metabolism of xenobiotics. Cellular GGT is necessary for the metabolism of xenobiotics conjugated with GSH. The section to the left of the dotted line shows the  $\gamma$ -glutamyl cycle. Cellular GGT is an indispensable enzyme in the metabolism of extracellular GSH. In this process, GGT releases glutamate (Glu) and the dipeptide cysteinylglycine (CG), which is subsequently cleaved into cysteine (Cys) and glycine (Gly) by plasma membrane dipeptidase (DP). CYP450, cytochrome P450



### Serum GGT activity as a cumulative exposure marker for various xenobiotics

Although the aforementioned study on Korean men [1] revealed much lower serum GGT activity than that observed in CARDIA and FinMONICA participants [2, 3], there was a striking secular trend in serum GGT [15]. After statistical adjustment, we concluded that the secular trend was not caused by changes in health behaviours or obesity, suggesting that other environmental factors play a role.

Another avenue of thought arose from the observation that serum GGT demonstrated dose–response relationships with blood concentrations of lead and urinary levels of cadmium [16]. This work brought into focus the important function of GSH as a conjugating ligand for the phase II reactions that occur during xenobiotic metabolism. Many xenobiotics are eliminated from cells by phase I biotransformation, followed by phase II conjugation to an anionic group, such as GSH, and transportation into the extracellular space [17]. The first necessary step for further metabolism of GSH conjugates is to break  $\gamma$ -carboxyl linkage of GSH by cellular GGT (Fig. 1, right of the dotted line) [18]. Thus, GGT activity may increase with an increased exposure to xenobiotics, including environmental pollutants, which require conjugation with GSH [17]. Our updated viewpoint on serum GGT as a marker of exposure to xenobiotics is a more comprehensive interpretation, which includes our earlier proposal that serum GGT reflects oxidative stress. Exposure of cells to xenobiotics directly increases the production of ROS [19]. ROS are also conjugated with GSH [17], and consumption of GSH by conjugation with xenobiotics is related to the depletion of intracellular GSH [17]. Based on these propositions, we hypothesised that the associations between serum GGT activity and type 2 diabetes might be explained by exposure to environmental pollutants.

### Serum GGT activity as a marker of exposure to POPs

For our hypothesis to be true, the environmental pollutants involved have to satisfy several conditions. First, human exposure to the presumed xenobiotics should be through food consumption, most likely meat. For example, the Korean secular trend of increasing serum GGT activity was similarly observed among all subgroups stratified by various factors, such as age, sex, smoking, alcohol drinking, obesity or job category [15], suggesting some common environmental exposures, such as food. Indeed, serum GGT was positively associated with meat intake in the CARDIA dataset [20]. Second, the xenobiotics should be associated with adipose tissue, because serum GGT activity is strongly associated with obesity. Considering the importance of adipose tissue in the pathogenesis of type 2 diabetes, pollutants stored in

adipose tissue could be important. Third, the xenobiotics should be metabolised by GSH conjugation.

Following this logic, POPs, endocrine disruptors stored in adipose tissue, represented the most plausible candidate. POPs include hundreds of different chemical compounds with common properties, such as long-term persistence in the environment and bioaccumulation through the food chain. POPs are detectable in virtually everyone, with exposure occurring through fatty animal food in particular [21]. Some POPs are conjugated to GSH for their metabolism [22–24], and exposure to high amounts of certain POPs in occupational or accidental settings increase serum GGT activity [25, 26].

We tested this hypothesis in the NHANES dataset and found graded associations between serum concentrations of POPs and serum GGT [27]. We also found strong dose–response relationships between POPs and the prevalence of type 2 diabetes [28]. Parallel to the interactions of obesity and diabetes with serum GGT activity, the association between POPs and type 2 diabetes was stronger among obese persons, but type 2 diabetes was nearly absent, irrespective of obesity when POPs concentrations were very low [28]. This observation led us to hypothesise that POPs stored in adipose tissue might be more critical than obesity itself to understanding the pathogenesis of type 2 diabetes.

The studies described above focused on type 2 diabetes risk, reflecting our original hypothesis. However, serum GGT activity within its normal range has also been shown to prospectively predict other clinical outcomes [29–33]. Similarly, in the NHANES dataset, serum POP concentrations were positively associated with the prevalence of the metabolic syndrome, insulin resistance, hypertension, and cardiovascular diseases [34, 36].

Mitochondrial dysfunction has recently emerged as a mechanism unifying the pathogenesis of insulin resistance and type 2 diabetes [37]. Interestingly, it has long been reported that POPs can decrease mitochondrial oxidative capacity in various organs [38, 39]. Even though POPs are mainly stored in adipose tissue, physiological fatty acid release from adipose tissue between meals may be accompanied by some release of POPs, and they may keep redistributing to various organs, such as muscle, liver, or pancreas, even under normal physiological control of adipose tissue [40]. Furthermore, the lipolytic potential of some POPs may disturb normal adipose tissue metabolism and lead to an excessive release of POPs [41]. Thus, continuous chronic exposure to POPs may diminish mitochondrial function in various organs, eventually leading to insulin resistance and type 2 diabetes.

### Critiques of the hypothesis

Counter to our hypothesis, type 2 diabetes is prevalent across countries with varying meat intakes. Although meat

may be the putative source of POPs, through bioaccumulation in the food chain, in developed countries where POPs were banned decades ago, this may not be the case in developing countries, where some POPs are still widely used [42]. If local agriculture utilises organochloride pesticides, the subclass of POPs that showed the strongest associations with type 2 diabetes and insulin resistance [28, 35], fruits and vegetables could be the main source of POPs. Furthermore, POP content differs across meats, with fish usually containing higher concentrations of POPs than mammals [43]. Cooking method is another important factor affecting POP absorption [44]. Thus, ecological comparisons of meat intake by country or time may not be fully relevant to our hypothesis.

