

Gamma Glutamyl Transferase – An Underestimated Marker for Cardiovascular Disease and the Metabolic Syndrome

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ABSTRACT- Gamma glutamyl transferase (GGT) is an enzyme in glutathione and cysteine metabolism. GGT is a standard liver enzyme test reflecting biliary tract involvement. It also has a prooxidant activity and a modulating influence on endothelial dysfunction. GGT is associated with the metabolic syndrome and is often elevated in patients with NAFLD. There is also a role for GGT activity in several aspects cardiovascular disease. There is an association between elevated GGT and cardiovascular mortality, atrial fibrillation, exacerbation of congestive heart failure. In addition there is an association with obstructive sleep apnea. We review the evidence available and suggest that there is a need for further assessing the use of GGT, together with the presence of the metabolic syndrome as a prognostic marker.

INTRODUCTION

Gamma glutamyl transferase (GGT) is a glycosylated protein embedded in the outer surface of the plasma membrane. It has a role in the homeostasis of both glutathione and cysteine (1).

GGT is a microsomal enzyme that is responsible for transferring the glutamyl groups from gamma glutamyl peptides to other peptides. With the exception of muscle cells, GGT is distributed in all other organs. GGT catalyzes the synthesis and transmembrane transport of proteins, counteracts oxidative stress by providing cysteine for regeneration of intracellular glutathione and contributes to the detoxication of ammonium of some drugs (2).

Cobbina and Akhlaghi (3) reviewed the possible mechanism of drug metabolizing system activation leading to nonalcoholic fatty liver disease (NAFLD) in human and experimental models. Induction of microsomal CYP2E1 also induces microsomal GGT activity.

GGT has been proposed to be a predictive biomarker for cellular antioxidant deficiency and several disease states (4). GGT makes a significant contribution to oxidant activity. In addition there are variations in GGT level based on gender, ethnic and regional differences and an upward trend in levels which suggest an environmental factor. Glutathione (GSH) is an important antioxidant defence and its failure results in impaired endothelial mediated vasodilatation due to an unbuffered increase in free radicals (5). The oxidative stress that occurs from both insufficient antioxidant defence and dysregulated glucose and lipid homeostasis, in the presence of vascular

inflammation results in endothelial dysfunction. Endothelial dysfunction is linked to atherogenesis and microvascular disease.

The liver, kidney, pancreas, bowel and prostate are rich in GGT (6). Despite the kidney having ten times more GGT activity than the liver, serum GGT is considered to be of biliary canalicular origin and has been used as a liver test for many years (2).

The liver isoenzyme, however, is especially important in diagnosing cholestatic liver disease and monitoring drug-induced liver damage. If the GGT is concomitantly elevated with alkaline phosphatase (ALP) it is almost certain that the ALP is of hepatic origin. As a result, cholestatic liver disease is the cause of ALP activity.

GGT is useful in evaluating the severity of liver disease in chronic alcoholics. Moreover, GGT is used to follow the evolution of alcoholic liver disease and assess the alcohol-drinking habits in chronic alcohol consumption.

As a microsomal enzyme GGT is activated in alcohol metabolism since the liver microsomes oxidize ethanol. In 1968, Lieber and De Carli described the microsomal ethanol-oxidizing system (MEOS) for the first time (7). MEOS represents an enzyme complex cytochrome P450 (CYP). The iso-enzyme CYP 2E1 plays a role in alcohol metabolism at high alcohol concentrations and microsomal oxidative stress.

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The oxidative stress generates the reactive oxygen species (ROS). CYP 2E1 and ROS are involved in the initiation and perpetuation of alcoholic liver injury. Histopathologically alcoholic liver disease (ALD) and non-alcoholic liver disease (NAFLD) present common cellular changes. Cyp2E1 is also activated in NAFLD and is modified by food (8-10).

GGT is elevated in hepatobiliary disease due to *de novo* synthesis, increased release from cell membranes secondary to the detergent effect of bile salts, backflow into the bloodstream, increased permeability and biliary epithelial cell damage (2).

