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Gamma-Glutamyl Transferase as a Marker of Alcohol Abuse

Effects of Moderate Drinking, Obesity
and Increasing Age on Reference Intervals



ACADEMIC DISSERTATION

To be presented, with the permission of
the Faculty of Medicine of the University of Tampere,
for public discussion in the auditorium of
Mediwest Health Technology Center, Koskenalantie 16, Seinäjoki,
on March 2nd, 2007, at 12 o'clock.

(Simultaneous video conference connection
in the small auditorium of Building K,
Medical School of the University of Tampere,
Teiskontie 35, Tampere)

UNIVERSITY OF TAMPERE

ACADEMIC DISSERTATION

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www.uta.fi/taju
<http://granum.uta.fi>

Cover design by
Juha Siro

Printed dissertation
Acta Universitatis Tamperensis 1211
ISBN 978-951-44-6858-2 (print)
ISSN 1455-1616

Electronic dissertation
Acta Electronica Universitatis Tamperensis 595
ISBN 978-951-44-6859-9 (pdf)
ISSN 1456-954X
<http://acta.uta.fi>

Tampereen Yliopistopaino Oy – Juvenes Print
Tampere 2007

To my family

Abstract

Excessive alcohol consumption is a major cause of health problems throughout the Western world, and the need for objective clinical tools for detecting alcohol abuse in its early phase has been widely acknowledged. Gamma-glutamyl transferase (GGT) is a liver-derived enzyme, which has long been used as a marker of excessive alcohol consumption, but the amount of drinking needed to elevate GGT levels has remained unknown. Also, it has been suggested that GGT levels may be elevated by factors such as obesity and increasing age in addition to alcohol consumption, although the magnitude of such effects have remained unclear.

The relationships between alcohol consumption, obesity, age and GGT values are studied here in a large number of heavy drinkers and apparently healthy reference individuals, classified as follows: subjects reporting no alcohol consumption (abstainers), subjects reporting 1–40 grams of ethanol consumed per day (moderate drinkers) and subjects reporting 40–540 grams of ethanol consumed per day (heavy drinkers). The reference population was further classified according to body mass index (BMI) into underweight (BMI<20), normal weight (BMI 20–25), overweight (BMI 25–30) and obese (BMI>30), and by age into those under 18 years, 18–30 years, 30–50 years, 50–70 years and over 70 years.

GGT activity was markedly higher in the heavy drinkers than in the moderate drinkers ($p<0.001$) or abstainers ($p<0.001$), and the values in the moderate drinkers also exceeded those for the abstainers, although the difference was significant only for men ($p<0.001$). GGT activities in the moderate drinkers also showed additive effects of overweight or obesity which were not present in the abstainers, although the changes occurring as a function of increasing BMI and moderate drinking were of a lesser magnitude in the women than in the men. Consequently, the upper normal GGT limits based on normal weight abstainers (men: 53 U/l; women: 45 U/l) were markedly lower than those based on the unselected reference population (men: 68 U/l; women: 50 U/l) and the analytical sensitivity of GGT as a marker of alcohol abuse showed significant variation as a function of BMI in the reference population. The rates of false positive values varied from 2 to 26% in the subgroups from low to high BMI, respectively. GGT activities also increased with age until after 70 years, although decreasing activities were noted in males who abstained from drinking ethanol. The heavy drinkers in the age groups 18–30, 30–50 and 50–70 years showed several-fold higher mean GGT activities than the abstainers and moderate drinkers of corresponding ages, and the values for moderate drinkers also exceeded those for abstainers in all age groups among the men, whereas for the women the difference was significant only among those aged 18–30 years.

The data show that serum GGT is a highly sensitive indicator of ethanol consumption, although its diagnostic value could be improved by using reference data based solely on abstainers of normal weight, or else BMI-specific reference intervals. Also, the fact that GGT activity responds to etha-

not in an age-dependent manner should be considered in the clinical use of GGT measurements for detecting alcohol consumption disorders.

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Abbreviations

ALD	Alcoholic liver disease
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
AUDIT	Alcohol Use Disorders Identification Test
BMI	Body mass index
CAGE	Cut down, Annoyed, Guilty, Eye-opener (acronym)
CDT	Carbohydrate-deficient transferrin
CHD	Coronary heart disease
CV	Coefficient of variation
EOA	Early onset alcoholics
FAE	Fetal alcohol effects
FAS	Fetal alcohol syndrome
GGT	Gamma-glutamyl transferase
GSH	Glutathione
HDL	High density lipoprotein cholesterol
IFCC	International Federation of Clinical Chemistry and Laboratory Medicine
NAFLD	Non-alcoholic fatty liver disease
NORIP	Nordic Reference Interval Project
LOA	Late onset alcoholics
MAST	Michigan Alcoholism Screening Test
MCV	Mean corpuscular volume
ROS	Reactive oxygen species
SD	Standard deviation
TLFB	Timeline follow-back method
WHO	World Health Organization

List of original publications

- I Hietala J, Puukka K, Koivisto H, Anttila P and Niemelä O (2005): Serum gamma-glutamyl transferase in alcoholics, moderate drinkers and abstainers: effect on GT reference intervals at population level. *Alcohol Alcohol* 40:511–514.
- II Puukka K, Hietala J, Koivisto H, Anttila P and Niemelä O (2006): Additive effects of moderate drinking and obesity on serum gamma-glutamyl transferase activity. *Am J Clin Nutr* 83:1351–1354.
- III Puukka K, Hietala J, Koivisto H, Anttila P, Bloigu R and Niemelä O (2007): Obesity and the clinical use of serum GGT activity as a marker of heavy drinking. *Scand J Clin Lab Invest*, in press.
- IV Puukka K, Hietala J, Koivisto H, Anttila P, Bloigu R and Niemelä O (2006): Age-related changes on serum GGT activity and the assessment of ethanol intake. *Alcohol Alcohol* 41:522–527.

The original articles are referred to in the text with the above Roman numerals.

1. Introduction

Both total alcohol consumption per capita and associated medical disorders have continued to increase in most Western countries over recent decades (WHO 2004, Stakes 2005). Simultaneously, the percentage of individuals abstaining from alcohol entirely has decreased. Excessive alcohol consumption causes a wide variety of medical and social problems and a considerable economic burden (Room et al. 2005). Economic prosperity, the increase in welfare and the better availability of alcohol have all had an impact on the growth in alcohol consumption as has the recent reduction in alcohol taxes in the case of Finland (WHO 2004, Stakes 2005).

The health problems caused by alcohol include liver diseases, gastrointestinal problems, cardiac problems, high blood pressure, diabetes and cancers (Diehl 1989, Rubin and Farber 1994, Lieber 1995, Bagnardi et al. 2001, Mukamal et al. 2005, Schneider and Singer 2005, Kodavali and Townsend 2006, Puddey and Beilin 2006, Welsch et al. 2006). Patients who have not yet developed any evident illness as a result of their alcohol consumption or addiction to alcohol are normally difficult to recognize in health care, and by the time the problem has advanced to the point where it is easy to identify, the prognosis is poor. Obviously, more attention should be paid to the possible use of ethanol in the early phase. It is also important to develop laboratory markers, because by monitoring test results, it may be possible to influence the will of the patient to reduce his or her alcohol consumption and also to evaluate the extent of the resultant tissue damage.

Gamma-glutamyl transferase (GGT) is a membrane-bound glycoprotein enzyme which catalyzes the transfer of the gamma-glutamyl moiety of glutathione to various peptide acceptors. Chronic ethanol consumption is known to induce a rise in serum GGT, and it has therefore been widely used as an index of excessive ethanol intake (Zein and Discombe 1970, Reyes and Miller 1980, Anton et al. 2002, Niemelä 2002, Conigrave et al. 2003). Although several studies have reported a positive correlation between the amount of alcohol consumed and serum GGT activities, the reported sensitivities of this marker have varied greatly in the literature, from 15 to 85% (Bagrel et al. 1979, Chick et al. 1981, Papoz et al. 1981, Persson et al. 1990, Anttila et al. 2004). Recent work by several groups of investigators has also emphasized obesity as an important factor which can increase serum GGT activities (Daepfen et al. 1998, Peters and Cook 2002, Lam and Mobarhan 2004, Colicchio et al. 2005, Lawlor et al. 2005). In addition, it has been suggested that GGT levels in the circulation may be affected by age (Daepfen et al. 1998, Sillanaukee et al. 1998, Conigrave et al. 2002, Stromme et al. 2004, Lee et al. 2004b). The magnitudes of the effects of drinking per se, overweight, or age on the clinical behaviour of GGT in the assessment of hazardous drinking practices have nevertheless remained poorly defined, and the effects of variables such as overweight or age on GGT normal ranges have not been explored.

The reference intervals and normal ranges for GGT determinations used in previous studies and in routine health care have been based on values obtained from mixed populations of apparently

healthy moderate drinkers and abstainers, whereas only limited attention has been paid to the exact amounts of ethanol consumed by these individuals (Stromme et al. 2004).

The present work set out to explore the relationships between ethanol consumption, obesity, increasing age and GGT activities in individuals with a wide variety of ethanol consumption patterns. The results indicate distinct effects of mild to moderate ethanol consumption on serum GGT levels, which should be considered in the clinical use of GGT as a marker of ethanol abuse and liver status and in the definition of GGT normal ranges.

2. Review of the literature

2.1. Alcohol consumption and health

Alcohol is the most common addictive substance and affects practically every organ in the human body, so that it ranks among the ten leading causes of disease and injury in the developed countries. Alcohol causes nearly 2 million deaths per year worldwide and the loss of 58 million disability-adjusted years of life. It has been estimated to be responsible for nearly 10% of the current disease burden in the developed countries. According to the World Health Organization (WHO), alcohol is the third most important risk factor for diseases and premature deaths among Europeans, after smoking and high blood pressure, which makes it as much as three-fold more important risk factor than diabetes and five-fold more important than asthma (WHO 2004).

Alcohol consumption in Finland has been growing steadily for many decades (Figure 1) and the current rate of growth is approximately 10% per year. Thus the Finns at present consume nearly 11 litres of absolute alcohol per capita per year, considering both the compiled statistics on consumption and estimated consumption (e.g. alcohol consumed abroad). In 2004 approximately 2800 people died because of excess alcohol consumption, which is over 20% more than in 2003 and the number of alcohol drinkers in Finland who regularly exceed the limit of risk consumption is estimated to be about 500 000 (Stakes 2005). This limit is about 280 grams of alcohol per week for men and 190 grams for women. There is about 12–15 grams of ethanol in one standard drink, 75 grams in a bottle of wine and 150 grams in a bottle of hard liquor (Sillanaukee et al. 1992).

Depending on both the amount and pattern of alcohol consumption, alcohol elevates the risk of health and social problems (WHO 2004), including liver diseases, neurological symptoms, cardiovascular diseases, many cancers, infectious conditions and hormonal and reproductive disorders (Lieber 1995, Pajarinen et al. 1996, National Institute on Alcohol Abuse and Alcoholism 2000a, Room et al. 2005). Mental disorders, injuries, poisonings and increased violence and suicides are frequently caused by excessive drinking (Gruenewald et al. 1995, Rossow and Amundsen 1995, Adrian and Barry 2003, Ahlm and Eriksson 2006). Different patterns of alcohol intake have distinctly different expected adverse health effects, e.g. the influence of chronic ethanol intake on health differs from that of acute (binge) drinking (Lieber 1995). Acute ethanol intake is clearly over-represented in cases of trauma, for instance (Cunningham et al. 2002, Savola et al. 2005) and in cases of embolic stroke (Hillbom et al. 1999), whereas chronic drinking may typically produce liver diseases or various neurological problems (Lieber 1995, Bonthius et al. 2006, Pan et al. 2006).

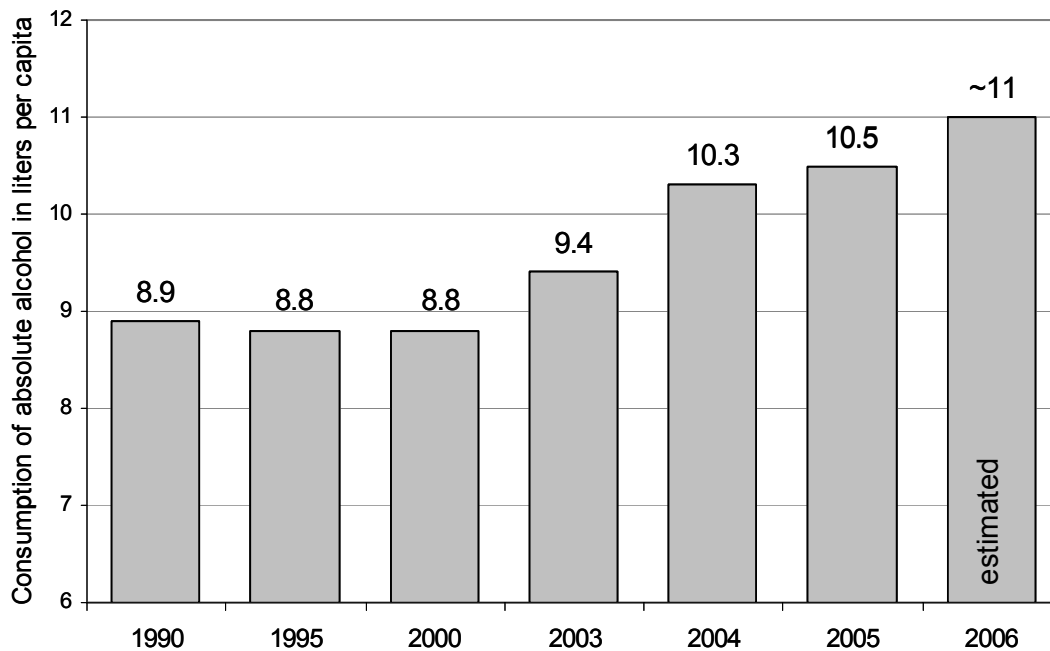


Figure 1. Mean ethanol consumption in Finland over the period 1990–2006 (Stakes 2005).

2.1.1. Definition of alcohol consumption patterns

Alcohol consumption in a population is measured by analysing the production and distribution statistics for alcoholic beverages as market commodities and by asking samples of the population about their drinking behaviour. The various stages of alcohol consumption patterns that are normally recognised include alcoholism, alcohol abuse, heavy drinking, moderate drinking and abstaining (Niemi 2002, Paille 2006). Alcoholism is a stage at which the consumption of alcohol is so high that it causes severe dependence and increased tolerance. Alcoholics can be further classified into two subgroups in terms of their age at the onset of drinking (Buydens-Branchey et al. 1989, Johnson et al. 2000, Enoch 2003, Wetterling et al. 2003): early onset alcoholics (EOA, type II alcoholics) and late onset alcoholics (LOA, type I alcoholics) (Johnson et al. 2003, Kranzler et al. 2003, Dom et al. 2006).

Alcohol abusers drink alcohol in such amounts that health or social problems or both are unavoidable and the patients suffer from mental or physical complications caused by alcohol even though the criteria for alcoholism may not be fulfilled. Heavy drinkers regularly consume over 40 grams of ethanol per day. Heavy drinking is a pattern that exceeds the standards of moderate drinking or social drinking and is often defined in terms of exceeding a certain daily volume or quantity per occasion or during daily drinking. The harmful level of alcohol consumption is over 280 grams per week (24 drinks) or 4–6 standard drinks on one occasion for men and over 190 grams per week (16 drinks) or 3–4 drinks on one occasion for women, which are the commonly accepted limits (Sillanauke et al. 1992, Niemi 2002). Moderate drinkers are individuals who consume less than the above limits and are able to control their drinking. Abstainers do not drink alcohol at all.

2.1.2. *Typical alcohol-related health effects*

2.1.2.1. *Liver effects*

Alcohol metabolism occurs mainly in the liver, which is therefore subjected to a variety of adverse effects of excessive ethanol intake. Ethanol-induced liver pathology is known to result in striking alterations in several laboratory parameters, which may therefore reflect liver status. In fact, many currently available alcohol markers are related to liver damage rather than to alcohol consumption. Alcohol is currently a leading cause of liver diseases, being responsible for a spectrum of alcoholic liver diseases (ALD) that includes fatty liver, alcoholic hepatitis, fibrosis and cirrhosis (Diehl 1989, Rubin and Farber 1994, Lieber 1995, Pan et al. 2006).

Only a few days' consumption of excess alcohol may cause fatty changes in the liver, although these are usually reversible. If the fatty liver is uncomplicated, patients usually lack clinical symptoms of liver disease (Rubin and Farber 1994). Alcoholic hepatitis is a clinically severe condition, with symptoms of malaise, right upper quadrant abdominal pain, jaundice, fever and mild leukocytosis and histological features that include necrosis of hepatocytes, cytoplasmic hyaline inclusions within hepatocytes, a neutrophilic inflammatory response and perivenular fibrosis (Lieber 1995, Niemelä 2002, Maraldi et al. 2006, Tsui et al. 2006). Fibrosis with a pericellular distribution is considered an early feature of ALD. Progressive fibrosis leads to the formation of fibrous septa surrounding the hepatocellular nodules, and typically develops in at least 15% of alcoholics. This state is characteristic of liver cirrhosis (Rubin and Farber 1994, Niemelä 2002). Women appear to be more sensitive to liver damage from alcohol than men, as they develop alcohol-related liver disease after a comparatively shorter period of heavy drinking and at lower levels of daily drinking than men.