The fact that an association between obesity and type 2 diabetes was observed as early as the 19th century [45, 46] provides evidence against our hypothesis. Although most POPs are man-made in the 20th century, dioxins can be created in natural events such as forest fires or volcanic eruptions [47]; and POPs may not be the only substances that can interact with adipose tissue to cause type 2 diabetes. Our hypothesis largely concerns the epidemic of type 2 diabetes after the Second World War.

Further evidence that might counter our hypothesis subsumes declining body burden of POPs in developed countries [48], even as the epidemic of type 2 diabetes increases. However, POPs may have increased potency at a lower body burden in an obesity epidemic, because the

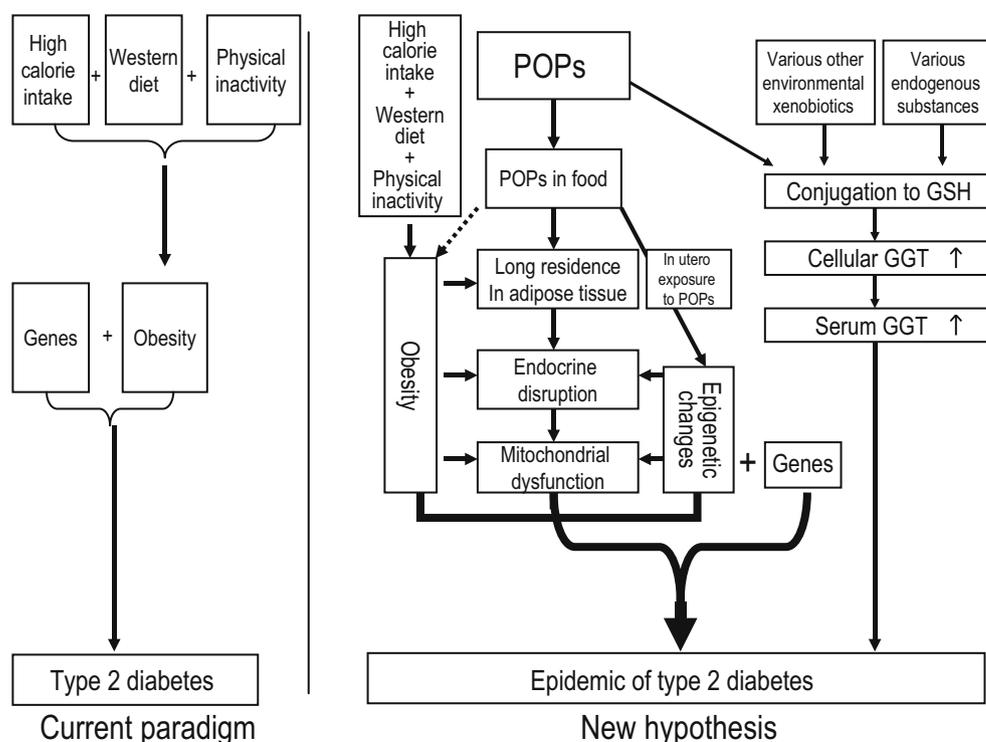
toxicity of POPs appears to increase among obese persons [29]. Additionally, other POPs, such as brominated flame retardants and perfluorinated compounds, which are still widely used today, may be as important as the POPs we studied in the pathogenesis of type 2 diabetes [49]. Furthermore, the current generation may be experiencing cumulative epigenetic changes due to POPs [50], and these changes could predispose individuals to diabetes.

**Conclusion**

Starting with epidemiological studies of serum GGT, we formed a two-part hypothesis: the association of serum GGT with type 2 diabetes reflects exposure to POPs, and POPs interact with obesity to cause type 2 diabetes. Although various researchers have recently commented on the predictive power of serum GGT [17–19], none considered the possibility that serum GGT was a cumulative biomarker of environmental pollutants.

Furthermore, several lines of evidence suggest that if people had no or very low exposure to POPs, reflected by very low serum GGT activity, the probability of developing type 2 diabetes could be low, even among the obese. In this sense, POPs stored in adipose tissue may interact with this tissue to cause type 2 diabetes. Our hypothesis linking obesity, serum GGT, POPs and type 2 diabetes considers the interactions between all the predisposing factors, suggests

**Fig. 2** A new hypothesis about the inter-relationships of serum GGT, POPs and type 2 diabetes. Serum GGT activity increases with increasing xenobiotic–GSH conjugation, including POPs. As endocrine disruptors, POPs can disturb glucose and lipid metabolism through mitochondrial dysfunction leading to an increase in the risk of type 2 diabetes. Obesity also contributes to type 2 diabetes by increasing the storage time of POPs in adipose tissue and enhancing toxicity of POPs. Some POPs may directly contribute to obesity. Epigenetic changes resulting from exposure to POPs over several generations may also be involved in the pathogenesis of type 2 diabetes. With genetic factors, the background exposure to POPs and their interactions with obesity can help to explain the recent epidemic of type 2 diabetes



an important role for the population-wide exposure to POPs (Fig. 2), and may help define the way in which obesity predisposes individuals to type 2 diabetes. Testing the hypothesis in prospective cohorts and in toxicological studies could help clarify whether and how specific POPs relate to type 2 diabetes.

**Duality of interest** The authors declare that there is no duality of interest associated with this manuscript.

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