

## Review

# Is Serum Gamma Glutamyltransferase a Marker of Oxidative Stress?

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Accepted by Professor B. Halliwell

(Received 4 February 2004; In revised form 8 March 2004)

The primary role of cellular gamma glutamyltransferase (GGT) is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. Paradoxically, recent experimental studies indicate that cellular GGT may also be involved in the generation of reactive oxygen species in the presence of iron or other transition metals. Although the relationship between cellular GGT and serum GGT is not known and serum GGT activity has been commonly used as a marker for excessive alcohol consumption or liver diseases, our series of epidemiological studies consistently suggest that serum GGT within its normal range might be an early and sensitive enzyme related to oxidative stress. For example, serum and dietary antioxidant vitamins had inverse, dose-response relations to serum GGT level within its normal range, whereas dietary heme iron was positively related to serum GGT level. More importantly, serum GGT level within its normal range positively predicted F2-isoprostanes, an oxidative damage product of arachidonic acid, and fibrinogen and C-reactive protein, markers of inflammation, which were measured 5 or 15 years later, in dose-response manners. These findings suggest that strong associations of serum GGT with many cardiovascular risk factors and/or events might be explained by a mechanism related to oxidative stress. Even though studies on serum and/or cellular GGT is at a beginning stage, our epidemiological findings suggest that serum GGT might be useful in studying oxidative stress-related issues in both epidemiological and clinical settings.

**Keywords:** Gamma-glutamyltransferase; Oxidative stress; Iron; Cardiovascular diseases

## CELLULAR GAMMA GLUTAMYLTRANSFERASE (GGT) AND OXIDATIVE STRESS: EXPERIMENTAL EVIDENCE

There is evidence that cellular GGT plays an important role in antioxidant defense systems.<sup>[1–3]</sup> This enzyme is widely distributed in the human body, especially in kidney and liver, and is frequently localized to the plasma membrane with its active site directed into the extracellular space.<sup>[4]</sup> The primary role of GGT ectoactivity is to metabolize extracellular reduced glutathione (GSH), allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis; in this way, a continuous “GSH cycling” across the plasma membrane occurs in a number of cell types.<sup>[5]</sup> Thus, ectoplasmic GGT favors the cellular supply of GSH, the most important non-protein antioxidant of the cell.

However, recent experimental studies<sup>[6–13]</sup> indicate that ectoplasmic GGT may also be involved in the generation of reactive oxygen species. This effect of GGT seems to occur when GGT is expressed in the presence of iron or other transition metals. Specifically, cysteinylglycine, which is one of the products of GGT action, has a strong ability to reduce  $\text{Fe}^{3+}$  to  $\text{Fe}^{2+}$ , which again promotes generation of free radical species. A GGT-mediated oxidative stress

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has been repeatedly reported, capable of inducing oxidation of lipids,<sup>[7,8]</sup> oxidation of protein thiols,<sup>[10]</sup> alterations of the normal protein phosphorylation patterns,<sup>[11]</sup> and biological effects such as the activation of transcription factors.<sup>[12,13]</sup>

## SERUM GGT AND CARDIOVASCULAR RISK FACTORS AND DISEASES

On the other hand, serum GGT activity has been used as a marker for excessive alcohol consumption or liver diseases in clinical practice.<sup>[14]</sup> A dose-response relationship is generally seen in which the mean GGT increases as the amount of self-reported alcohol consumption increases. Nevertheless, considerable variation in GGT exists among subjects who report the same amount of alcohol consumption, and even for those who report no alcohol consumption.<sup>[15,16]</sup> For a long time, serum GGT has been used as an objective indicator of excess alcohol consumption, with the result that the association between GGT and disease has been interpreted as a surrogate of that of alcohol consumption with disease.<sup>[17–19]</sup> However, the existence of a positive association of GGT with myocardial infarction leads to a question about whether this is the proper interpretation of the role of GGT, given that moderate alcohol consumption is usually associated with decreased, rather than increased, cardiovascular risk.<sup>[19]</sup>

Recently, emerging evidence has shown that serum GGT is more than a marker of alcohol consumption. Population-based studies<sup>[20–23]</sup> have observed a strong association between serum GGT levels and many cardiovascular disease risk factors. After adjusting for alcohol consumption, the factors showing a positive association with elevated serum GGT level in the population studies include: old age, male gender, body mass index, smoking, lack of exercise, high blood pressure, heart rate, high blood cholesterol, high blood fasting triglycerides, high blood LDL cholesterol, low blood HDL cholesterol, high fasting glucose, and, among women, menopause and use of oral contraceptive.<sup>[20–23]</sup> These relationships have been shown to be strong even within the normal range (as defined by clinical chemists) of GGT levels. In addition, several prospective studies<sup>[22–28]</sup> have shown that baseline serum GGT level is an independent risk factor for the development of heart disease, hypertension, stroke, and type 2 diabetes, regardless of alcohol consumption.