2.1.2.2. *Extrahepatic tissues*

Excessive ethanol consumption may create several distinct types of health problems in virtually all tissues, such as gastrointestinal problems, cardiomyopathy, increased blood pressure, neurological problems, ischaemic strokes and cancer risks (Bagnardi et al. 2001, Mukamal et al. 2005, Schneider and Singer 2005, Kodavali and Townsend 2006, Puddey and Beilin 2006, Shukla and Aroor 2006, Welsch et al. 2006). Blood pressure is elevated by regular consumption of alcohol, but increased blood pressure is not related to the type of alcoholic beverage (Kodavali and Townsend 2006, Puddey and Beilin 2006). Chronic pancreatitis is also often associated with excessive alcohol consumption (Schneider and Singer 2005). Alcohol and its metabolites can alter the metabolic pathways involved in inflammatory responses and carcinogenesis in several ways, and other risk factors for cancers, such as genetic, dietary, environment and lifestyle factors, can be modulated by alcohol. This may lead to acute or chronic pancreatitis or diabetes mellitus, for instance, and finally to the development of pancreatic cancer (Welsch et al. 2006). It has also been shown that alcohol increases the risk of cancer of the upper gastrointestinal tract, stomach, colon and rectum (Yokoyama et al. 1996, Bagnardi et al. 2001, Salaspuro 2003).

Alcohol has complex effects on the nervous system, because it interacts with many different neurotransmitter systems, and also because the effects on these systems may be dose-dependent and variable between individuals. The long-term effects of heavy alcohol consumption lead to brain injury, most often seen in the cerebellum. Alcohol also acts as a central nervous system depressant (Ogilvie et al. 1998, Ryabinin et al. 2002). The molecular mechanisms and neuronal interactions implicated in the effects of alcohol are still unclear, however (Haddad 2004).

2.1.2.3. Suggested beneficial effects

It has been proposed that small amounts of ethanol may have a positive health effect, mainly in the form of a reduced risk of coronary heart disease (CHD), although this protection is restricted to middle-aged and older individuals in populations with a high risk of CHD, and protection may even be confined to certain subgroups within these populations. It has not been shown, however, that alcohol is necessary for health. Studies of lifetime abstainers have indicated that they have a longer life expectancy than alcohol consumers (Ellison 2002, Mukamal et al. 2005, Tolstrup et al. 2006).

There is also evidence of other possible benefits of moderate alcohol consumption in conditions such as peripheral vascular diseases (Pai et al. 2006), gallstones, cognitive functioning and dementia. Moderate alcohol consumption may reduce the risk of diabetes, perhaps through the effects of alcohol on insulin sensitivity (Rimm et al. 1995, Conigrave and Rimm 2003, Ting and Lutt 2006). It is difficult, however, to determine the limit of alcohol consumption which is still healthy (Ellison 2002). The same amount of alcohol may not have the same outcome in different persons, for many reasons, including genetic differences, personality, behavioural features and environment (Heath et al. 1994, Prescott et al. 1997, National Institute on Alcohol Abuse and Alcoholism 2000a, Enoch 2003, Fromme et al. 2004, McBride et al. 2004). For some people moderate drinking may also have some beneficial effects on mental health, causing an effect of reduced anxiety. Improvements in mood and social adjustment may be achieved by light to moderate drinking, and non-problem drinkers may obtain help in coping with stress or other negative emotional states (Ashley et al. 2000).

2.1.3. Gender-dependent consequences of ethanol intake

Men drink more than women, although the gap has narrowed at least to some extent in recent decades in relation to both the amount of alcohol consumed and the resulting health problems. There has been a particularly marked increase in the number of heavily drinking women in Europe, where the role of women in society has changed, possibly bringing about changes in attitudes and behaviour (WHO 2004).

Alcohol influences men and women largely in the same ways, but there are many cases where women may run a greater risk, and there are several problems specific to women. Drinking increases the probability of medical and psychosocial problems, and there is compelling evidence that women suffer from these at lower levels of consumption than men (Bradley et al. 1998a). Light to moderate alcohol consumption may even have a protective effect against CHD in middle-aged and elderly men (Maraldi et al. 2006), and there is some evidence that light drinking also has a protec-

tive effect in women. It seems, however, that alcohol intake may be the primary determinant of the inverse association between drinking and the risk of CHD among women, whereas among men, the frequency of drinking rather than alcohol intake as such may be more important (Mukamal et al. 2005, Tolstrup et al. 2006).

The optimum amount of alcohol that should be consumed is lower for women than for men since the higher ratio of fat to water in the body means that women are less able to dilute the alcohol and therefore have higher concentrations of alcohol in their blood after drinking the same amount. Women have a lower risk of heart diseases, but their susceptibility to liver damage is higher (Becker et al. 1996, Doll 1997). The acute effects of alcohol last longer in women because their alcohol metabolism in the stomach is slower, while the rate of ethanol oxidation in the liver, creating acetaldehyde and other toxic products, is higher (Baraona et al. 2001). Women are generally more vulnerable to alcohol-related diseases than men, and the alcohol-attributable reduction in life expectancy is more drastic in women (John and Hanke 2002).

Alcohol consumption among women is associated with an increased risk of breast cancer by about 10% for each additional daily drink (Longnecker 1994, Jain et al. 2000, Gonzalez 2006, Key et al. 2006). Alcohol drinking during pregnancy can result in damage to the unborn child (White 2001, Eustace et al. 2003), the features of fetal alcohol syndrome (FAS) being craniofacial abnormalities, growth deficiency and deficits in intellectual functioning (National Institute on Alcohol Abuse and Alcoholism 2000b, Fiorentino et al. 2006). The actual association between ethanol intake and the risk of damage to the fetus has remained unclear, although it is thought that damage may start to increase significantly after the level of 1–2 daily drinks, or binge drinking of 5 or more drinks on any single occasion (Streissguth et al. 1990, Forrest et al. 1991, Olsen 1994, Larroque et al. 1995, Larroque and Kaminski 1998, Stoler et al. 1998, Eustace et al. 2003). Children whose mothers drink moderate amounts of alcohol during pregnancy, may have mild symptoms of FAS representing a condition known as fetal alcohol effects (FAE), which may have a several-fold higher incidence than FAS (National Institute on Alcohol Abuse and Alcoholism 2000b).

2.2. Assessment of ethanol consumption

Since it is difficult to detect alcohol abuse reliably in clinical work, as typical clinical manifestations may not be apparent until the patient has reached an advanced stage of dependence, a wide range of structural questionnaires, include both self-reported measures and clinical interview strategies, have been developed to screen for alcohol problems. There are also a wide variety of laboratory markers, as described later in section 2.3. below. Such questionnaires have been considered uncertain because of prevailing attitudes towards drinking, but they are useful for obtaining information on the pattern of drinking, which is important, since many of the adverse consequences of ethanol intake are related to heavy drinking on occasions, whereas others are found in individuals with continuous consumption (Niemelä 2002, Aalto et al. 2003, Room et al. 2005).

Patients' drinking habits are most typically estimated through self-reports. Some studies support the hypothesis that self-reports are valid for evaluating the outcome of abstinence treatment (Mundle et al. 1999), whereas others have reported that alcoholic patients tend to underestimate

their consumption when monitored (Orrego et al. 1979, Peachey and Kapur 1986, Fuller et al. 1988). The reliability of self-reporting decreases if the patient has memory problems, difficulties in understanding questions, problems in performing mental calculations to quantify drinking or a tendency for intentional dissimulation (Allen et al. 1992, Laatikainen et al. 2002). One study on college students has tested whether they were able to estimate their alcohol consumption correctly in terms of standard drinks (White et al. 2003), and another has assessed the factors which affect interviews (Vinson et al. 2003). It appears that students tend to overestimate the size of a standard drink (beer: 25%; mixed drinks: 80%; shots: 26%) (White et al. 2003). The latter study further indicated that interviews can be conducted either in person or by telephone, because this does not have any influence on the outcome.

The Alcohol Use Disorders Identification Test (AUDIT) (Saunders et al. 1993, Reinert and Allen 2002, Aalto and Seppä 2005) is a specific questionnaire developed to screen early phase alcohol abuse. It was primarily developed for adults, but nowadays it is also used among students. Because of its good sensitivity and specificity, both over 80% (Aertgeerts et al. 2001, Gomez et al. 2006), it has been recommended as a first-line method for screening. Its length has been considered a possible problem for a busy practice, however, and a more accurate measure for assessing binge drinking could be achieved by asking directly for the largest number of drinks consumed in a single session (Matano et al. 2003). The timeline follow-back (TLFB) procedure is widely used to estimate patients' patterns in the most common styles of daily drinking, weekend or holiday drinking or drinking on special occasions (Allen et al. 1992). This method differs from traditional self-reports in that it relies on specific recollection rather than asking the patient to estimate his or her average drinking over a given time. Other useful questionnaires are CAGE (Cut down, Annoyed, Guilty, Eye-opener: acronym) (Buchsbaum et al. 1991, Bradley et al. 1998b) and the Michigan Alcoholism Screening Test (MAST) (Storgaard et al. 1994).

2.3. Biomarkers of ethanol consumption

Several laboratory markers of different kinds have been developed for the biomonitoring of alcohol abuse. Such markers are important in helping clinicians to raise the issue of excessive drinking as the underlying cause of health problems, and they are also needed in the follow-up of patients, who are willing to reduce their ethanol intake. The list of commonly used laboratory markers includes blood ethanol, gamma-glutamyl transferase (GGT), carbohydrate-deficient transferrin (CDT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and mean corpuscular volume (MCV) of erythrocytes.

2.3.1. Ethanol concentration in blood

Ethanol measurements can be used to indicate recent intoxication, and these can also be helpful in determining long-term alcohol consumption when combined with clinical observations (Savola et al. 2004). Alcoholism can be inferred when the concentration of alcohol in the blood or breath exceeds 150 mg/l (1.5‰) without any obvious evidence of intoxication or 300 mg/l (3‰) at any time (National Council on Alcoholism 1972). These criteria may also help to distinguish between acute

and chronic alcoholism. The short half-life of ethanol, which is also influenced by drinking practices, nevertheless limits its usefulness as a marker to measure only very recent intake (Rosman and Lieber 1992, Niemelä 2002).

2.3.2. *Gamma-glutamyl transferase (GGT)*

Gamma-glutamyl transferase is an enzyme derived from the liver, the changes in the activities of which have been used for several decades to monitor excessive alcohol consumption. GGT has in fact been the most commonly used laboratory marker of drinking. Serum GGT concentration may also be elevated for other reasons, however, such as the use of certain drugs or the presence of certain diseases, including biliary tract disease, severe heart and kidney diseases, trauma and hyperthyroidism (Cushman 1992, Allen et al. 2000), increasing age (Daepfen et al. 1998, Sillanaukee et al. 1998, Conigrave et al. 2002, Stromme et al. 2004, Lee et al. 2004b) or obesity (Daepfen et al. 1998, Peters and Cook 2002, Conway and Rene 2004, Lam and Mobarhan 2004, Colicchio et al. 2005, Lawlor et al. 2005).

GGT is mainly found in liver cells and is involved in the transport of amino acids and peptides into cells. Its usefulness as an alcohol marker is based on the pharmacological effects of ethanol on the liver, so that it may show different characteristics in patients without liver disease from those observed in patients with liver disease (Nalpas et al. 1997). A more detailed account of the characteristics of the GGT enzyme and its clinical use is given in section 2.6.

2.3.3. *Carbohydrate-deficient transferrin (CDT)*

Research over the past three decades has shown that the desialyated isoforms of transferrin in biological fluids increase in amount as a result of alcohol consumption, and although the data on the accuracy of carbohydrate-deficient transferrin (CDT) as an alcohol abuse marker have remained conflicting, it has been increasingly used for this purpose, showing a sensitivity varying from 20% to 90% (Stibler 1991, Nyström et al. 1992, Allen et al. 1994, Bean et al. 1997, Salaspuro 1999, Scouller et al. 2000, Arndt 2001, Helander et al. 2001, Sillanaukee et al. 2001, Conigrave et al. 2002). Relatively poor sensitivity has usually been found among women, however (Anton and Moak 1994, Löf et al. 1994, Reif et al. 2001). CDT has been considered especially useful as a variable for detecting changes in alcohol intake in chronic alcohol abusers (Burke et al. 1998, Whitfield et al. 1998, Hock et al. 2005), but not for screening alcohol consumption at the population level. A combination of CDT with GGT (GGT-CDT) has recently been shown to offer better sensitivity than CDT or GGT measurements alone for detecting alcohol use disorders (Anttila et al. 2004, Hietala et al. 2006).

2.3.4. *Serum aminotransferases*

Although the serum aminotransferase enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are more directly related to liver status than to ethanol consumption per se, they are still commonly used as laboratory markers of excessive alcohol consumption. ALT is an

enzyme normally present in liver cells and is released into the bloodstream when the liver is damaged (O'Connor et al. 1997, Clark 2006, Ioannou et al. 2006). Some medications can also raise ALT levels, however. AST is an enzyme found in red blood cells, liver and heart cells and in muscle tissue, and it is also found in other organs such as the pancreas and kidneys (Rej 1989, Panteghini 1990).

When ALT and AST are used and interpreted together they may give more specific information on the alcoholic aetiology of liver disease (Niemelä 2002, Majhi et al. 2006). An AST to ALT ratio of over 2 has a positive predictive value for alcoholic liver damage about 80%, since for patients with non-alcoholic liver disease the ratio normally is below one (Rosman and Lieber 1992, Rosman and Lieber 1994).

2.3.5. Mean corpuscular volume (MCV)

Mean corpuscular volume (MCV, red blood cell size) is known to increase as a result of ethanol consumption (Baral et al. 2005, Koivisto et al. 2006) and can also be used to measure injury to the cells that manufacture red blood cells. As an alcohol marker, MCV may be more sensitive in women than in men (Morgan et al. 1981, Seppä and Sillanaukee 1994). Some studies even suggest that it is superior to all the other markers when assessing female patients (Mundle et al. 2000). The use of MCV for monitoring heavy drinkers is limited by its long normalization time (2 to 4 months), and its specificity as an alcohol marker is also limited in patients with vitamin B12 or folic acid deficiency, liver diseases, several haematological diseases, hypothyroidism or reticulocytosis (Niemelä 2002).

2.3.6. Serum lipid profiles

Excessive alcohol consumption is one of the most common causes of hypertriglyceridaemia, which is often associated with alcoholic fatty liver. Even a moderate consumption of alcohol will lead to significantly increased triglyceride levels. High HDL (high density lipoprotein cholesterol) concentrations are more often associated with alcohol consumption than are elevated triglyceride levels and these may alert clinicians to investigate the patient's recent alcohol consumption (Szegeedi et al. 2000). As the metabolism of alcohol in the liver inhibits the oxidation of fatty acids, alcohol leads to increased triglyceride synthesis. The use of HDL as an alcohol marker is nevertheless limited by the high variability of serum HDL in the normal population and the complexity of the net effects of alcohol on HDL metabolism (Rosman and Lieber 1992). Other indices affecting the serum HDL concentration are age, sex, smoking, exercise, oestrogens, severe liver disease and certain drugs (Cushman 1992).

2.4. Obesity and associated health problems

2.4.1. Prevalence

The prevalence of obesity and overweight has increased rapidly worldwide (Halsted 1999, Conway and Rene 2004). In many European countries the prevalence of such problems has tripled since the 1980s, and over 1 billion adults globally currently suffer from overweight and of these at least 300 million are obese. The increasing incidence of child obesity is a special object of concern, and has been linked to environmental and behavioural changes such as economic development, modernization and urbanization (WHO 2000). About 20% of employed people in Finland are obese and over 40% are classified as overweight (Sosiaali- ja terveystieteiden ministeriö 2006). Obesity is a matter of concern in both developed and developing countries.

Childhood obesity is already epidemic in the developed countries and is on the increase elsewhere. In Thailand, for example, the prevalence of obesity in children between 5 and 12 years of age rose from 12% to 16% between years 1991–1993. Nowadays about 22 million children under the age of five years are estimated to be overweight worldwide, and the US Surgeon General has estimated that the number of overweight children has doubled and the number of overweight adolescents trebled in the U.S. since 1980. The prevalence of obese children aged from 6 to 11 years in the U.S. has more than doubled since the 1960s, while obesity in young people aged 12–17 years increased from 5% to 13% in boys and from 5% to 9% in girls between 1966–1970 and 1988–1991 (WHO 2000).

While the reasons for the increasing prevalence of obesity have not been established, it appears that when incomes rise and populations become more urban, diets come to include higher proportions of fats, saturated fats and sugars. At the same time physically demanding work is decreasing worldwide and people are physically less active because of the increasing use of automated transport, technology in the home and more passive leisure pursuits (WHO 2000).