GGT determination as a biomarker of increased glutathione demand presents several advantages. These include low cost, sample stability and easy preparation, as well as the availability of clinical laboratory measurement (11).

The metabolic syndrome consists of truncal obesity, hypertension, impaired glucose tolerance and hyperlipidemia. It is associated with multi-organ disease. Non-alcoholic fatty liver disease is the hepatic manifestation of the metabolic syndrome (12).

This is the leading cause of hepatic pathology including cirrhosis and hepatocellular carcinoma worldwide and is of epidemic proportions. GGT is often elevated in NAFLD (13).

Other organ systems are involved in NAFLD related to the other components of the metabolic syndrome. These include kidney, cardiovascular and gastrointestinal systems. There is evidence involving the heart not just as a direct result of the cardiac risk factors that make up the metabolic syndrome. For example, diastolic dysfunction has been shown to be linked with NAFLD independently of the factors of the metabolic syndrome (14). It is the purpose of this review to examine the relationship between GGT and the metabolic syndrome and the use of measurements of GGT as a prognostic indicator in several disease states.

GGT is associated with the metabolic syndrome (MS).

The Framingham offspring study conducted between 1978 and 1982 included data for cardiac risk factors including weight, BMI, smoking and alcohol use, serum lipid values, blood glucose and serum creatinine, CRP and the presence of diabetes. The total follow-up period was 20 years, with an interim assessment after 8 years for the presence of metabolic syndrome. The onset of MS was related to the baseline GGT (15). In addition, there was a dose-response relationship for the GGT level to MS over all four quartiles. After

adjusting for age, gender and alcohol use, the hazard ratio for developing MS was elevated by 2.54 in the fourth quartile ($p \leq 0.001$). At the end of the follow-up period (mean of 19 years), those subjects in the highest GGT quartile had an adjusted 67% increase in the incidence of cardiovascular disease. Another study on this cohort with a mean follow-up of 23.6 years found that baseline GGT levels above the gender median had a 1.71 times increase risk of developing heart failure compared to those with GGT below the gender median (16). Thus a one-time determination of GGT may be a predictive marker for MS, CVD and heart failure. In addition, GGT may be decreased by dietary manipulation (17).

An increase in mortality in patients with an elevated baseline GGT has been found in an Austrian population. For men with a GGT > than 36 U/L and women > 36 U/L the mortality rate was increased by 100%. The increased mortality was due to cancer deaths and cardiovascular deaths (18). A further study, from Finland, also showed a higher hazard ratio for both non-fatal myocardial infarction and fatal coronary heart disease for subjects with elevated GGT (19).

Cardiovascular diseases associated with GGT. Cardiovascular morbidity and mortality.

GGT may have a role in predicting the development and outcome of cardiovascular morbidity and mortality. A study of more than 3500 patients with coronary heart disease comparing the patients with a GGT level above the median to those with a GGT level below the median found an increase in all cause mortality (HR 1.32) after multi-variate analysis but the presence of the metabolic syndrome was not included in the analysis (20). GGT has been shown to be a predictor of cardiovascular risk in 200 patients with angiographically proven coronary artery disease. 59.5% of these patients had the metabolic syndrome (21). Multivariate analysis was not performed in this study.

A study of 123 patients with acute coronary syndrome and a newly diagnosed ejection fraction less than 45% found that a GGT level greater than 49 u/mL had a 81% sensitivity for predicting hospitalization (22)

A study from Iran of 367 patients undergoing elective angiography (men ≤ 45 years of age and females < 55 years of age) found an elevated GGT to be significantly associated with premature coronary artery disease (CAD) (23). Another group from India has also reported an association between premature CAD and elevated GGT (24). Serum GGT has also been linked to CAD in a Chinese population (25). A recently published

systematic review and meta-analysis found elevated serum GGT levels to be an independent predictor of both cardiovascular mortality and all-cause mortality in patients with CAD (26).