The mechanism underlying these associations remains largely unknown. Insulin resistance syndrome is a potential unifying factor, because it connects GGT with obesity, fat distribution, hypertension, dyslipidemias, smoking, exercise

and cardiovascular and cerebrovascular risk, as reviewed by Whitfield.<sup>[4]</sup> Fatty liver changes have also been discussed as a possible mechanism to explain GGT-disease relationships<sup>[29,30]</sup> even though fatty liver changes is recently regarded as one component of the insulin resistance syndrome by some researchers.<sup>[31,32]</sup> However, the strong dose-response relationship between baseline serum GGT within its normal range and diabetes was not explained by any liver damage, as assessed by the minimal association in the same data between diabetes and other, more specific liver enzymes.<sup>[22,23]</sup>

## SERUM GGT AND OXIDATIVE STRESS: EPIDEMIOLOGICAL EVIDENCE

The relationship between serum GGT and cellular GGT is not known. Specifically, it is not known whether serum GGT levels simply correlates with cellular GGT expression or whether other factors regulate the fraction of GGT that is directed to the cell surface for cellular secretion. However, our series of epidemiological studies with Coronary Artery Risk Development in Young Adults (CARDIA) subjects (a longitudinal, multicenter epidemiological study with 5115 black and white men and women aged 17–35 years at baseline) consistently suggest that serum GGT within its normal range might be an early and sensitive enzyme related to oxidative stress, supporting experimental findings.

First, circulating concentrations of beta-carotene, alpha-carotene, beta-cryptoxanthin, zeaxanthin/lutein, and alpha-tocopherol were related inversely to serum GGT level within its normal range in dose-response manners.<sup>[33]</sup> For example, subjects who had lower concentrations of serum antioxidant vitamins at baseline had higher concentrations of serum GGT in the future, although serum GGT at baseline did not predict the future levels of these blood antioxidant. The inverse association between serum antioxidants and GGT was confirmed among a sample of the US population using the third National Health and Nutrition Examination Survey.<sup>[34]</sup>

Second, when we examined associations between dietary factors and serum GGT, a higher consumption of fruit inversely predicted the future serum GGT level in a dose-response manner and various plant foods also appeared to be inversely associated with serum GGT level.<sup>[35]</sup> Among nutrients contained in plant foods, dietary antioxidants such as vitamin C and beta-carotene showed inverse associations with serum GGT level, though these same compounds in isolation (taken as supplements) were positively related to GGT. There are two studies<sup>[36,37]</sup> which reported an inverse association

between fruit intake and serum GGT, but did not further examine nutrients.

Third, in addition to alcohol consumption, meat consumption was the only other food group which positively predicted future serum GGT level in a dose-response manner.<sup>[35]</sup> When possible nutrients contained in meat were examined, heme iron, but not saturated fat, was strongly associated with serum GGT level. This finding may be helpful in understanding the mechanism by which serum GGT relates to poorly controlled oxidation, because free iron is a critical catalyst in generating oxidative stress<sup>[38–40]</sup> even though it is uncertain that higher intake of heme iron in the general population is directly related to the presence of free iron. Several population-based studies<sup>[37,41]</sup> have reported a positive association between serum GGT and ferritin, a conventional marker of stored body iron, regardless of alcohol consumption. Furthermore, patterns of serum GGT and serum ferritin are similar across age and sex groups.<sup>[42–48]</sup> The well-known elevation of serum GGT after alcohol drinking might be also interpreted as reflective of oxidative stress; acute and chronic ethanol consumption under experimental conditions increased production of reactive oxygen species.<sup>[49]</sup>