2.4.2. Obesity as a health problem

Obesity is considered a chronic disease in its own right (Greenway and Smith 2000). Furthermore, it is a major contributor to the global burden of chronic disease and disability and the increased risk of premature death, all of which reduce the overall quality of life. Although it is a complex disease of multifaceted aetiology, with its own disabling capacities, pathophysiologies and co-morbidities, it also has a huge impact on other diseases.

Obesity is associated with an increased incidence of type 2 diabetes, cardiovascular disease, hypertension, stroke, osteoarthritis, gallbladder disease and various cancers (Must et al. 1999, Conway and Rene 2004, Rohrer et al. 2005). The prevalence of type 2 diabetes and osteoarthritis increases among both overweight and obese persons, and the prevalence of gallbladder disease increases with increasing body weight but is not similar for overweight men and women. It has been shown that men under 55 years have gallbladder disease with increasing weight, but the influence of weight among older men is not so obvious. For women, gallbladder disease increases with weight in both

age groups (Must et al. 1999). In addition, excess weight can also cause high blood pressure and high blood cholesterol levels and impair mental health. High blood pressure is the most common health condition related to overweight and obesity in both men and women. High blood cholesterol levels are found for both sexes, but they do not increase with weight, although high cholesterol levels are more probable in overweight persons than in those of normal weight (Must et al. 1999, Ford et al. 2001). Immunological protection mechanisms are also altered in obesity. Obese individuals have a high risk of sepsis, respiratory tract infections, bacteraemia, and delayed wound healing (Baik et al. 2000, Martí et al. 2001, Samartín and Chandra 2001, Lamas et al. 2002). The non-fatal but debilitating health problems associated with obesity include respiratory difficulties, chronic musculoskeletal problems, skin problems and infertility.

Obesity has already been estimated to be responsible for 7% of health care costs in the U.S. (Colditz 1999) and 10–13% of deaths in different parts of the Europe. The costs are almost certainly much greater, because not all diseases caused by obesity are classified into these categories (WHO 2000).

2.4.3. Obesity and alcohol

The adipose tissue is a source of fatty acids that are supplied to the liver, whereas the liver produces triglycerides and releases them into bloodstream, the adipose tissue acting as a storage depot for them. The effects of ethanol on the adipose tissue include increased expression of leptin and cytokines and reduced concentrations of adiponectin (Xu et al. 2003), and alcohol consumption may be a risk factor for developing overweight and obesity. Interestingly, overweight may enhance all stages of alcohol-induced liver diseases, including fatty liver and fibrosis (Raynard et al. 2002, Diehl 2004). The prevalence of hepatic steatosis increases with both heavy drinking and obesity, and thus the risk of non-alcoholic disease may be even higher for obese persons than the risk of alcoholic disease is for heavy drinkers (Bellentani et al. 2000). Naveau and her co-workers (1997) have also proposed that excess weight may be an independent risk factor for the development of alcoholic cirrhosis and acute alcoholic hepatitis, and have also suggested that overweight may have effects on ethanol ingestion. It has also been postulated that obesity-induced non-alcoholic fatty liver disease (NAFLD) may constitute a first hit to the liver, which may then become susceptible to "second hits" such as alcohol (Jones 2005).

2.5. Assessment of obesity using the body mass index (BMI)

Calculation of the body mass index (BMI), defined as weight in kilograms divided by the square of height in metres (kg/m^2), is a commonly used method of estimating the prevalence of overweight and obesity. Typical BMI categories for adults (over 18 years) are summarized in Table 1.

Table 1. Categories of body mass index (BMI) and the risk of associated diseases.

Categories	BMI (kg/m ²)	Risk of associated diseases
underweight	<18.5	
normal weight	18.5–25	
overweight	25–30	increased
obese	≥30	high
morbid obese	≥40	very high

The average BMI value in Europe is over 25 kg/m², while mean BMI levels in Africa and Asia are 22–23 kg/m². Figures of 25–27 kg/m² are prevalent across North America and in some Latin American, North African and Pacific Island countries. In Finland mean BMI levels in 2002 were 27 kg/m² for employed men and 26 kg/m² for women (Laatikainen et al. 2003). Increased BMI is especially common amongst middle-aged and elderly people, who have the greatest risk of health complications. In countries which are undergoing nutrition transition, overnutrition may often co-exist with undernutrition (WHO 2000).

2.6. Gamma-glutamyl transferase (GGT)

2.6.1. Enzyme properties

Gamma-glutamyl transferase (GGT) is a heterodimeric protein which has two subunits, each consisting of a single polypeptide chain (Tate and Meister 1976). It is located on the cytoplasmic membrane of many cells in the body and its active centre faces outwards. The luminal surfaces of cells have either a secretory or an absorptive function, and they are particularly rich in GGT. The basolateral surfaces of renal tubular cells also contain GGT (Goldberg 1980). GGT belongs to a group of peptidases which catalyze the transfer of amino acids from one peptide to another and thus act as amino acid transferases. It reacts with peptides or peptide-like compounds containing a terminal glutamate residue joined to the remainder of the compound through the terminal carboxyl. It also plays an important role in the metabolism of inflammatory mediators and in metabolizing carcinogens and toxic xenobiotics (Lieberman et al. 1995).

The GGT enzyme cleaves significant amounts of glutathione (GSH) and its conjugates. Gamma-glutamyl cysteinyl glycine is formed intracellularly and translocated to the extracellular luminal side of the cell membrane, where it is cleaved by GGT into cysteinyl glycine and a gamma-glutamyl residue. This sequence of events can be referred to as the gamma-glutamyl cycle (Figure 2) (Speisky et al. 1990, Lieberman et al. 1995, Ristoff and Larsson 2003).

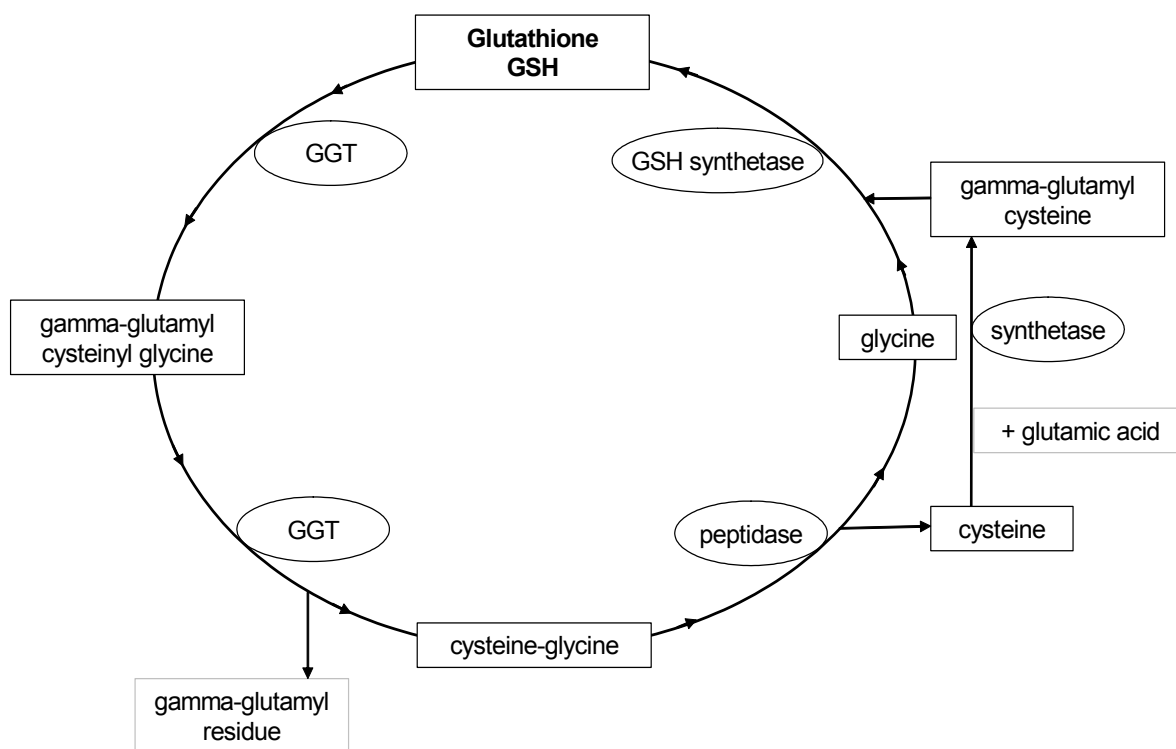


Figure 2. Schematic representation of the gamma-glutamyl cycle.

Serum GGT originates from the liver and is mostly bound by lipoproteins, particularly HDL but also the larger low-density lipoproteins. A smaller water-soluble fraction of 84 kDa resembles the GGT released by proteases from the liver cell membrane (Wenham et al. 1985). GGT which is bound by HDL predominates in non-icteric liver diseases, while the GGT bound by low density lipoproteins is elevated in cholestasis and the water-soluble form in a variety of liver diseases. GGT is removed from the plasma mainly via the liver, but a small fraction is degraded by the kidneys. It is then eliminated from the liver with the bile and from the kidneys with the urine (Welbourne and Dass 1982, Whitfield 2001). In the fetal liver GGT is distributed evenly in the lobules, both dissolved in the hepatocytes and bound to the cell membrane, while in the adult liver it is located mainly at the periphery of the lobules. The major fraction in a healthy liver is membrane-bound, particularly to the canalicular and sinusoidal portions of the hepatocyte membrane and the epithelial membrane of larger bile ducts, whereas only small activities are detectable in the hepatocytes (Köttgen et al. 1976).

2.6.2. Clinical use of GGT

Since chronic alcohol consumption readily leads to an increase in serum GGT activity, it has commonly been used as a marker of alcohol abuse (Zein and Discombe 1970, Reyes and Miller 1980, Anton et al. 2002, Niemelä 2002, Conigrave et al. 2003). It has been suggested that heavy drinkers require two to four weeks of abstinence for their GGT levels to return to the normal range (Anton et al. 2002, Hietala et al. 2006). This makes serum GGT useful for monitoring abstinence in recover-

ing alcoholics. Diagnostic sensitivities varying from 15 to 85% have reported for GGT (Bagrel et al. 1979, Chick et al. 1981, Papoz et al. 1981, Persson et al. 1990, Anttila et al. 2004). GGT may already be elevated before the appearance of liver damage, and this finding may need to be interpreted together with serum aminotransferase levels.

In addition to the detection of alcoholism and alcoholic liver damage and the monitoring of alcohol abstinence, GGT can be used to differentiate between cholestasis and cell-membrane damage. The evaluation criteria are its behaviour in relation to the aminotransferases (in patients with jaundice this ratio is a measure of the extent of cholestasis in relation to cell membrane damage), the level of its activity and its relation to other cholestasis enzymes. Recent data have further suggested that serum GGT can also be regarded as a general marker of oxidative stress (Lim et al. 2004, Lee et al. 2004a). In addition to chronic alcohol consumption, serum GGT concentration may be elevated as a result of liver disease, smoking, obesity, trauma and certain drugs, and also diseases such as biliary tract disease, severe heart and kidney diseases, hyperthyroidism and hypertension (Cushman 1992, Allen et al. 2000, Whitfield 2001, Niemelä 2002, Nakanishi et al. 2003, Stranges et al. 2005). Increasing age may also influence GGT activity (Daepfen et al. 1998).

Cholestasis, chronic alcohol consumption and therapeutic dosages of various drugs can all induce the synthesis of GGT in the liver (Goldberg 1980, Niemelä 2002, Sotil and Jensen 2004), as a result of which the membrane-bound form of the enzyme spreads, mainly periportally, from the canalicular membranes to other parts of the cell membrane facing Disse's space between the endothelium and the hepatocytes. The GGT activity in serum increases after induction of the enzyme, and the possibility of parenchymal damage should always be considered if the levels have increased to more than twice the upper reference limit or if the increase is coupled with increases in the other liver enzymes. The increases in GGT found in bile excretion disorders can be due to increased formation of GGT by the hepatocytes. This seems to be the main reason for the increased GGT levels in hepatoma cells, in cells compressed by a liver tumor and in regeneration areas in a cirrhotic liver. Solubilization of GGT by the bile acids can also increase its activity (Lieberman et al. 1995). Increased GGT activity is nearly always a sign of liver damage if another liver-specific enzyme such as ALT is also pathological, whereas isolated elevation of GGT can be divided clinically into the following categories: drug-related induction of GGT synthesis, fatty liver, subclinical biliary obstruction, space-occupying processes in the liver, chronic liver congestion in heart disease and an alcohol aetiology.

2.6.3. GGT assays

GGT catalyzes the transfer of the glutamyl residue from L- γ -glutamyl-3-carboxy-4-nitroanilide to glycylglycine with liberation of 4-nitroanilide. Under standardized conditions the increase in the concentration of this compound, measured as the change in absorbance at 410 nm, is proportional to the GGT activity in the reaction mixture. A method for its determination has been proposed by the International Federation of Clinical Chemistry (IFCC) (Shaw et al. 1983) which, although optimized for 30°C, can also be used at 37°C (Schumann et al. 2002). The method is initiated by adding a substrate to the reaction mixture under the conditions described in Table 2. In essence, after the reaction solution (dissolved glycylglycine) and sample are equilibrated to 37.0°C, mixed thoroughly

and incubated for 180 s. At the end of the incubation time the temperature will have reached 37.0°C. The start reagent solution (dissolved L-γ-glutamyl-3-carboxy-4-nitroanilide) is added, and solution is mixed. After waiting 60 s, the absorbance is measured at least 6 times at intervals of 180 s. The change in absorbance at a wavelength of 410 nm is proportional to the GGT activity.

Table 2. Conditions recommended by the IFCC for the measurement of GGT (Schumann et al. 2002).

Parameter	
Temperature	37.0°C ± 0.1°C
Wave length	410 nm ± 1 nm
Band width	≤2 nm
Light path	10.00 mm ± 0.01 mm
Incubation time	180 s
Delay time	60 s
Measurement interval	180 s
Readings (measurement points)	≥6

2.7. Reference intervals

2.7.1. Concept, definition

The concept of reference values was originally introduced by Gräsbeck and Saris (1969). According to the IFCC, the current concept of reference values can be considered at various levels, including terms such as *reference individual*, *reference value*, *observed value*, *reference population*, *reference sample group*, *reference distribution*, *reference limit* and *reference interval*.

A *reference individual* is selected for comparison on certain defined criteria. It is important to know the state of health of such individuals, as they are expected to represent healthy persons. A *reference value* is measured in the reference individual. An *observed value* is a measured value produced with aim of making a medical decision, and can be compared with reference values. The *reference population* consists of all the reference individuals and a *reference sample group* is an adequate number of reference individuals representing the reference population. A *reference distribution* is the statistical distribution of reference values, whereas a *reference limit* is derived from the reference distribution and used for descriptive purposes. The *reference interval* is the interval between and including two reference limits (Solberg 1987).

Reference intervals, which give a range of acceptable values for healthy individuals, serve as a basis for laboratory testing and are useful for determining whether the patient is healthy or not. If

the result is not within the reference interval, the value is flagged and the patient should be examined further (Horn and Pesce 2003, Gräsbeck 2004).

Reference intervals are usually calculated from data extracted from the samples available to the particular laboratory. When estimating the endpoints of the reference interval the sample size should be enough to yield a reasonable degree of precision, otherwise the number of incorrect decisions may increase, leading to higher costs and unnecessary examinations, and may even endanger the safety of patients (Gräsbeck 1990). When abnormal values are used to determine reference intervals, there may also be cases referred to as outliers, representing recording errors or laboratory errors. The most common method of determining whether there are outlier effects is to estimate the Gaussian or normal distribution of the data. Since the reference interval will be widened by the presence of outliers, which may permit more false negative results, outlier detection should be performed prior to calculating the reference interval (Horn and Pesce 2003).

2.7.2. Methods for determining reference intervals

A reference interval can be determined from a healthy population, and non-parametric estimates for the 95% reference interval can be obtained by leaving out 2.5% of the data at each end of the distribution. Most laboratories use this method, so that their results exhibit similar precision, employ identical units, are related and correlate well with each other. Methodological bias can be eliminated using the reference interval width. Horn and Pesce (2003) proposed that the logarithmic ratio of reference interval widths is a good estimate for the variability between groups.

Normal calculations estimate reference intervals (between 2.5% and 97.5%) using a mean and two standard deviations (SD) of the data for a Gaussian population. If the distribution is not Gaussian, the data can be adjusted using a logarithmic or square-root transformation (Gräsbeck 2004). The mean \pm 2SD is then calculated from the transformed data and reference intervals are obtained by transforming these back to the original units. This method can be used only if the transformation to a normal population is successful. The mean \pm 2SD is not valid if the distribution of the data or transformed data is not normal, which can be caused by the influence of outliers on the sample mean and standard deviation (Horn and Pesce 2003).