There is a link between inflammation and cardiovascular disease. This is reflected by a link to elevated levels of C reactive protein (CRP) (27). The strong association between GGT and CRP has been found in both genders and various ethnic categories (28). There is also a correlation with the Framingham risk score (29). In addition, there is a correlation between a higher GGT quartile and severity of coronary calcification(30). A study comparing 138 patients with coronary plaques to 121 without found higher levels of GGT (35.7 with acute coronary syndrome compared to controls/L with plaques versus 19.6 U/L without) (31).

In addition, GGT elevation and coronary plaques were linked to smoking, diabetes as judged by Hemoglobin A1c (HBA1c), as well as, hypertension. Biochemical markers such as hyperlipidemia, creatinine, triglycerides, uric acid, HBA1c and CRP have been utilized. Many of these biomarkers are components of the metabolic syndrome although this was not examined as a separate entity.

Serum GGT levels have been found to be higher in patients with acute coronary syndrome compared to control patients. In addition there was no difference between the diabetic and non-diabetic subgroups (32).

A systematic review and meta-analysis of 35 prospective observational studies including 571,511 subjects and 72,196 cases of mortality, found that elevated GGT was linked to an increase in all-cause mortality and cardiovascular mortality, even at physiological levels (33). In addition, there was an increase in cardiovascular mortality in patients with coronary artery disease and type 2 diabetes mellitus.

Atrial fibrillation

There is also a connection between atrial fibrillation (AF) and GGT. A study of 809 patients with atrial fibrillation on admission out of 5501 CAD patients showed that GGT activity was independently associated with AF (34). However, a prospective study of 1428 men aged 60-79 years of age who developed AF did not show a relationship between AF and change in GGT after adjusting for N-terminal pro-brain natriuretic peptide (35). A study from Korea from a national database of 266,550 people with a mean age of 53 who underwent a national insurance health check up found an increase in AF incidence with increasing quartiles of GGT and after adjusting for BMI the GGT was a good predictor of AF only in

the non-obese population (36). Serum GGT has also been shown to be elevated in nonvalvular AF (37). Patients with hepatic steatosis on ultrasound have not been found to have increased AF whereas increased levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and especially GGT have been linked with AF.

Catheter ablation is increasingly being employed for treatment of atrial fibrillation. A study of 102 patients with paroxysmal atrial fibrillation undergoing cryoballoon catheter ablation found that elevation of GGT to >23.5U/L was an independent predictor for recurrence of AF after ablation (38).

Heart failure

A study of 323 heart failure patients, found that higher central venous pressure was related to an increase in serum GGT, and elevated ALT, AST or direct bilirubin were associated with higher CVP and a low cardiac index (39). A study of 1033 consecutive ambulatory heart failure patients with a mean follow up of 34.4 months found that increased GGT was associated with increasing severity of heart failure in terms of NYHA class, LV ejection fraction and BNP. In addition increased GGT was an independent predictor of death or cardiac transplantation (40). A Chinese study of 165 patients with heart failure after percutaneous coronary intervention (PCI) from a cohort of 5638 patients found that a baseline serum GGT level of less than 19.6 U/L or more than 32.9 U/L increased the risk for developing heart failure following PCI (41). However, a smaller prospective study of 60 patients with heart failure from India did not find a predictive role for serum GGT but did for BNP and tumor necrosis factor (TNF)- α (42).

In patients admitted with acute coronary syndrome (ACS), GGT may help in predicting those patients with new onset heart failure in those with left ventricular systolic dysfunction. A study from Turkey of 123 patients with ACS and a systolic function less than 45% were followed up for a mean period of 15 months. An admission GGT of more than 49 U/L had a sensitivity of 81.7% and specificity of 65.2% for predicting hospitalization. On multivariate analysis GGT > 49 U/L and hypertension were independent predictive factors (22). A similar conclusion of an elevated GGT as a poor prognostic factor, with an increased risk for mortality, in patients with reduced systolic function, was reported from another group in Turkey (43).