An increase of serum GGT in the above CARDIA findings<sup>[33,35]</sup> might be interpreted as a defense mechanism reflecting induction of cellular GGT under oxidative stress. If this were the case, serum GGT might eventually minimize oxidative stress and consequent pathologic change due to oxidative stress by facilitating regeneration of intracellular GSH. However, in the CARDIA study,<sup>[23]</sup> serum GGT level within its normal range positively predicted F2-isoprostanes, a highly-regarded marker of oxidative stress, which was measured 5 and 15 years later, in a dose-response manner. This association was similarly observed among non-drinkers, drinkers and subjects with normal level of other liver enzyme. Moreover, serum GGT also predicted fibrinogen and C-reactive protein, markers of inflammation.<sup>[23]</sup> These associations suggest that the net result of higher serum GGT activity was increased, rather than decreased, oxidative stress. In addition, prospective cohort studies have shown that baseline serum GGT predicted development of many disease outcomes, especially diabetes,<sup>[22–28]</sup> adding to the evidence that the increase in serum GGT is not successful as a defense against oxidative stress. Furthermore, cellular GGT activity is elevated in a number of human tumors, e.g. ovary, colon, lung, liver, prostate, sarcoma, melanoma and leukemias, especially in their metastatic forms,<sup>[12]</sup> although the role of GGT in carcinogenesis is unclear. Taken together, these findings suggest that serum or cellular GGT might be

involved, whether as an active participant or indirectly, in the development of oxidative stress or various diseases.

In this context, experimental findings<sup>[6–9]</sup> that cellular GGT changes its role from an antioxidant to a pro-oxidant in the presence of a transition metal such as iron are of interest because iron can increase cellular GGT during oxidative stress. In addition to the CARDIA finding of a higher intake of heme iron increasing serum GGT,<sup>[35]</sup> one experimental study<sup>[50]</sup> reported that iron intake may lead to increased level of cellular GGT. In that study, rats consumed iron mixed in rat chow for 10 weeks. Hepatic GGT activity increased 6-fold and was co-located with iron; GGT mRNA also increased. As iron itself could be considered one of the oxidative stressors, iron overload leads to GSH depletion, and, in turn, causes GGT induction. GGT elevation induced by a pro-oxidant effect of iron, unlike a pro-oxidant effect of other oxidative stressors, may lead to a vicious cycle because GGT itself may then also be involved in generating oxidative stress.

GGT is a multifunctional protein. In addition to its transpeptidase enzyme activity, the physiological role of GGT has been demonstrated by development of GGT-deficient knockout mice.<sup>[51]</sup> Such mice appear normal at birth but grow slowly, they are about half the weight of wild type mice, are sexually immature, develop cataract and die by 6–8 weeks of age. Plasma and urine GSH levels are elevated 6- and 2500-fold, respectively, demonstrating the essential role of GGT in cellular salvage of extracellular GSH. Recently, it was also demonstrated in the same knockout mice that GGT regulated osteoclast biology, bone remodeling and longitudinal growth.<sup>[52]</sup> In addition to its gamma glutamyltranspeptidase activity it is also essential for leukotriene biosynthesis and bone metabolism as demonstrated in GGT deficient humans.<sup>[53]</sup>

## SERUM GGT AS A USEFUL BIOMARKER OF OXIDATIVE STRESS IN FUTURE STUDIES

There are various biological markers that are used to assess oxidative stress, that includes F2 isoprostanes, 8-hydroxydeoxyguanosine and protein carbonyls.<sup>[54]</sup> These biomarkers give a view of oxidative damage to, respectively, lipid, DNA and protein, but individually do not give a global view of whole body stress.<sup>[54]</sup> Furthermore, these tests are expensive and difficult to perform well. A simple and inexpensive test of overall oxidative stress would be very useful for researchers investigating oxidative stress in relation to aging and chronic degenerative diseases. Although, it is unclear to what extent cellular or serum GGT reflects systemic stress, at least, measurement

of serum GGT is reliable, easy, and inexpensive. In one study<sup>[55]</sup> on stability of serum GGT, the activity of the enzyme was essentially unchanged in repeated tests during 40 weeks when frozen-stored samples were thawed and then frozen again after each testing.

In conclusion, based on current epidemiological and experimental studies, we suggest that elevated serum GGT within its laboratory normal range might be an early and sensitive marker for oxidative stress. Serum GGT might be useful in studying oxidative stress-related issues in both epidemiological and clinical settings.

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