2.7.2.1. Non-parametric methods

A non-parametric method makes no assumption about the distribution of the data. The data items are simply ranked by ordering them from lowest to highest and the 2.5 and 97.5 percentiles in the sample are used to form the 95% reference interval. Non-parametric methods can be used to determine the reference interval for a population of at least 120 individuals. If the reference interval is calculated from a small number of samples, the non-parametric statistic may use extreme values, even though these represent outliers (Whitley and Ball 2002, Horn and Pesce 2003).

2.7.2.2. Truncation methods

Truncation methods assume (ad hoc) the percentage of outliers in the data set and eliminate the smallest and largest 10%, for example, giving these zero weight and using the middle 80% to derive a 95% reference interval. If the 2.5 and 97.5 percentiles of the central 80% of the data are used when there are no outliers, the resulting interval will cover only 0.95×0.80 , or 76%, of the reference population (Horn and Pesce 2003).

2.7.2.3. Robust methods

Robust statistical methods have been developed to deal with the problem of deviations of statistical models from ideal conditions and to estimate the centre of a symmetric distribution. In such methods the further values are from the centre of the sample the more they are downweighted to resist outlier influence. Robust methods are more tolerant of outliers if the data come from a Gaussian population. These methods do not require as large a sample size as the non-parametric calculation method and the data do not have to follow a Gaussian distribution. They are also efficient in cases where the distribution is heavy-tailed (Horn et al. 1998, Horn et al. 1999, Maronna et al. 2006).

2.7.3. Assessment of biological influences on reference intervals

It may often be difficult to determine a reference interval for a healthy population, as gender, age, race, exceptional exercise, diet, obesity, or non-healthy status may have an effect on the outcome and should be considered carefully (Gräsbeck 1990, Horn and Pesce 2003, Rustad et al. 2004, Stromme et al. 2004). Similarly, it is not clear whether there should be separate reference intervals for different demographic groups such as males and females. The standard mathematical test for deriving separate reference intervals is that introduced by Harris and Boyd (1990). If the reference interval has to be divided into subgroups, partitioning testing can be used (Lahti 2004), and if the sample size is large, it is possible to use partitioning of the reference individuals into subgroups according to demographic descriptors such as gender, age or ethnic background (Horn and Pesce 2002, Lahti et al. 2002).

It is easier to consider a few groups of reference intervals rather than reference intervals divided into several subgroups. There are certain advantages in combining different groups in order to derive a single reference interval, e.g. it allows a laboratory to obtain a large body of data more easily, but the combining of two subgroups may increase the probability of misclassification, because they may differ in the distributions of their analytical values. Harris and Boyd (1990) recommended that two groups can be combined if their means and/or deviations do not exceed appropriate predetermined thresholds.

2.7.4. The Nordic Reference Interval Project (NORIP)

A Nordic Reference Interval Project (NORIP) has been initiated recently to obtain data on apparently healthy individuals from 102 Nordic clinical chemical laboratories (Rustad et al. 2004). A

total of 3036 persons participated in this trial, the aim of which was to establish standard reference intervals for the 25 most common clinical biochemical analyses. A reference material consisting of a frozen pool of liquid serum with values traceable to reference methods was measured in each laboratory by routine methods, the bias in each routine method being eliminated by use of common sets of reference material measured in each of the participating laboratories. Only results obtained with measuring systems compatible with that of the International Federation of Clinical Chemistry (IFCC) were selected for determination of the reference intervals (Rustad et al. 2004).

3. Aims of the present research

Excessive alcohol consumption and obesity are both creating rapidly growing health problems in our society. Serum GGT is a liver-derived enzyme which has previously been suggested as being sensitive to the effects of alcohol abuse and overweight. Although serum GGT measurements are widely used as markers of heavy drinking, it has remained unclear how the amount of drinking, increased body weight and advancing age influence GGT activity and the definition of its normal ranges.

The aims of the present work were as follows:

1. To explore the effect of moderate drinking on GGT values and its reference intervals.
2. To explore the relationship between ethanol consumption, obesity and GGT values in a large number of apparently healthy individuals.
3. To study the effects of increasing body mass index in the reference population on the sensitivity and specificity of GGT for detecting heavy drinkers.
4. To compare the effect of age on GGT activities among individuals with different levels of ethanol intake.

4. Materials and methods

4.1. Patients and control subjects

The series reported on in paper I included 103 heavy drinkers (90 men, 13 women) and 92 moderate drinkers or abstainers (54 men, 38 women), who underwent detailed personal interviews on the amounts and patterns of their alcohol consumption using a timeline follow-back technique. The heavy drinkers were patients who had been admitted for detoxification and had a history of continuous ethanol consumption or binge drinking, their mean consumption being in the range 40–540 grams of ethanol per day during the 4 weeks prior to sampling. In addition, there were 30 abstainers and 62 moderate drinkers with a mean daily ethanol consumption ranging from 1 to 40 grams per day over the month prior to sampling.

The data for paper II applied to 2490 apparently healthy individuals involved in the NORIP survey for establishing enzyme reference intervals for use in the Nordic countries. They were classified as either abstainers (n=1160: 479 men, 681 women) or moderate drinkers (n=1330: 705 men, 625 women), and also into BMI categories as follows: BMI<19 (underweight), BMI 19–25 (normal weight), BMI 25–30 (overweight) and BMI>30 (obese). The moderate drinkers consumed less than 40 grams of ethanol/day and the maximum amount consumed during the twenty-four hours prior to sampling had been two standard drinks. The survey excluded individuals who had clinical or laboratory evidence of current or recent illnesses or infections, were pregnant, had donated blood during the past five months or had taken any prescription drugs during the preceding week. Smoking had not been allowed for one hour prior to sampling.

The serum samples for paper III were collected from 208 heavy drinkers (174 men, 34 women) who had been admitted for detoxification. Personal interviews showed these patients to have a history of continuous ethanol consumption or binge drinking, their mean ethanol consumption over the past month being 128 grams per day. They were all devoid of any clinical or laboratory evidence of apparent liver disease, however. None of them had taken any prescription drugs known to induce GGT activities, such as barbiturates or anticoagulants. In order to assess the effectiveness of BMI in correctly classifying this population of heavy drinkers, the data were compared with the GGT data on either moderate drinkers (n=1147: 590 men, 557 women), whose consumption had been below 40 grams per day, or abstainers (n=449: 168 men, 281 women). This reference population was also classified according to BMI as follows: BMI<20 (underweight), BMI 20–25 (normal weight), BMI 25–30 (overweight), BMI>30 (obese).

The population of heavy drinkers in paper IV was essentially the same as in paper III, and the reference group the same as in paper II. The patients were categorized here according to age, as follows: 18–30 years: 16 heavy drinkers, 328 moderate drinkers and 281 abstainers; 30–50 years:

111 heavy drinkers, 449 moderate drinkers and 301 abstainers; 50–70 years: 81 heavy drinkers, 391 moderate drinkers and 314 abstainers; age>70 years: 162 moderate drinkers and 264 abstainers.

All the serum samples were stored at -70°C until analysis. The procedures were approved by the institutional review boards and informed consent was obtained from the participants. The research was carried out according to the provisions of the Declaration of Helsinki.

4.2. Measurements of GGT activities

Serum GGT in the heavy drinkers was measured by standard clinical chemical methods in an accredited laboratory (SFS-EN 17025, ISO/IEC) at Seinäjoki Central Hospital, Finland. The GGT measurements for the reference individuals representing the NORIP material were carried out in several Nordic laboratories with measuring systems compatible with that of the International Federation of Clinical Chemistry (IFCC).

4.3. Statistical methods

Values are expressed as mean \pm SD. Comparisons between two groups were made with the Mann-Whitney test and comparisons between three or more groups with the one-way analysis of variance (ANOVA) together with Bonferroni's method for multiple comparisons. If a Gaussian distribution or equal variances for the values could not be achieved even after transformations, the comparisons were carried out using the Kruskal-Wallis test. Dixon's test was used for detecting outliers, as recently recommended by Horn and Pesce (2003).

Correlations were calculated with Pearson product-moment correlation coefficients for continuous non-skewed parameters or with the Spearman rank correlation, as required, and the differences between correlations were assessed with the z-test for correlation coefficients. Reference intervals for GGT were calculated as mean \pm 2SD after logarithmic transformation of the data.

The analyses were carried out using GraphPad Prism, version 3.03 (GraphPad Software, San Diego, CA, USA) statistical software. The SPSS 12.0 for Windows statistical software (Chicago, Illinois, USA) was used for the two and three-factor analyses following natural logarithmic transformation of the GGT values to obtain symmetrical distributions. A p-value<0.05 was considered statistically significant.

5. Results

5.1. Effect of various levels of drinking on GGT and its reference intervals

Serum GGT concentrations (mean \pm SD) in the groups of heavy drinkers ingesting either 40–80 grams (68 ± 54 U/l) or over 80 grams (167 ± 254 U/l) of ethanol per day significantly exceeded the levels of both the abstainers ($p < 0.001$) and the moderate drinkers ($p < 0.001$). Interestingly, the GGT values for the moderate drinkers, with a daily consumption of 1–40 grams (28 ± 23 U/l), also exceeded those for the group of abstainers (24 ± 17 U/l) ($p < 0.001$). The correlation between ethanol consumption and GGT values, as calculated for the individuals interviewed using the timeline follow-back method, was also significant ($r = 0.35$, $p < 0.001$).

The estimated GGT reference intervals as calculated for this population of moderate drinkers were 66 U/l (men) and 40 U/l (women), whereas the values for the abstainers were 45 U/l and 35 U/l, respectively. The upper normal limits were found to be 43% higher when the individuals with moderate drinking were contrasted with the population of abstainers.

The choice of reference population had a significant effect on the estimated diagnostic sensitivity of GGT as a marker of excessive ethanol consumption. When the heavy drinkers were contrasted with the abstainers, 69% of the former were correctly classified, whereas if the reference interval and definition of normal values had been based on moderate drinkers, the sensitivity would have remained at 56%. The corresponding percentages in separate analyses by sex were 68% and 54% for men and 77% and 69% for women, respectively.

5.2. Interactions between moderate drinking, sex, obesity and serum GGT activities

GGT activities in a large population of moderate drinkers were also found to be significantly higher than those in abstainers (Figure 3), the values for the male moderate drinkers in particular (34 ± 24 U/l) differing significantly from those for the abstainers (28 ± 19 U/l) ($p < 0.001$). The difference between the female moderate drinkers (23 ± 20 U/l) and abstainers (21 ± 14 U/l) was not significant.

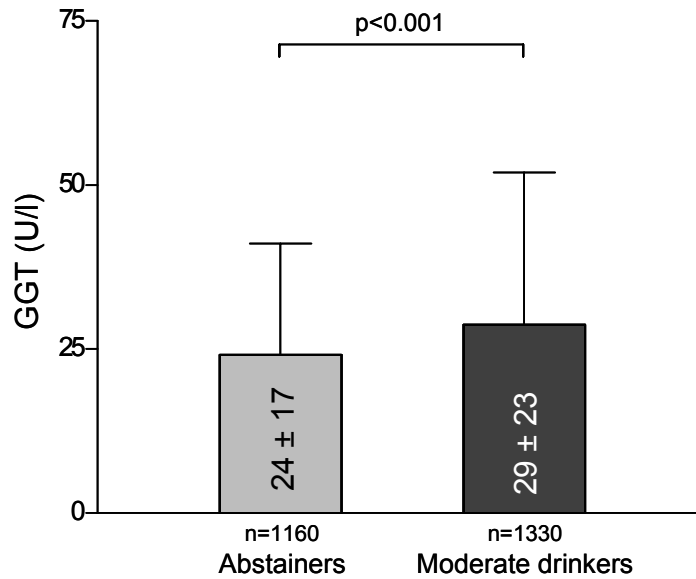


Figure 3. GGT values (mean \pm SD) in abstainers and moderate drinkers.

There was a significant main effect of gender ($p < 0.0001$), drinking habit ($p < 0.01$), and BMI ($p < 0.001$) on serum GGT activities, and the GGT activities were also found to increase significantly with increasing BMI. The highest values were found to occur in those with moderate drinking combined with overweight (men: 36 ± 22 U/l; women: 26 ± 25 U/l) or obesity (men: 54 ± 33 U/l; women: 33 ± 23 U/l). Although the magnitude of the GGT changes in men appeared to exceed that observed in women, the three-factor interaction between the effects of all these factors (sex \times BMI \times drinking status) on GGT as the dependent variable was not found to be significant, nor did any of the two-factor interactions (sex \times drinking status, sex \times BMI, or drinking status \times BMI) reach the level of significance, either.

The correlations between GGT and BMI were significant for both men, $r = 0.24$ ($p < 0.0001$) and women, $r = 0.15$ ($p < 0.0001$), and the correlation observed for men was also significantly different from that for women ($p < 0.05$). Computation of the partial correlation coefficients while controlling for the effects of sex, drinking status, or both, also yielded significant correlations ($r = 0.25$, $p < 0.0001$; $r = 0.31$, $p < 0.0001$; $r = 0.26$, $p < 0.0001$, respectively).

5.3. Obesity and the clinical use of serum GGT as a marker of heavy drinking

Serum GGT activities (177 ± 309 U/l) measured in 208 heavy drinkers significantly exceeded the levels obtained for both moderate drinkers (28 ± 23 U/l) ($p < 0.001$) and abstainers (23 ± 16 U/l) ($p < 0.001$), the latter two groups also being significantly different from each other ($p < 0.001$). When the reference population was further classified in terms of BMI, the mean GGT activities in those with obesity were found to be significantly higher than those in the corresponding groups with nor-

mal weight (Table 3). There was also a high degree of overlapping between the values obtained for the heavy drinkers and the obese individuals (Table 3).

Table 3. Serum GGT activities (mean \pm SD) in the population of abstainers and moderate drinkers, classified by BMI categories, as compared with the activities found in heavy drinkers.

	n	GGT U/l (mean \pm SD)	
		Men	Women
Heavy drinkers	208	190 \pm 330 (n=174)	111 \pm 151 (n=34)
Abstainers			
BMI<20 (underweight)	41	25 \pm 18 (n=6)**	17 \pm 7 (n=35)***
BMI 20–25 (normal weight)	258	24 \pm 13 (n=94)***	20 \pm 13 (n=164)***
BMI 25–30 (overweight)	124	27 \pm 14 (n=61)***	22 \pm 14 (n=63)***
BMI>30 (obese)	26	31 \pm 15 (n=7)	30 \pm 14 (n=19) ^a
Moderate drinkers			
BMI<20 (underweight)	86	21 \pm 16 (n=17)*** ^b	18 \pm 8 (n=69)*** ^c
BMI 20–25 (normal weight)	696	29 \pm 20 (n=313)***	22 \pm 19 (n=383)***
BMI 25–30 (overweight)	335	37 \pm 22 (n=238)*** ^a	25 \pm 16 (n=97)*** ^c
BMI>30 (obese)	30	54 \pm 36 (n=22) ^a	35 \pm 24 (n=8) ^c

*** p<0.001, ** p<0.01, * p<0.05, when compared with the values for the heavy drinkers.

^{a, b, c} significantly different from the corresponding group with normal weight, ^a p<0.001, ^b p<0.01, ^c p<0.05.

Calculation of the relative differences in mean GGT activities in the reference population of moderate drinkers and abstainers indicated 125% increases in male obese moderate drinkers and 75% increases in female obese moderate drinkers relative to the corresponding values in normal weight abstainers. Consequently, the upper normal limits were found to increase by up to 189% (men) and 122% (women) with BMI, the most striking elevations being noted in values based on moderate drinkers with obesity.

The effect of BMI on the diagnostic sensitivity of GGT in correctly classifying heavy drinkers was found to vary between 32% and 67% in men and between 29% and 56% in women. Thus a markedly higher percentage of alcohol abusers was detected when contrasted with normal or underweight individuals.

The effect of overweight or obesity on GGT specificity with cut-offs defined based on the data from normal weight abstainers was calculated for all the subgroups. The rates of false positive values were 27% in men and 25% in women among the obese moderate drinkers, whereas the corresponding values among the obese abstainers were 14% and 11%, respectively. False positive rates between 5% and 14% were found to occur in overweight individuals (BMI 25–30).

5.4. Age-related changes in serum GGT activity

The GGT activities calculated for the heavy drinkers, moderate drinkers and abstainers grouped according to age are summarized in Table 4. The mean values in the age groups 18–30, 30–50 and 50–70 of heavy drinkers were 2.7, 8.0 and 6.9-fold higher than those for the respective abstainers, and the GGT activities among the moderate drinkers also exceeded those for the abstainers in all the age groups. While there was a continuous increase in GGT activities with advancing age among the consumers of alcohol, the abstainers were found to have decreased GGT activities when aged above 70.

Table 4. GGT activities (U/l) in the heavy drinkers, moderate drinkers and abstainers, by age.