Obstructive sleep apnea (OSA) and NAFLD

Obstructive sleep apnea (OSA) is characterized by repeated partial or total airway collapse during sleep. It affects 4% of men and 2% of women. OSA increases the risk for cardiovascular disease and also for NAFLD^[44]. This is accompanied by systemic inflammation and endothelial dysfunction. OSA with excessive daytime sleepiness has also been shown to be associated with NAFLD independently of the amount of abdominal visceral fat (45).

In addition OSA has been shown to be linked to more severe liver fibrosis in patients with NAFLD (46).

OSA is also associated with increased levels of GGT. A study from Turkey of 320 patients with OSA found that serum GGT levels were associated with and increased apnea-hypopnea index. In this study increased GGT was also linked to cardiovascular disease (47)

Thus there is a link between elevated GGT and OSA with both OSA and cardiovascular pathology. Table 1 summarizes the data regarding the link between GGT and CVS disease.

Table 1. Author: Provide a self-explanatory legend, and in a footnote define abbreviations. ED.

<i>GGT</i>	Result	Result	References
Met S onset	related to baseline GGT	OR 2.54 upper quartile	15
CVS	Increased	OR 1.67 upper quartile	15
Heart failure	Increased	1.71	16
All cause Mortality	Increased	2	18
non fatal MI and fatal CHD	Increased	OR 1.57 men, OR 1.44 women	19
<i>GGT CVS</i>			
All cause mortality	Increased	1.32	20,26
Cardiovascular risk	Predictor of CVS risk		21
Hospitalization		81% predictive sensitivity	22
Premature CAD		10 unit increase GGT, OR 13.34	23
		highest tertile, OR 2.1	24
		coronary complexity	25
Coronary calcification		independent risk factor	30
Coronary plaques		Higher GGT, independent risk factor	31
ACS		Higher GGT levels and also in diabetics	32
<i>GGT AF</i>	Association	OR 1.66	34
		no relationship after adjusting for BNP	35
	only non-obese		36
	non-valvular		37
	recurrence after catheter ablation		38
<i>GGT Heart failure</i>			
higher CVP	Higher GGT		39
	P<0.001		
low CI	Higher GGT		39
	P<0.001		
NYHA	Increase in NYHA class, decrease EF		40
BNP	Increase BNP		40
Post PCI	increased risk for developing HF		41
Hospitalization	GGT >49	HR 2.663, p=0.047	22
<i>GGT OSA</i>			
increased GGT levels	linked to severe OSA	p<0.001	47

Liver disease and cardiovascular events

Cirrhotic patients are known to have more extensive and severe CAD as compared to a control group of patients referred with new-onset chest pain (48), although in this study the majority of the patients had cirrhosis due to alcohol consumption. Recently, GGT activity has been shown to be associated with coronary artery atherosclerotic plaque vulnerability (49). Furthermore the serum GGT level has been found to be linked to significant coronary atherosclerosis and the level of GGT increased with the increasing severity of arterial obstruction (50). As in previous studies the metabolic syndrome was not included as a variable in the multivariate analysis.

Elevated GGT is well known to be a consequence of NAFLD (51). In addition GGT is linked to an increased risk of type 2 diabetes mellitus (52). A systematic review of 24 cohort studies with 177,307 patients, of whom 11,155 had type 2 DM found a non-linear relationship between type 2 DM and elevated GGT level with an odds ratio of 1.34 for the upper third of GGT levels.

NAFLD scans a spectrum of disease from steatosis through steatohepatitis and resulting in fibrosis, cirrhosis and its complications including hepatocellular carcinoma (12, 53). Liver fibrosis has been shown to be linked to long-term outcomes of patients with NAFLD. A retrospective study of 619 patients diagnosed with NAFLD from 1975 to 2005 in the United States, Europe and Thailand, found liver fibrosis to be the only histological feature associated with long term outcomes (54). In this study the mean BMI was 30.7 kg/m² and 37.5% of the population had diabetes mellitus. No data was available regarding GGT in this study. Furthermore 38.3% of the patients died as a result of cardiovascular disease. Another long term population study from Sweden (55) of 229 patients with biopsy proven NAFLD followed up for a mean of 26.4 years, found a HR of 1.29 for NAFLD patients, with increased risks of cardiovascular disease, HCC and cirrhosis. Cardiovascular mortality was the cause of 43% of the deaths, whereas HCC and cirrhosis were only responsible for 5% and 4% of mortality respectively.