Age (years)	Heavy drinkers	Moderate drinkers	Abstainers
18–30	52 ± 34	23 ± 13	19 ± 9
30–50	183 ± 341	27 ± 20	23 ± 17
50–70	194 ± 288	33 ± 26	28 ± 21
>70	–	35 ± 33	26 ± 17

–, no observations

When the reference population was divided by both age and gender, the moderate drinkers were found to have higher GGT activities than the abstainers in all the age groups among the men (18–30 years: $p < 0.001$; 30–50 years: $p < 0.05$; 50–70 years: $p < 0.05$; >70 years: $p < 0.01$), whereas among the women a statistically significant difference occurred only in those aged 18–30 years ($p < 0.05$). The youngest age group of men (18–30 years) showed significantly lower GGT activities than the other age groups within both the moderate drinkers and abstainers ($p < 0.001$), whereas in the women the GGT activities remained essentially similar over the period 18–50 years and were significantly lower than in those above 50 years of age ($p < 0.001$).

The upper normal limits for GGT activity, defined as mean \pm 2SD, in the different age groups and their relative changes as a function of moderate drinking were found to be up to 10–42% (men) and 8–27% (women) higher when the normal ranges were based on the data for moderate drinkers than in the case of abstainers. The sensitivities of GGT for correctly classifying heavy drinkers in age-matched comparisons were found to vary between 49 and 74% (men) and between 41 and 56% (women), the highest sensitivities being found in the youngest age group. By contrast, in comparisons without age matching, the youngest age group of heavy drinkers was detected with poor sensitivity, only 41% (men) and 50% (women). The moderate drinkers had a 5–9% incidence of elevated values without age matching, whereas in the age-matched comparisons the incidence of elevated GGT activities was found to be up to 21% in men and 10% in women.

When the analysis was controlled for the effect of BMI, no significant interaction was observed. The correlation between GGT activities and age between 18 and 70 years was positive and significant for all the subgroups, as follows: heavy drinkers ($r = 0.21$, $p < 0.01$), moderate drinkers ($r = 0.22$, $p < 0.0001$) and abstainers ($r = 0.24$, $p < 0.0001$). In those older than 70, however, the correlation be-

tween GGT and age became negative both in the moderate drinkers ($r = -0.18$, $p < 0.01$) and in the abstainers ($r = -0.16$, $p < 0.001$).

6. Discussion

6.1. Moderate drinking and GGT reference intervals

The present study indicates that even a moderate ethanol consumption can influence serum GGT concentrations, and that this may significantly affect the interpretation and establishment of common reference intervals for GGT measurements in health care. The data support the view that in order to improve the diagnostic potential of GGT for detecting excessive ethanol consumption and early-phase liver induction, the reference intervals should be based on healthy individuals who abstain from ethanol. Since no gold standard for a bona fide social drinker currently exists, the concepts of moderate drinking and social drinking should be defined more accurately and the proportions of abstainers and moderate drinkers considered separately when selecting reference individuals.

According to the present findings, the estimated upper normal limits for GGT measurements could be 40% higher if the data applying to moderate drinkers were used as the basis for the reference population instead of abstainers. In accordance with this view, the NORIP survey for the Nordic countries showed markedly increased GGT reference values when a mixed population of moderate drinkers and abstainers was used (Stromme et al. 2004). These new reference limits have also been widely adopted for routine use in most Nordic laboratories. It appears, however, that the diagnostic utility of GGT measurements as a marker of excessive ethanol consumption would improve if reference intervals were to be based on the data for abstainers alone. The present data indicate that 13% of alcoholics would escape detection if moderate drinkers were used as the reference population. Thus, there may be a need for revising the reference range in a downward direction (Kornhuber et al. 1989). It maybe argued, however, that setting a lower limit could worsen the specificity of GGT assays and lead to a high number of false positive values. In the present material, about 11% of the moderate drinkers would have shown increased values under such circumstances. There may nevertheless be some individuals who are close to the upper limit of social drinking, and since the data are based on self-reporting, occult alcohol abuse in these subjects can not be ruled out at this time.

6.2. Interactions between moderate drinking, sex, obesity and serum GGT

Only a few studies have previously been available on the combined effects of moderate ethanol consumption and obesity on biochemical parameters reflecting health status (Daeppen et al. 1998, Stranges et al. 2004, Rohrer et al. 2005). The present data derived from a large population of abstainers and moderate drinkers indicate distinct additive effects of moderate alcohol consumption

and overweight on serum GGT activities. These findings are consistent with the view that obesity together with ethanol may lead to an increased metabolic burden and risk of forthcoming liver problems. The main function of hepatic GGT is to metabolize extracellular reduced glutathione, and it has been proposed that the enzyme also has a role in the generation of reactive oxygen species (ROS) (Lee et al. 2004a), so that it could be considered a marker of oxidative stress. Enhanced oxidant stress has recently been linked with obesity-associated metabolic syndrome and the generation of fatty liver in connection with this condition (Browning and Horton 2004, Furukawa et al. 2004, Lim et al. 2004, Lee et al. 2004a). In addition to alcohol and adiposity, GGT activity may also increase via similar mechanisms in conditions such as diabetes (Nakanishi et al. 2003). Interestingly, coffee consumption may reduce serum GGT activities, and regular consumption of coffee seems to be protective against liver disease, particularly that attributable to alcohol (Klatsky et al. 2006).

High-fat diets together with ethanol have been shown in experimental animals to enhance oxidative stress and aggravate alcoholic liver injury (Niemelä 2001), while studies on both experimental animals and humans have indicated that ethanol-inducible cytochrome enzyme, CYP2E1, may be induced by obesity alone, due to free fatty acids serving as substrates (Weltman et al. 1996, Weltman et al. 1998, Leclercq et al. 2000, Niemelä et al. 2000, Lieber 2004). This may create oxidative stress and also render the liver more susceptible to oxidative stress and associated cellular injury (Albano 2006). Thus the generation of fatty changes in alcoholic livers and in non-alcoholic fatty liver disease would appear to share common pathogenic features with respect to both cytochrome enzyme induction and the generation of ROS (Weltman et al. 1996, Weltman et al. 1998, Niemelä et al. 2000, Diehl 2004, Lieber 2004, Carmiel-Haggai et al. 2005).

Obesity has been shown to potentiate the severity of alcohol-induced liver damage in humans, although the mechanisms of the interaction between adiposity and ethanol remain unknown (Halsted 1999, Neuschwander-Tetri and Caldwell 2003, Diehl 2004). It is possible that enhanced serum GGT activities could represent a sign of generalized metabolic induction and activation of the body's defence mechanisms in the face of the metabolic burden (Speisky et al. 1990, Nakanishi et al. 2000b, Kevil et al. 2004). GGT is an ectoenzyme that can be shed from the cell membrane without cell death. In the light of previous findings reported by Speisky and co-workers (1990), GGT on the sinusoidal side of hepatocytes could provide a mechanism whereby the efflux of glutathione from the periportal hepatocytes is broken down into readily oxidizable cysteine, which replenishes this glutathione precursor for pericentral hepatocytes. The pericentral area is known to be the primary site of alcohol-induced CYP2E1 induction and the generation of ROS in the liver. Thus, increases in GGT may provide a compensatory hepatic mechanism to protect the pericentral hepatocytes. Since ROS also increase in fatty liver and obesity, similar mechanisms may apply to both the alcohol-induced and obesity-induced increases in GGT and their potentiation.

There also seem to be distinct differences in serum GGT responses as a result of moderate alcohol consumption and overweight between men and women. While there may be sex-dependent differences in the metabolic consequences of ethanol intake and fat accumulation, the present three-factor analysis of the interaction between sex, BMI and drinking status with GGT as the dependent variable did not show these to be significant. Sex steroids have previously been shown to play a role in cytochrome enzyme expression and the regulation of oxidant stress status in the liver (Niemelä et al. 1999, Wolbold et al. 2003), and it is possible that GGT values in women may increase at lower levels of alcohol consumption and be associated with women's increased vulnerability to the toxic

effects of alcohol (Anton et al. 1998). It should also be noted that although no information was available here on the type of alcoholic beverage consumed by the subjects, recent work among individuals consuming over 5 drinks per day has indicated a lower risk of alcoholic cirrhosis in wine drinkers (Becker et al. 2002). Future studies appear warranted to address the possibility that moderate drinking could vary in terms of serum GGT responses depending on whether it consisted primarily of wine, beer, or liquor.

6.3. Obesity and the clinical use of serum GGT as a marker of heavy drinking

Where earlier studies have established the clinical value of serum GGT as a sensitive clinical marker of alcohol abuse (Bagrel et al. 1979, Chick et al. 1981, Papoz et al. 1981, Bernadt et al. 1982, Mundle et al. 2000, Whitfield 2001, Anttila et al. 2004), the present data show that a moderate ethanol consumption can elevate serum GGT activity, particularly when it co-occurs with obesity. While the correlation observed between self-reported alcohol consumption and GGT has typically been of the order of 0.3–0.4 (Bagrel et al. 1979, Chick et al. 1981, Papoz et al. 1981, Bernadt et al. 1982, Anton and Moak 1994, Anttila et al. 2004) it is shown here that a somewhat similar correlation ($r=0.2-0.3$) may be achieved between GGT and BMI.

Thus overweight and moderate ethanol consumption should both be considered in the interpretation of GGT activity as a clinical marker of heavy drinking. The effects of increasing body weight may also complicate the establishment of common reference intervals (Nakanishi et al. 2003, Diehl 2004, Lam and Mobarhan 2004, Stranges et al. 2004, van Hoek 2004, Rohrer et al. 2005, Seitz 2006). The data support the view that in order to improve the diagnostic potential of GGT measurements, reference intervals should be based on healthy individuals who abstain from ethanol and are not significantly overweight, and a further improvement might be achieved by using specific BMI-based reference intervals. This approach could also open up new perspectives for clinical applications of GGT measurements and a possibility for monitoring smaller changes in GGT activity in a clinically realistic manner. In the light of previous observations showing that the diagnostic sensitivity of GGT may be lower for women than for men (Anton and Moak 1994, Yersin et al. 1995, Mundle et al. 2000), the present data further suggest that these findings may in part be explained by the definition of the reference intervals.

The upper normal GGT limits appear to become over 20% higher when based on data that include normal weight moderate drinkers, and perhaps more than 100% higher when based on moderate drinkers who are overweight. On the other hand, approximately 20–30% of obese subjects and 10% of those who are overweight show higher GGT activities than those seen in normal weight abstainers, and especially among moderate drinkers. Future studies appear to be warranted in order to determine whether the follow-up of such individuals would reveal increased risks of morbidity related to conditions such as metabolic syndrome and cardiovascular complications, as suggested by recent findings indicating a predictive value for serum GGT activities (Lee et al. 2006).

6.4. Age-related changes in serum GGT

The present data also indicate distinct age-related effects on GGT activities, which could create population variability in assessments of excessive ethanol consumption and liver induction. GGT activities were found to increase with age until after 70 years, when a decrease was noted in men who did not consume alcohol. While previous studies have also noted increased GGT activity with age (Daepfen et al. 1998, Sillanaukee et al. 1998, Conigrave et al. 2002, Stromme et al. 2004, Lee et al. 2004b), the decrease specifically among male abstainers in old age has not previously been acknowledged. The correlation between age and GGT values appears in fact to become a negative one in those over 70 who abstain from ethanol. The incidence of heavy alcohol consumption is also known to decline naturally with increasing age (Karlmanngla et al. 2006), as reflected in the present material, which contained no heavy drinkers above the age of 70 among over 200 consecutive admissions for detoxification.

Moderate drinking seems to increase GGT activity to higher levels than in abstainers in all age categories of men and in women under 30 years, suggesting that these age groups may also show more sensitive liver induction. There may also be both age and gender-dependent susceptibility to ethanol-induced hepatotoxicity. It has been proposed that young adults may be more resistant to the damaging effects of alcohol (Chan et al. 1989). On the other hand, women are usually more vulnerable to the development of alcoholic liver disease (Schenker 1997, Ashley et al. 2000). Thus increasing age together with ethanol consumption and/or obesity could create an interaction triad which synergistically increases the metabolic burden and the risk of liver injury. It is also of interest to note that coffee consumption could inhibit the inducing effects of ageing on serum GGT activities (Nakanishi et al. 2000a).

The sensitivity of GGT as a clinical marker of alcohol abuse has previously been shown to be especially disappointing in studies dealing with young adults (<30 years) (Chan et al. 1989, Nyström et al. 1993, Sillanaukee et al. 1998, Conigrave et al. 2002), even when they show alcohol dependence (Bisson and Milford-Ward 1994). It is therefore significant that the present reference populations with or without age matching provided markedly different views of GGT sensitivity.

7. Conclusions

The data indicate that ethanol consumption, even in rather moderate amounts, in conjunction with obesity, may lead to increased activity of serum gamma-glutamyl transferase, GGT.

Since both the mean alcohol consumption and the prevalence of obesity in our society are increasing, this may lead to increasing mean GGT activities at the population level, which may come to be accepted as normal in routine health care. A critical re-evaluation of GGT reference intervals therefore appears to be necessary.

Excessive ethanol intake and obesity may together create cumulative pathogenic effects, which should be considered in studies on the pathogenesis of ethanol-induced oxidative stress and liver injury.

Increasing age also has a significant effect on GGT activity, suggesting that specific BMI and age-categorized reference intervals should also be considered. Analogously, it might also be possible to formulate age and body mass index based guidelines for safer alcohol consumption.

It remains to be established whether GGT measurements could also be extended to wider clinical applications for monitor the metabolic burden and oxidant stress status *in vivo*.

Acknowledgements

This research was carried out at the Department of Clinical Chemistry, Seinäjoki Central Hospital, during the years 2004-2007. I extend my sincere gratitude to my supervisor, Professor Onni Niemelä, M.D., Ph.D, for giving me the possibility to work in his research group. His guidance, enthusiasm and wide knowledge of science have encouraged me during these years. It has been a privilege to have such a dedicated and supportive supervisor.

I would like to acknowledge Professor Olavi Pelkonen, Head of the Department of Pharmacology and Toxicology, University of Oulu, and Docent Timo Koivula, Tampere University Hospital, for their expert review and valuable comments on my work. I also would like to thank Malcolm Hicks for carefully revising English language of this thesis.

I owe special thanks to my collaborators and co-authors Johanna Hietala and Heidi Koivisto for all their help and support during these years and to Taana Sandman for expert secretarial assistance and for many enjoyable moments shared over these years. It has been a great pleasure to work with you all. I would also like to thank Petra Anttila, Ph.D., for collaboration, and Mr Risto Bloigu for his crucial help in the field of statistics. I am deeply indebted to my colleagues Aira Sorto, Markku Latva-Nevala, Kristina Eichholz and Katja Viitala for sharing the world of science and the life of a specialising hospital chemist. I also express my deepest gratitude to the whole staff of the Central Hospital Laboratory for the support and friendship that they have given me throughout this work.

I also thank Dr. Matti Rekiaro and Docent Hannu Puolijoki for their interest and support and Jaakko Pihlajamäki, Head of the Hospital District, also deserves special thanks for his positive attitude towards the project. I am grateful to Professor Pål Rustad, Først Medical Laboratory, Oslo, Norway, for providing data on GGT measurements from the Nordic NORIP Survey for establishing enzyme reference intervals.

Finally, I owe my deepest gratitude to my parents, Raija and Matti, for their never-ending support and encouragement, and to my brothers, Ville and Santtu, for their support and lifetime competition in all fields of life and sports. Their support has enabled me to carry out this work and made this the most rewarding and enjoyable phase of my life.

Part of this research was supported financially by the Finnish Foundation for Alcohol Studies and the Foundation for the Promotion of Laboratory Medicine.

Seinäjoki, January 2007

Katri Puukka

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SERUM GAMMA-GLUTAMYL TRANSFERASE IN ALCOHOLICS, MODERATE DRINKERS AND ABSTAINERS: EFFECT ON GT REFERENCE INTERVALS AT POPULATION LEVEL

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(Received 2 June 2005; first review notified 15 July 2005; accepted in final revised form 8 August 2005)

Abstract — **Aims:** To clarify in the association between amount of ethanol consumption and serum gamma-glutamyl transferase (GT) levels. **Methods:** GT values were measured from 195 individuals with a wide variety of well-documented ethanol consumption assessed by detailed personal interviews using a time-line follow-back technique. These included 103 heavy drinkers (90 men, 13 women) and 92 healthy volunteers (54 men, 38 women) who were either abstainers ($n = 30$) or moderate drinkers ($n = 62$). For comparisons, data were collected from GT measurements for establishing GT reference intervals from 2485 healthy volunteers including 1156 abstainers and 1329 moderate drinkers. **Results:** GT values in the individuals whose mean ethanol consumption exceeded 40 g of ethanol per day were significantly higher than those in the moderate drinkers with a mean consumption of 1–40 g/day ($P < 0.001$) or in abstainers ($P < 0.001$). The GT values in the group of moderate drinkers also exceeded those of the abstainers ($P < 0.001$). The upper normal GT limits obtained from the data from abstainers were markedly lower (men 45 U/l, women 35 U/l) than those obtained from the population of moderate drinkers (men 66 U/l, women 40 U/l). **Conclusions:** Serum GT concentrations may respond to relatively low levels of ethanol consumption, which should be considered when defining GT reference intervals. The continuous increase in alcohol consumption at population level may lead to increased GT cut-off limits and hamper the detection of alcohol problems and liver affection in their early phase.

INTRODUCTION

Gamma-glutamyl transferase (GT) is a commonly used laboratory parameter for detecting excessive alcohol consumption (Zein and Discombe, 1970; Reyes and Miller, 1980). Although several studies have reported a positive correlation between the amount of alcohol consumed and serum GT levels, the reported sensitivities of this marker in previous literature have varied greatly, from 15 to 85% (Bagrel *et al.*, 1979; Chick *et al.*, 1981; Papoz *et al.*, 1981; Persson *et al.*, 1990; Leino *et al.*, 1995; Anttila *et al.*, 2004).

Over the past decades, both the total ethanol consumption per capita and associated medical disorders have continued to increase. Simultaneously, the percentage of individuals fully abstaining from ethanol has decreased. In previous studies and in routine health care, reference intervals for GT determinations have been based on values obtained from mixed populations of apparently healthy moderate drinkers and abstainers, whereas only limited attention has been paid on the exact amounts of ethanol consumption in these individuals.

In this work we explored the relationship between ethanol consumption and GT values in individuals with a wide variety of ethanol consumption. Our data indicate distinct effects of mild to moderate ethanol consumption on serum GT levels, which should be considered in the clinical use of GT measurements as a marker of ethanol abuse and liver status.

METHODS

Study protocol

Serum GT was first measured from a sample of 195 individuals (103 heavy drinkers: 90 men, mean age 42 ± 10 years; 13 women, mean age 40 ± 7 years, and 92 moderate drinkers

or abstainers: 54 men, mean age 41 ± 16 years; 38 women, mean age 44 ± 19 years) who underwent detailed personal interviews using a time-line follow-back technique (interview sample). The heavy drinkers had a history of continuous ethanol consumption or binge drinking, the mean consumption being in the range of 40–539 g/day during the period of 4 weeks prior to sampling. In addition, this interview sample included 30 abstainers and 62 moderate drinkers with a mean daily ethanol consumption between 1 and 40 g/day. Measurements of GT levels were carried out using standard clinical chemical methods in an accredited (SFS-EN 45001, ISO/IEC Guide 25) laboratory of Seinäjoki Central Hospital, Finland. For comparisons, data from a survey on 2485 apparently healthy individuals (1174 men, age 47 ± 18 years; 1311 women, age 47 ± 18 years) collected for establishing reference intervals in Nordic countries were also used as kindly provided by the project coordinator, Professor Pål Rustad, Først Medical Laboratory, Oslo, Norway. These subjects were classified as either abstainers ($n = 1156$: 471 men, age 49 ± 19 years; 685 women, age 49 ± 19 years) or moderate drinkers ($n = 1329$: 703 men, age 46 ± 17 years; 626 women, age 45 ± 16 years). In these subjects, the maximum amount of alcohol consumption during the 24 h period prior to sampling had been 24 g (two standard drinks). The survey excluded individuals who had clinical or laboratory evidence of current or recent illnesses or infections, who were pregnant, had donated blood during the past 5 months, or had used any prescription drugs during the preceding 1 week. Smoking was not allowed for 1 h prior to sampling. All GT measurements were carried out with homogeneous International Federation of Clinical Chemistry (IFCC) compatible measuring systems.

Ethical considerations

The procedure was approved by the institutional review board. Informed consent was obtained from the participants and the

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study was carried out according to the provisions of the Declaration of Helsinki.

Statistical methods

Values are expressed as mean \pm SD. Comparisons were made with Kruskal-Wallis test and Dunn's Multiple Comparison Test or Mann-Whitney test when comparing two groups. Correlations were calculated with Pearson product-moment correlation coefficients. Reference intervals were calculated after logarithmic transformation as previously described (Horn and Pesce, 2003). A *P*-value <0.05 was considered statistically significant.

RESULTS

In the total population, serum GT concentrations (mean \pm SD) in the groups of heavy drinkers drinking 40–80 g (68 ± 54 U/l) or >80 g (167 ± 254 U/l) of ethanol per day significantly exceeded the levels of both abstainers ($P < 0.001$) and moderate drinkers ($P < 0.001$) (Fig. 1). Male alcoholics had slightly higher GT values (166 ± 267 U/l) than female alcoholics (130 ± 163 U/l), although the difference between genders did not reach significance. Interestingly, GT values in the group of moderate drinkers with a daily consumption of 1–40 g (28 ± 23 U/l) also exceeded the values obtained from the

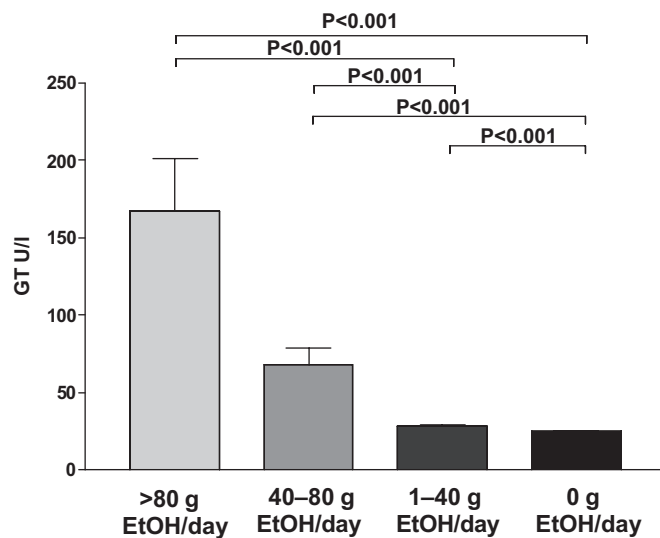


Fig. 1. GT values in individuals with different levels of ethanol consumption. The values in the groups with ethanol consumption over 80 g (167 ± 254 U/l) or 40–80 g (68 ± 54 U/l) are significantly higher than those in the abstainers (24 ± 17 U/l) or moderate drinkers consuming a mean of 1–40 g/day (28 ± 23 U/l). The difference between the group of moderate drinkers and abstainers was also significant ($P < 0.001$).

group of abstainers (24 ± 17 U/l) ($P < 0.001$). The correlation between ethanol consumption and GT values, as calculated from the individuals interviewed with the time-line follow-back method, was significant ($r = 0.35$, $P < 0.001$).

Figure 2 demonstrates the previously established changes in national GT reference intervals in Finland in comparison with the yearly changes in ethanol consumption at population level. The data on GT reference intervals, as calculated from the individuals classified according to ethanol consumption data in the detailed personal interviews, are summarized in Table 1. The upper normal limits were found to be on average 43% higher, when the individuals with moderate drinking are contrasted with the population of abstainers. The upper normal limits for men were higher than those for women and the age group ≥ 40 years had higher levels than the age group 18–39 years in both genders. However, the correlation between GT levels and age *per se* did not reach significance ($r = 0.097$).

The effect of the choice of the reference population on the estimated diagnostic sensitivity of GT as a marker of excessive ethanol consumption is demonstrated in Fig. 3. When the heavy drinkers are contrasted with abstainers, 69% of heavy drinkers become correctly classified. If the reference interval and definition of normal values would be based on moderate drinkers, the sensitivity remains at a level of 56%. In separate analyses of men and women, the corresponding percentages were 68 and 54% for men and 77 and 69% for women, showing essentially similar changes.

DISCUSSION

Alcohol abuse and alcoholism rank as one of the most serious health problems in most Western countries (Room *et al.*, 2005). Therefore, a high priority should be given to reduction in the prevalence of alcoholism through more effective diagnosis and early intervention. Objective methods for detecting excessive ethanol consumption in health care are necessary for a majority of heavy drinkers who have not self-identified as having alcohol problems.

Studies in the past have shown that a number of biochemical parameters are altered in alcoholics, of which serum GT has emerged as one of the most efficient tests (Bagrel *et al.*, 1979; Chick *et al.*, 1981; Papoz *et al.*, 1981; Bernadt *et al.*, 1982; Leino *et al.*, 1995; Anttila *et al.*, 2004). The present study indicates that even moderate amounts of ethanol consumption influence serum GT concentrations at population level and this phenomenon may significantly affect the interpretation and the establishment of common reference intervals for GT measurements in health care. The data support the view that in order to improve the diagnostic potential of laboratory markers of excessive ethanol consumption and liver status, the reference intervals of each test should be based on healthy

Table 1. GT reference intervals based on the data from the groups of individuals classified according to ethanol intake in the interview sample

	All men	Men 18–39	Men ≥ 40	All women	Women 18–39	Women ≥ 40
Moderate drinkers						
Alcohol consumption (1–40 g/day)	8–66	7–63	11–66	6–40	6–31	6–64
Moderate drinkers and abstainers						
Alcohol consumption (0–40 g/day)	9–61	8–57	12–62	7–39	7–30	8–44
Abstainers						
Alcohol consumption (0 g/day)	10–45	9–37	11–52	8–35	7–30	9–37

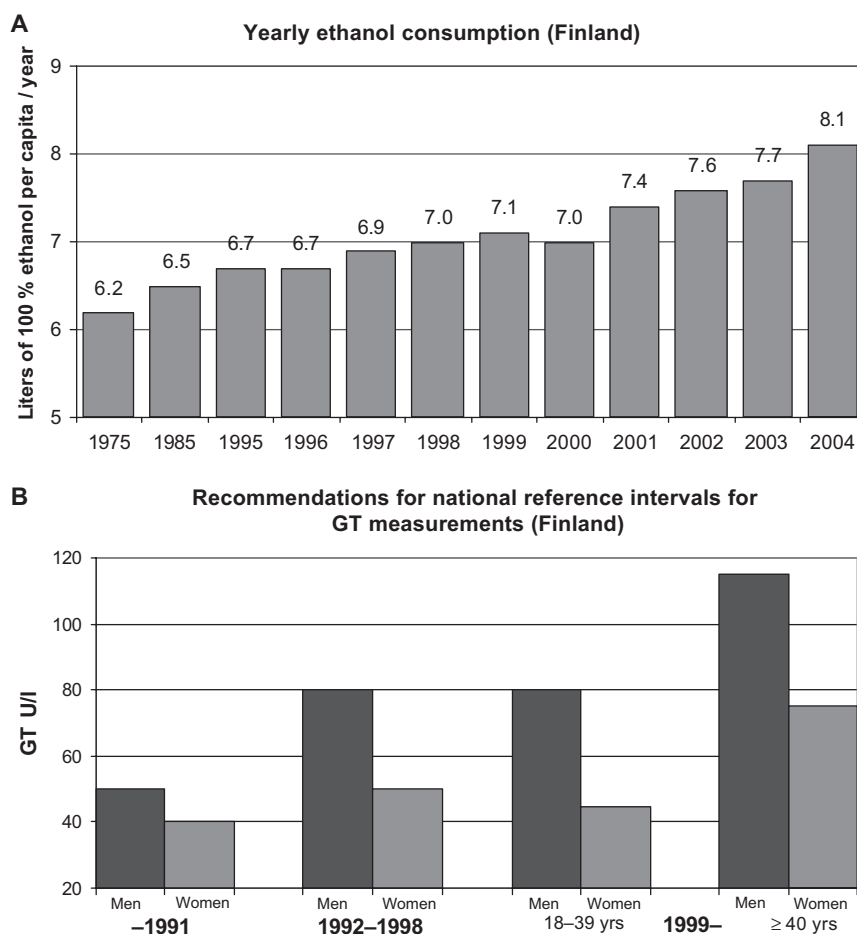


Fig. 2. (A) Mean ethanol consumption in Finland over the years 1975–2004. (B) The changes in recommended reference intervals for GT measurements based on surveys on apparently healthy individuals.

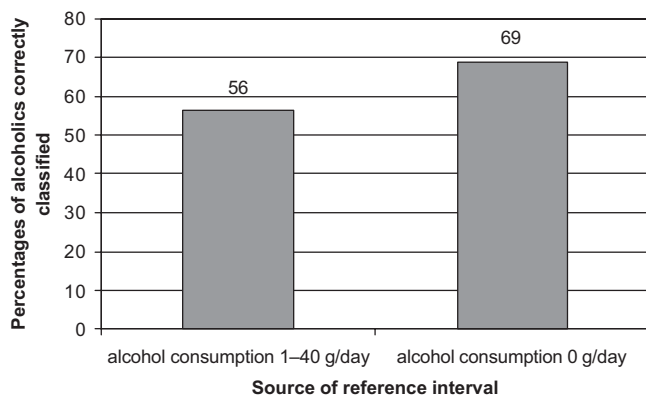


Fig. 3. Effect of the source of reference intervals on the sensitivity of detecting problem drinking with GT measurements. When reference intervals are based on values from abstainers, 69% of the heavy drinkers are detected. When the reference population consists of moderate drinkers, only 56% of the heavy drinkers become correctly identified.

individuals who abstain from ethanol. Since a gold standard for a bona fide social drinker currently does not exist, the concepts of moderate drinking and social drinking should also be defined more accurately and the proportions of abstainers and

moderate drinkers considered separately when selecting reference individuals in future studies.

The present data indicate that the estimated upper normal limits for GT measurements would be ~40% higher if the data based on moderate drinkers would be used as the basis of the reference population instead of abstainers. In accordance with this view, a recent NORIP survey from the Nordic countries showed markedly increased GT reference values (Stromme *et al.*, 2004). The diagnostic sensitivity of GT measurements as a marker of excessive ethanol consumption would obviously improve if reference intervals would be based on the data from abstainers. This work indicates that 13% of alcoholics would escape detection if moderate drinkers are used as the reference population, instead of abstainers. Thus, there may be a need for revising the reference range downwards. It may, however, be argued that setting a lower limit could worsen the specificity of GT assays and lead to a high number of false positive values. According to this work, ~11% of the moderate drinkers would have shown increased values. However, there may be individuals who are in the upper range of the limits of social drinking. Since the data are based on self-reports, we cannot rule out occult alcohol abuse in these subjects. It should be noted, however, that future studies are clearly warranted to explore the independent effect of various possible sources of unspecificity on GT

values, such as obesity or diabetes in individuals reporting either moderate drinking or no drinking. Our preliminary analyses on moderate drinkers with different degrees of obesity have indicated potentiation of GT activities in individuals with significant obesity (data not shown). The associations between GT, moderate drinking, and obesity have previously been examined by Kornhuber *et al.* (1989) who also concluded that the definition of GT normal values may need to be readdressed. The correlation ($r = 0.35$) between alcohol consumption *per se* and GT values in this study is consistent with previous observations (Bagrel *et al.*, 1979; Chick *et al.*, 1981; Papoz *et al.*, 1981; Leino *et al.*, 1995; Anttila *et al.*, 2004). The correlation was, however, essentially similar in women ($r = 0.36$) and men ($r = 0.32$), though some earlier studies have reported higher correlations in populations consisting of men only (Papoz *et al.*, 1981; Anton and Moak, 1994; Sillanaukee *et al.*, 1998). The diagnostic sensitivity of GT has usually been shown to be lower for women than for men (Anton and Moak, 1994; Yersin *et al.*, 1995; Mundle *et al.*, 2000). Based on the present data, these findings may in part be explained by the definition of reference intervals. Furthermore, GT values in women may increase at lower levels of alcohol consumption as a result of women's increased vulnerability to the toxic effects of alcohol (Anton *et al.*, 1998).

The advent of carbohydrate-deficient transferrin (CDT) testing has recently imposed a new challenge to the use of GT measurements because CDT has shown higher specificities than GT in several trials. Although CDT has become an increasingly important tool for assessing excessive ethanol consumption, it appears that CDT and GT frequently increase in different individuals (Anton *et al.*, 2002; Anttila *et al.*, 2003; Neumann and Spies, 2003). Some studies have concluded that GT is more efficient in identifying female alcoholics than CDT (Anton and Moak, 1994, Anton *et al.*, 2002). Therefore, it seems at this time that CDT alone does not cover all the needs for an alcohol marker in routine clinical practice, and other markers, especially GT are needed as well.

Taken together, the present data indicate that the changes in drinking behaviour at population level may parallel increases in recommended GT cut-offs, which may subsequently lead to problems in recognizing excessive alcohol consumption in its early phase. Therefore, a critical re-evaluation of reference intervals even in the use of the well-established biochemical markers of alcohol consumption may be necessary in order to improve the assessment and treatment of patients with early-stage alcohol problems.

Acknowledgements — The help of professor Pål Rustad, Først Medical Laboratory, Oslo, Norway, for providing data on GT measurements in the Nordic NORIP Survey for establishing reference intervals is gratefully acknowledged. The studies were supported in part by a grant from the Finnish Foundation for Alcohol Studies.

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See corresponding CME exam on page 1448.

Additive effects of moderate drinking and obesity on serum γ -glutamyl transferase activity^{1–3}

Katri Puukka, Johanna Hietala, Heidi Koivisto, Petra Anttila, Risto Bloigu, and Onni Niemelä

ABSTRACT

Background: γ -Glutamyl transferase (GGT) is a widely used index of liver induction and a marker of alcohol overconsumption. Obesity has also been suggested to elevate serum GGT activities.