Furthermore, data regarding smoking, presence of the metabolic syndrome and medication was not available for the study group. Since approximately one third of the Swedish population is obese it is highly likely that there were lots patients with DM in the control group. Overall mortality was increased with patients with F3-4 fibrosis. Once more no data was presented regarding GGT. Chronic kidney disease has also

been shown to be linked to the presence and severity of NAFLD (56).

NAFLD has been shown to be connected with an increased risk of 1 year all-cause mortality and cardiac rehospitalization in elderly patients admitted with acute heart failure (57). In addition NAFLD has been shown to be linked to aortic valve sclerosis in patients with type 2 DM (58).

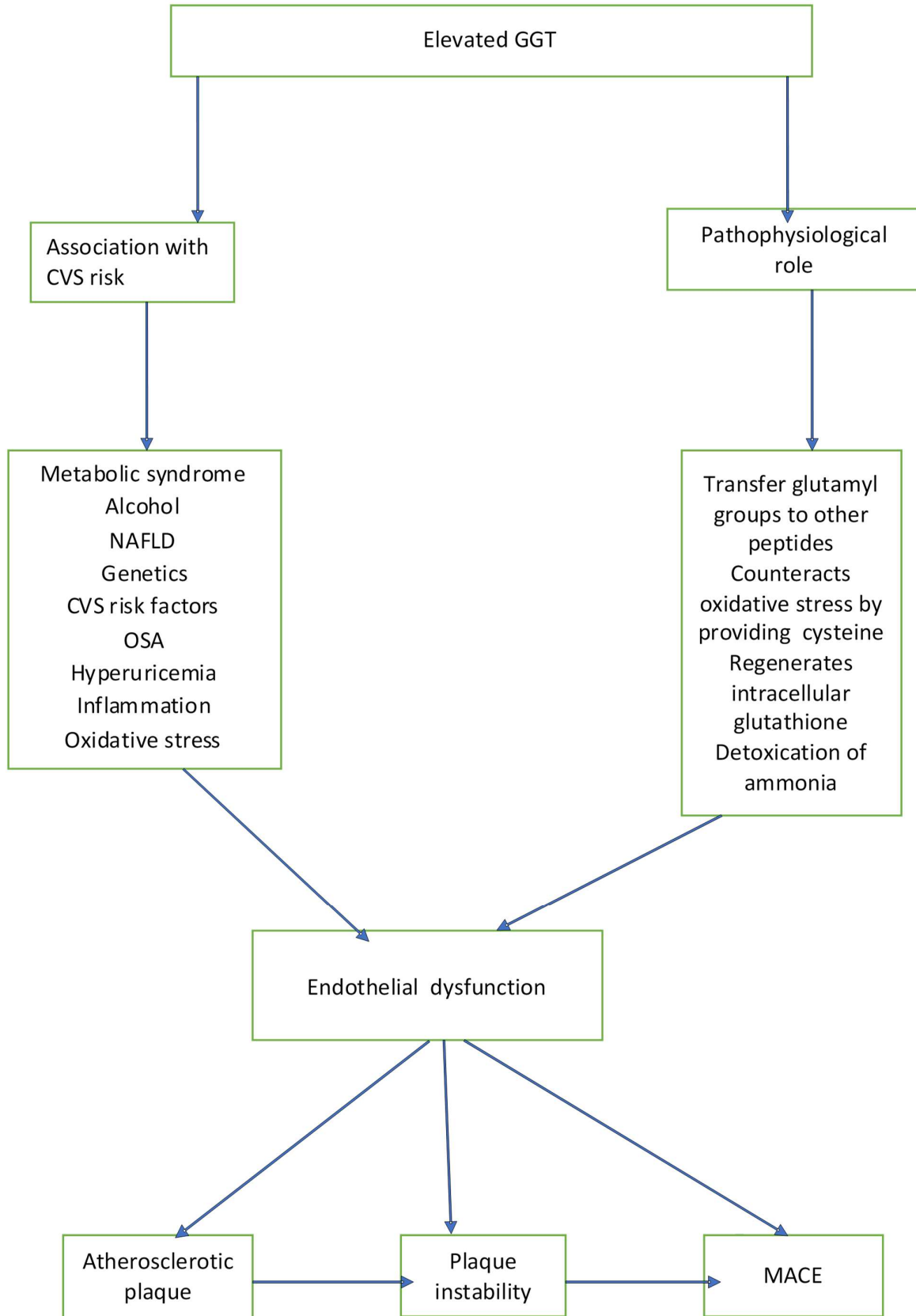
In addition although it is clear that in NAFLD fibrosis is a major determinant of mortality it is not clear if this is independent of the components of the metabolic syndrome. This is of more than academic interest since billions of dollars are being spent in drug development which can decrease hepatic fibrosis as an end point in trials. This may not show benefit in reduction of overall mortality or decreasing the associated cardiovascular morbidity.