Objective: The aim was to examine the links between moderate ethanol consumption, obesity, and GGT activities.

Design: GGT values were recorded from 2490 persons (1184 men and 1306 women) who reported either no alcohol use (abstainers) or 1–40 g ethanol consumption per day (moderate drinkers). The study population was additionally classified according to body mass index (BMI; in kg/m²) as follows: <19 (underweight), \geq 19 and <25 (normal weight), \geq 25 and <30 (overweight), and \geq 30 (obese).

Results: Significant main effects of sex ($P < 0.0001$), drinking habits ($P < 0.01$), and BMI ($P < 0.001$) on serum GGT activities were observed. The values were higher in the men than in the women and higher in those with higher BMIs. The highest activities were found to occur in persons with moderate drinking combined with overweight or obesity. A significant positive correlation between GGT and BMI ($P < 0.0001$) was observed, which was stronger for the men ($r = 0.24$) than for the women ($r = 0.15$, $P < 0.05$ for the difference between correlations).

Conclusion: The data indicate that serum GGT activities may respond to moderate drinking and overweight in an additive manner; this should be considered in the clinical use of GGT measurements and when defining normal GGT values in health care. *Am J Clin Nutr* 2006;83:1351–4.

KEY WORDS Ethanol, obesity, lipid peroxidation

INTRODUCTION

γ -Glutamyl transferase (GGT) is commonly used as a biological marker for excessive alcohol consumption and as an index of liver induction due to ethanol, drug, or xenobiotic use (1–4). Several studies have reported a positive correlation between the amount of ethanol ingestion and serum GGT activities (5–8). Recent data have additionally suggested that serum GGT can also be considered a marker of oxidative stress (9).

Obesity is an increasingly common nutritional disorder in many industrialized countries affecting a major proportion of the adult population (10, 11). Over the past decades, although there has been an epidemic increase in the incidence of obesity, there has also been a simultaneous increase in the total per capita ethanol consumption and associated medical disorders (12). A growing body of evidence has indicated that obesity may also

lead to increased serum GGT activities (13–17). However, the associations between body mass index (BMI), sex, alcohol consumption, and the interpretation of serum GGT activities in this context have remained unclear. The present study set out to explore the relations between ethanol consumption, overweight, and GGT activities in a large number of apparently healthy abstainers and moderate drinkers who were classified according to sex and BMI.

SUBJECTS AND METHODS

Study protocol

Data from 2490 apparently healthy persons (1184 men, mean (\pm SD) age: 47 ± 18 y; 1306 women, aged 46 ± 18 y) collected for a survey for establishing enzyme reference intervals in Nordic countries were used. The study population was classified into either alcohol abstainers ($n = 1160$; 479 men aged 48 ± 19 y, and 681 women aged 48 ± 19 y) or moderate drinkers ($n = 1330$; 705 men aged 46 ± 17 y, and 625 women aged 45 ± 16 y) and further classified according to BMI (in kg/m²), as summarized in **Table 1**. In moderate drinkers, the amount of alcohol consumed varied between 1 and 40 g ethanol/d, and the maximum amount of alcohol consumed during the 24 h before sampling was 2 standard drinks. The survey excluded persons who had clinical or laboratory evidence of current or recent illnesses or infections, were pregnant, had donated blood in the past 5 mo, or had used any prescription drugs during the preceding week. Smoking was not allowed for 1 h before sampling. All GGT measurements were carried out with International Federation of Clinical Chemistry-compatible measuring systems.

The study was approved by the Seinäjoki Central Hospital institutional review board. Informed consent was obtained from the participants, and the study was carried out according to the provisions of the Declaration of Helsinki.

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² Supported in part by a grant from the Finnish Foundation by Alcohol Studies (to ON).

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Received September 9, 2005.

Accepted for publication January 12, 2006.

TABLE 1

Main characteristics of the study population¹

	Age	Underweight (BMI <19 kg/m ²)	Normal weight (BMI ≥19 and <25 kg/m ²)	Overweight (BMI ≥25 and <30 kg/m ²)	Obese (BMI ≥30 kg/m ²)
	y	n (%)	n (%)	n (%)	n (%)
Abstainers (n = 1160)					
Men (n = 479)	48 ± 19 ²	7 (1)	270 (56)	182 (38)	20 (4)
Women (n = 681)	48 ± 19	27 (4)	442 (65)	175 (26)	37 (5)
Moderate drinkers (n = 1330)					
Men (n = 705)	46 ± 17	6 (1)	386 (55)	285 (40)	28 (4)
Women (n = 625)	45 ± 16	35 (6)	460 (74)	121 (19)	9 (1)

¹ Two-factor interactions and the test of between-subjects effects (sex × BMI × drinking status) were not significant, $P > 0.05$.² $\bar{x} \pm SD$ (all such values).

Statistical methods

Values are expressed as means \pm SDs. Comparisons were made with Kruskal-Wallis test and Dunn's multiple comparison test or with Mann-Whitney test in a comparison of 2 groups. Correlations were calculated with Pearson product-moment correlation coefficients and the differences between correlations with z tests for correlation coefficients. The analyses were carried out with GRAPHPAD PRISM version 3.03 (GraphPad Software, San Diego, CA) statistical software. SPSS version 12.0 for Windows statistical software (SPSS Inc, Chicago, IL) was used for the 2- and 3-factor analyses after natural logarithmic transformation of GGT values to obtain symmetrical distributions. A P value < 0.05 was considered statistically significant.

RESULTS

Significant main effects of sex ($P < 0.0001$), drinking habits ($P < 0.01$), and BMI ($P < 0.001$) on serum GGT activities were observed. Data for the groups of abstainers and moderate drinkers classified further according to BMI are shown in **Figure 1**. GGT activities were found to significantly increase with increasing BMI. The highest values occurred in those with moderate drinking combined with overweight or obesity. Although the magnitude of GGT changes in men appeared to exceed those observed in women, a 3-factor interaction (sex \times BMI \times drinking status) on the between-subjects effects for all of these factors with GGT as the dependent variable was not significant. The 2-factor interactions (sex \times drinking status, sex \times BMI, or drinking status \times BMI) were also not significant.

The correlations between GGT and BMI were significant for both men ($r = 0.24$, $P < 0.0001$) and women ($r = 0.15$, $P < 0.0001$). The correlation observed for men was also significantly different from that of women ($P < 0.05$). Computing the partial correlation coefficients with control for sex, drinking status, or both, also yielded significant correlations ($r = 0.25$, $P < 0.0001$; $r = 0.31$, $P < 0.0001$; and $r = 0.26$, $P < 0.0001$, respectively).

DISCUSSION

Morbidity and mortality related to excessive ethanol consumption and obesity are increasingly pervasive public health problems that extend across the entire spectrum of body weights and different levels of ethanol consumption. However, only few studies have investigated the combined effects of moderate ethanol consumption and obesity on the biochemical variables that reflect health status (14, 18, 19).

The present data in a large population of abstainers and moderate drinkers indicated distinct additive effects of moderate alcohol consumption and overweight on serum GGT activities. These findings are consistent with the view that obesity together with ethanol consumption may lead to an increase in metabolic burden and risk of liver problems. Although the primary role of hepatic GGT is to metabolize extracellular reduced glutathione, recent studies have indicated that GGT enzyme induction may also be closely associated with the generation of reactive oxygen species (ROS) (20). Enhanced oxidant stress has been recently linked with obesity-associated metabolic syndrome and generation of fatty liver (21, 22). Interestingly, recent studies conducted both in experimental animals and in humans have indicated that the ethanol-inducible cytochrome enzyme, CYP2E1, may also be induced by obesity alone because free fatty acids can serve as substrates (23–27). This may create oxidative stress and render the liver more susceptible toward oxidative stress and associated cellular injury. Thus, the generation of fatty change in livers of alcoholic persons and in patients with nonalcoholic fatty liver disease appears to share several common pathogenic features both on cytochrome enzyme induction and generation of ROS (24, 25, 28, 29). In experimental animals, high-fat diets together with ethanol consumption enhanced oxidative stress and aggravated alcoholic liver injury (30). In humans, obesity has been shown to potentiate the severity of alcohol-induced liver damage, although the mechanisms of interaction by adiposity and ethanol are still unknown (11, 29, 31). It is possible that enhanced serum GGT activities could be regarded as a sign of generalized metabolic induction and activation of the body's defense mechanisms against the metabolic burden (32–34). GGT is an ectoenzyme that can be released from the cell membrane even without cell death. In light of previous findings by Speisky et al (34), GGT on the sinusoidal side of hepatocytes could provide a mechanism whereby glutathione released from periportal hepatocytes is broken down into readily oxidizable cysteine, replenishing this glutathione precursor for pericentral hepatocytes. The pericentral area is known to be the primary site of alcohol-induced CYP2E1 induction and of generation of ROS in the liver. Thus, increases in GGT may provide a compensatory hepatic mechanism to protect the pericentral hepatocytes against oxidative damage. Because ROS also increase in fatty liver and obesity, similar mechanisms may apply in both the alcohol- and obesity-induced increases in GGT and in the possible synergistic effects of alcohol abuse and overweight.



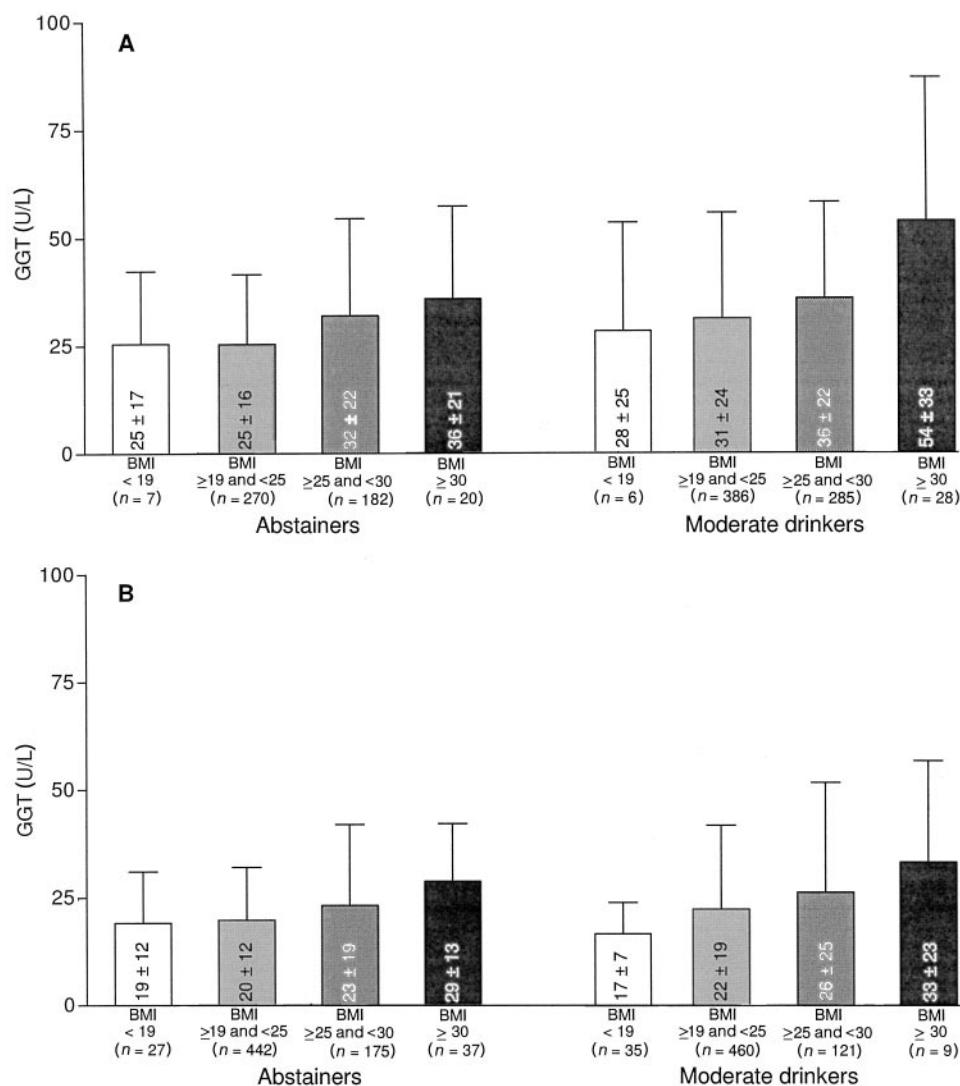


FIGURE 1. Mean (\pm SD) distributions of γ -glutamyl transferase (GGT) activities in men (A) and women (B) classified according to alcohol consumption and body mass index (BMI; in kg/m^2). A significant main effect of sex ($P < 0.0001$), drinking status ($P < 0.01$), and BMI ($P < 0.001$) was observed. Neither the 3-factor interaction (sex \times BMI \times drinking status) nor the 2-factor interactions (sex \times BMI, sex \times drinking status, or BMI \times drinking status) were significant.


Interestingly, the present data suggest distinct differences in serum GGT responses as a result of moderate alcohol consumption and overweight in groups consisting either of men or women. Although there may be sex-dependent differences in the metabolic consequences of ethanol intake and fat accumulation, the 3-factor analysis and 2-factor analyses on the interactions between sex, BMI, and drinking status with GGT as the dependent variable were not significant. Sex steroids have been shown to play a role in cytochrome enzyme expression and regulation of oxidant stress status in the liver (35, 36). Unfortunately, information on the type of alcoholic beverages consumed by the study subjects was not available in the present study. Because recent studies conducted in persons who consume >5 drinks/d have indicated a reduced risk for alcoholic cirrhosis in wine drinkers (37), future studies should address whether GGT responses would differ based on moderate drinking of wine, beer, or liquor. Increasing age also influences GGT activities, and, therefore, studies conducted in populations with wide age distributions should also explore the biological significance of such responses.

Studies in the past decades have shown that serum GGT is a sensitive clinical marker of alcohol abuse (5–8, 38–40). These data show that even moderate amounts of ethanol consumption elevate serum GGT activities, especially when occurring together with obesity. Although the correlation observed between self-reported alcohol consumption and GGT values has typically been in the order of 0.3–0.4 (5–8, 41), the present data show that a nearly similar correlation ($r = 0.2$ –0.3) may be achieved between GGT and BMI. It should, however, be noted that a correlation of 0.7 can be reached between GGT activities and alcohol consumption when the amount of ethanol ingestion is monitored for prolonged periods with daily ethanol analyses (42).

The effects of obesity on GGT activities should also be considered in the interpretation and establishment of common reference intervals for GGT measurements in health care. Upper normal limits for such assays have usually been based on values obtained from mixed populations of apparently healthy moderate drinkers and abstainers. The present data supports the view that to improve the diagnostic potential of GGT measurements,



reference intervals should, in fact, be based on healthy persons who abstain from ethanol consumption and are not significantly overweight. Additional improvement might be achieved if BMI-based reference intervals could be made available.

In conclusion, the present data supports the view that changes in drinking behavior and the continuing increase in the prevalence of obesity may elevate mean serum GGT activities at the population level. Future studies to address the prognostic implications of such responses as possible indicators of enhanced oxidative stress and whether it is possible to formulate BMI-based guidelines for safer alcohol consumption appear warranted (29). A critical reevaluation of reference intervals for GGT measurements as a biochemical marker of alcohol consumption and liver induction may also be necessary. 

We thank Pål Rustad, Først Medical Laboratory, Oslo, Norway, for providing data on GGT measurements from the Nordic NORIP Survey on enzyme reference intervals. We thank Timo Marjomäki, University of Jyväskylä, for help with the statistical analyses.

KP and JH contributed equally to this work and were involved in the study design, material collection, data analyses, and drafting the manuscript. HK and RB were involved in the data analyses. PA was involved in the material collection. ON was involved in the study design and writing the manuscript. None of the authors had any conflicts of interest.

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Obesity and the clinical use of serum GGT activity as a marker of heavy drinking

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Running title: BMI and GGT normal ranges

ABSTRACT

Objective: Gamma-glutamyl transferase (GGT) is a widely used clinical marker of alcohol abuse. However, although obesity may also elevate serum GGT activities, the effects of overweight on the interpretation of GGT testing have remained poorly defined.

Material and methods: GGT activities from 1147 moderate drinkers and 449 abstainers, who were classified according to body mass index (BMI), were compared with those of 208 heavy drinkers admitted for detoxification.

Results: GGT upper normal limits, as defined based on normal weight abstainers (men 53 U/L; women 45 U/L) were lower than those based on moderate drinkers (men 68 U/L; women 50 U/L). The relative increases in GGT activities in male moderate drinkers with overweight (54 %) or obesity (125 %) exceeded the corresponding changes found in women (25 % and 75 %, respectively). The BMI-dependent variation on the sensitivity of GGT for correctly classifying heavy drinkers ranged from 29 to 67 %. The rates of false positive values in the subgroups from low to high BMI varied from 0 to 27 %, respectively.