The gold standard for assessing fibrosis is liver biopsy. This is of course an invasive procedure with a small risk of complications including hemorrhage. It is not easily available as a method for performing long-term follow up of NAFLD patients to assess fibrosis progression. There are several methods for assessing fibrosis in NAFLD that do not require biopsy (59). These include the NAFLD fibrosis score, the BARD score, the FIB-4 score, the fibrometer NAFLD and the hepascore. In addition there is the AST:platelet ratio index (APRI), although this is of more use in identifying those patients unlikely to have advanced fibrosis (60). The FIB4 index appears to be superior to the other simple, inexpensive tests available for measuring hepatic fibrosis (61).

In addition the GGT:platelet ratio has been found to be predictive of advanced fibrosis in Chinese patients with both chronic HBV hepatitis and NAFLD (62). FIBI-4 index has been shown to be an independent predictor of all cause mortality in patients with heart failure (63) and the NAFLD fibrosis score has been shown to be linked with unfavorable outcomes in patients with CHF (64). The interactions between GGT and cardiovascular end-points are summarized in the Figure 1.

There is little data in the literature regarding the GGT levels in different ethnic populations. GGT levels are influenced by weight, alcohol consumption, and exposure to toxins. Very few studies have reported data on different ethnic populations in healthy people after controlling for these variables.

In the Framingham study the upper limit of normal for GGT was < 50 iU/L in men and < 40 iU/L in women (65). After excluding patients with the metabolic syndrome the mean GGT was 24.9 iU/L for men and 18.9 iU/L for women.



This suggests that in a similar fashion to ALT the reference numbers for GGT need to be adjusted downwards. The British Womens' Heart and Health Study of 3511 women with no history of stroke or coronary heart disease (66) gave no numbers for GGT just the quintiles of the distribution .

A study from Iran comparing GGT levels in 3 different ethnic groups, found a mean BMI of 28 kg/m² and a GGT value that was significantly different between the 3 regions.

An additional study from Korea (67) (5th National Health and Nutrition Examination Survey) with 8863 participants from 2010-2011 reported a mean GGT level of 33.9 iU/L and women 18.8 iU/L . In this study, however, 18% of the men had moderate alcohol consumption vs 3% of the women. Furthermore based on the NHANES data from 1999-2010 it appears that coffee consumption decreases liver enzymes. In this study 65.1% of the participants were non-Hispanic whites and 15.3% non-Hispanic blacks. 28.7% of the participants consumed less than one drink per week (68). Thus, the data are not available to enable clear conclusions regarding the effect of ethnicity on serum GGT levels and this will need to be addressed by further studies.

In view of the data presented above linking elevated GGT to worsened cardiovascular events and the link between GGT and MS, there is a need to examine if the presence of the metabolic syndrome could be an independent marker for poor outcomes in patients with cardiovascular pathology including heart failure. If this were to be the case , it would underline the need to have rigid cardiovascular outcomes in ongoing trials of treatment of NAFLD.

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