Conclusions: The data indicate that the diagnostic value of serum GGT testing could be improved by using reference data derived from databases of abstainers with normal weight or BMI-based categorization of reference ranges.

Key words: biomarkers, BMI, ethanol, gamma-glutamyl transferase, liver, reference values, sensitivity, specificity

Abbreviations: BMI = body mass index, GGT = gamma-glutamyl transferase, IFCC = International Federation of Clinical Chemistry and Laboratory Medicine.

INTRODUCTION

Gamma-glutamyl transferase (GGT) enzyme catalyzes the transfer of the gamma-glutamyl moiety of glutathione to different peptide acceptors. Heavy ethanol intake commonly induces a rise in serum GGT activities and therefore, it is currently perhaps the most widely used laboratory marker for detecting alcohol use disorders [1-3]. However, in clinical materials conflicting data have appeared on the sensitivities and specificities for serum GGT measurements [3-9].

Several lines of recent evidence have indicated that GGT activities in circulation may also be induced as a result of obesity [10-15]. The prevalence of obesity is increasing in an epidemic manner and a major proportion of the population in most industrialized countries currently suffers from overweight or obesity [16]. The impact of overweight as a confounding factor in serum GGT testing has remained unclear and in the definitions of assay normal ranges only limited attention has so far been paid on the exact amounts of ethanol consumption and the presence or absence of overweight in the reference populations.

We have recently demonstrated that moderate drinking and obesity may create additive effects on serum GGT activities [15]. In order to gain further insight on the effects of these phenomena on the sensitivities and specificities of GGT in detecting alcohol abuse we have compared here GGT activities from a reference population consisting of either moderate drinkers or abstainers, as further classified according to body mass index, to the data obtained from a population of heavy drinkers.

MATERIAL AND METHODS

Data from a survey including 1596 apparently healthy individuals (758 men and 838 women, mean age 41 ± 14 years, range 18–70 years) collected for establishing reference intervals for common enzyme determinations in Nordic countries were used [17]. All subjects underwent detailed assessments, in which age, gender, height, weight, health status and dietary habits were recorded using specifically designed questionnaires. The subjects in whom homogeneous interview data on the amount of alcohol consumption was available were classified to either moderate drinkers ($n = 1147$: 590 men, age 41 ± 14 years; 557 women, age 41 ± 13 years) or abstainers ($n = 449$: 168 men, age 40 ± 15 years; 281 women, age 41 ± 14 years). In moderate drinkers, the mean amount of recent alcohol consumption ranged from 1 to 40 grams of ethanol per day, the maximum amount during the past twenty-four hours prior to sampling being 24 grams (two standard drinks). Those, whose current alcohol consumption had been 0 drinks per week for the past few months were categorized as abstainers. The population was further classified according to body mass index (BMI) expressed as kg/m^2 (weight in kilograms divided by the square of height in meters) into subgroups as follows: BMI < 20 (low weight), BMI 20–25 (normal weight), BMI 25–30 (overweight), BMI > 30 (obese). The above subjects represented primarily hospital personnel and their relatives or acquaintances, new blood donors, or blood donors who seldom donate blood. The survey excluded individuals who had clinical or laboratory evidence of current or recent illnesses or infections, who were pregnant, had donated blood during the past five months or had used any prescription drugs during the preceding one week. Smoking had not been allowed for one hour prior to sampling.

Serum GGT activities were also measured from 208 heavy drinkers (174 men, mean age 43 ± 10 years; 34 women, mean age 42 ± 10 years; range 19–67 years), who had been admitted for detoxification. Detailed personal interviews on the patterns and amounts of alcohol consumption

using a time-line follow-back technique were carried out from all patients. They showed a history of continuous ethanol consumption or binge drinking, the mean ethanol consumption during the period of 4 weeks prior to sampling being 128 grams (range 40–540 grams) per day. All patients were, however, devoid of clinical and laboratory evidence of apparent liver disease. The mean number of days of abstinence prior to sampling was 2 days (range 0-7 days). None of these patients had used any prescription drugs, which are known to induce GGT activities, such as barbiturates or anticoagulants. The patients were also found to be negative for hepatitis B surface antigen or hepatitis C serology.

All GGT measurements were carried out with homogenous IFCC compatible measuring systems. The procedure was approved by the institutional review board. Informed consent was obtained from the participants and the study was carried out according to the provisions of the Declaration of Helsinki.

Statistical methods

Values are expressed as mean \pm SD. Reference intervals were calculated as mean \pm 2SD after logarithmic transformation of the GGT raw data to obtain symmetrical distributions and Dixon's test for detecting outliers, as recently recommended by Horn and Pesce [18]. Comparisons between groups were made with Kruskal-Wallis test and Dunn's Multiple Comparison Test or Mann-Whitney test when comparing two groups. Multiple regression analysis was used to measure proportions of variability. The analyses were carried out using SPSS version 14.0 for Windows statistical software (SPSS Inc, Chicago, IL). A p-value $<$ 0.05 was considered statistically significant.

RESULTS

Serum GGT activities (177 ± 309 U/L, mean \pm SD) in the heavy drinkers significantly exceeded the levels obtained from both moderate drinkers (28 ± 23 U/L) ($p < 0.001$) or abstainers (23 ± 16 U/L) ($p < 0.001$). The values in moderate drinkers were also higher than those of abstainers ($p < 0.001$).

Figure 1 shows the relative differences in mean GGT activities in the population of moderate drinkers and abstainers, as classified according to both gender and BMI. An increasing trend was observed both due to adiposity and alcohol consumption, the changes being more prominent in men. Table 1 summarizes the estimated lower and upper normal GGT limits and their relative changes with respect to BMI. The medians and ranges of GGT values are also given. The estimated upper normal limits increased with increasing BMI by up to 189 % (men) and 122 % (women), the most striking elevations occurring in the groups representing moderate drinkers with obesity.

The effect of BMI on the diagnostic sensitivity of GGT in correctly classifying the population of heavy drinkers is demonstrated in Figure 2. In men the sensitivities were found to vary between 32 % and 67 % and in women between 29 % and 56 %. The effect of overweight or obesity on GGT specificity with cut-offs defined from the data of normal weight abstainers is demonstrated in Figure 3. In obese moderate drinkers the rates of false positive values were 27 % in men and 25 % in women, whereas in obese abstainers the corresponding values were 14 % and 11 %, respectively. False positive rates between 5 % and 14 % were found to occur in individuals with overweight (BMI 25–30 kg/m²).

Since increasing age has also been shown to affect GGT levels, multiple regression analysis was carried out to address the impact of age as a predictor among the multiple values in the present

material. The data indicated a lack of significant interaction between age, GGT, and drinking status, or BMI (multiple $R^2=0.03$ for men, and 0.02 for women).

DISCUSSION

The present data in a large population of moderate drinkers and abstainers indicates that both overweight and moderate ethanol consumption induce serum GGT activities, which is in accordance with previous observations by us [15] and by other groups of investigators [10-14]. The present study further addresses the extent to which such phenomena could also influence the definition of normal ranges for GGT measurements. Excessive ethanol consumption [19] and obesity [16] are both rapidly increasing in our society and it appears that these conditions may also create cumulative pathogenic effects, such as an increased risk for liver injury [13, 15, 20-24] or diabetes [25]. Therefore, in health care it may also be important to revisit the concept of GGT reference intervals, as well as perhaps for other biochemical parameters which may be sensitive to ethanol consumption.

It appears that the clinical value of serum GGT testing could be markedly improved by establishing new reference values derived from well-defined databases of abstainers with normal weight. Current data indicates that when compared to abstainers with normal weight, the upper normal GGT limits would become over 20 % higher based on the data including moderate drinkers with normal weight into the reference population and even over 100 % higher based on moderate drinkers with overweight. The changes appear to be more striking in men indicating distinct gender-dependent differences in GGT responses towards ethanol and obesity. It may further be suggested, that there is an ongoing population trend towards increasing mean GGT activities as recently observed for South Korean men during the years 1996-2003 [26]. In accordance with this view, the recent NORIP

survey on reference intervals for common enzyme determinations in Nordic countries concluded that GGT values up to 110 U/L could be considered normal among middle-aged men representing a mixed population of moderate drinkers and abstainers [26]. Based on several lines of earlier observations it appears, however, that particular caution should be exerted with the definition of GGT normal ranges due to a high rate of morbidity in individuals with mildly elevated GGT levels [11, 23-25, 28].

In the present material, approximately 20–30 % of the obese individuals and 10 % of those with overweight showed elevated GGT activities in comparisons with normal weight abstainers, especially among moderate drinkers. In light of recent observations linking GGT enzyme induction with the generation of reactive oxygen species to such an extent that GGT could be considered a marker of oxidative stress [29, 30], future studies appear warranted to address the possibility whether follow-up of such individuals would also reveal increased risks for morbidity. It might also be possible to define adjusted biological models to more exactly evaluate the quantitative contributions of obesity and alcohol drinking as predictors of GGT responses. Previously, it has been suggested that diabetes may also be associated with elevated GGT levels and that the association between diabetes and obesity may be mediated by liver pathology (11). On the other hand, coffee consumption has recently been shown to be inversely related to serum GGT and regular coffee consumption seems to be protective against liver disease, particularly due to alcohol [33]. It remains to be established to what extent the above observations could be related to the physiological role of GGT enzyme in breaking glutathione into cysteine and regulation of cellular redox status [34].

Increased GGT activities have also been linked with increasing age, use of certain drugs, hyperthyroidism, hypertension and several types of liver diseases [2, 3, 25, 31, 32]. Such conditions

should not, however, create significant confounding variables in the present material of apparently healthy reference individuals. Furthermore, multiple regression analyses indicated that age-related effects account for only less than 3% of the variation in GGT.

In this work the population of heavy drinkers was not subgrouped according to BMI, because our scope was to contrast BMI-categorized reference individuals with a consecutive unselected sample of heavy drinkers. It should also be noted that possible differences in the decay of GGT activity should not significantly influence the interpretation of the data in this work since GGT enzyme in circulation has a half-life of approximately three weeks [3,9] and the time of abstinence prior to sampling in all heavy drinkers was found to be relatively similar (range 0-7 days).

Taken together, the present work emphasizes the need for BMI-categorized reference intervals derived from databases of abstainers for GGT determinations in health care. Consequently, BMI-based guidelines for more safe alcohol consumption could perhaps also be established with the help of using GGT as an index of the individual metabolic burden.

Acknowledgements: The help of Professor Pål Rustad, Først Medical Laboratory, Oslo, Norway, for providing data on GGT measurements in the Nordic NORIP Survey is gratefully acknowledged. The studies were supported in part by a grant from the Finnish Foundation for Alcohol Studies.

Table I. GGT medians, ranges and estimated normal limits in subgroups divided according to body mass index and drinking status.

Study group	Age (yr, mean \pm SD)		GGT median and range (U/L)		GGT lower normal limit (U/L)		GGT upper normal limit (U/L)	
	Men	Women	Men	Women	Men	Women	Men	Women
Abstainers								
BMI < 20 (low weight)	34 \pm 12	34 \pm 14	18 (15–62)	14 (8–42)	7 (–13 %)	8 (+14 %)	62 (+17 %)	33 (–27 %)
BMI 20–25 (normal weight)	37 \pm 15	42 \pm 14	21 (9–88)	17 (8–90)	8	7	53	45
BMI 25–30 (overweight)	44 \pm 15	44 \pm 14	22 (10–59)	18 (8–84)	10 (+25 %)	8 (+14 %)	59 (+11 %)	51 (+13 %)
BMI > 30 (obese)	47 \pm 15	43 \pm 16	26 (16–56)	29 (14–69)	11 (+38 %)	12 (+71 %)	73 (+38 %)	63 (+40 %)
Moderate drinkers								
BMI < 20 (low weight)	34 \pm 15	36 \pm 14	18 (10–78)	15 (5–45)	7 (–13 %)	7 (\pm 0 %)	49 (–8 %)	36 (–20 %)
BMI 20–25 (normal weight)	41 \pm 14	41 \pm 13	24 (10–168)	17 (7–110)	9 (+13 %)	7 (\pm 0 %)	68 (+28 %)	50 (+11 %)
BMI 25–30 (overweight)	43 \pm 13	45 \pm 13	31 (9–199)	19 (8–112)	12 (+50 %)	8 (+14 %)	86 (+62 %)	57 (+27 %)
BMI > 30 (obese)	43 \pm 13	43 \pm 13	41 (17–133)	25 (15–83)	13 (+63 %)	9 (+29 %)	153 (+189 %)	100 (+122 %)

GGT, gammaglutamyl transferase (U/L), BMI, body mass index (kg/m²). The percentages in brackets after each normal limit indicate the relative

change from the corresponding value in normal weight abstainers.

FIGURE LEGENDS

Figure 1. The relative changes (%) in mean serum GGT activity in the moderate drinkers and abstainers as further classified according to BMI. The mean GGT activity obtained from the corresponding group of abstainers with normal weight is used as baseline, as indicated in the y-axis. BMI < 20 kg/m², low weight; BMI = 20–25 kg/m², normal weight; BMI 25–30 kg/m², overweight; BMI > 30 kg/m², obese.

Figure 2. The sensitivities of serum GGT measurements in correctly classifying the population of heavy drinkers in comparisons with the reference populations of either abstainers or moderate drinkers classified according to BMI. The cut-offs obtained from each reference population are indicated under the data columns.

Figure 3. The rates of false positive GGT values in abstainers and moderate drinkers with different BMIs. Cut-offs were defined based on the data from normal weight abstainers (men 53 U/L, women 45 U/L).

Figure 1

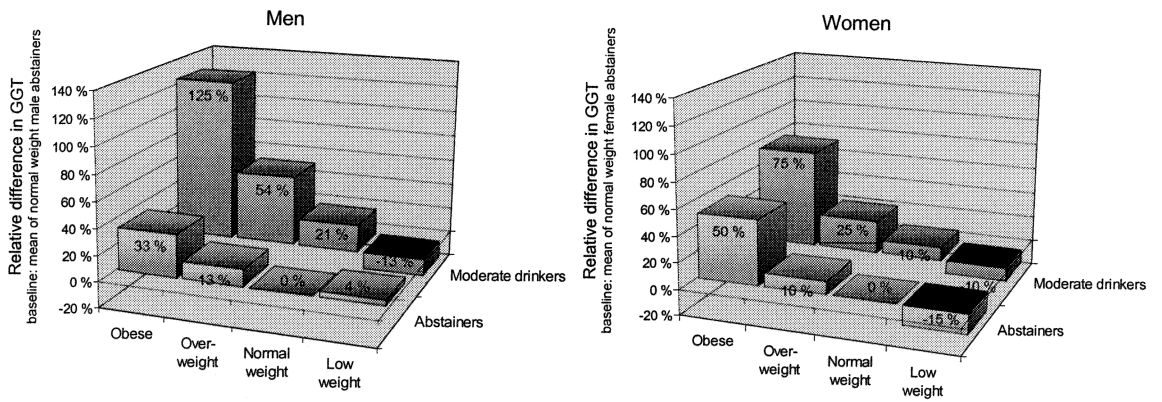


Figure 2

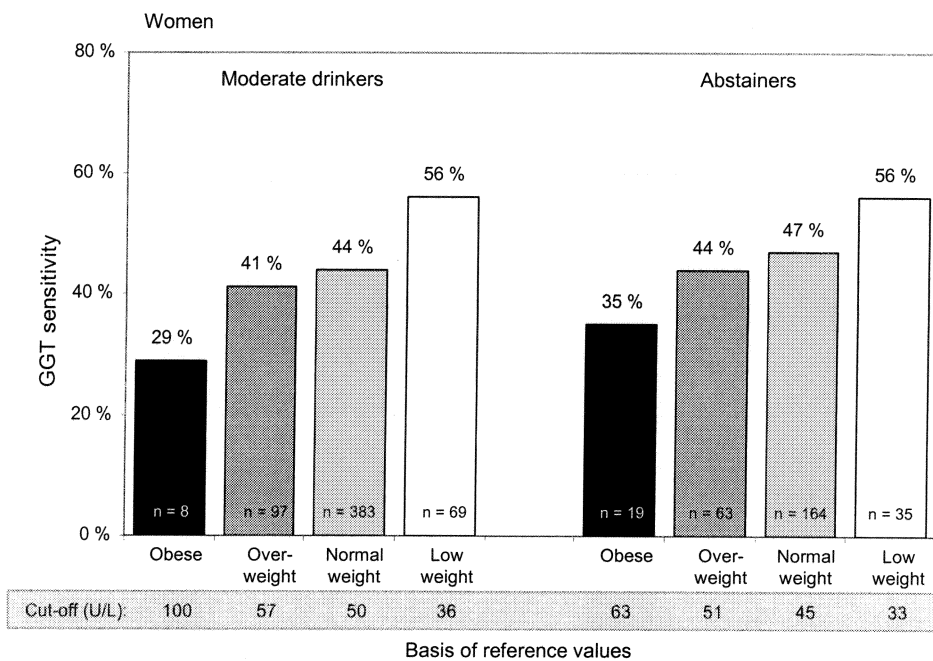
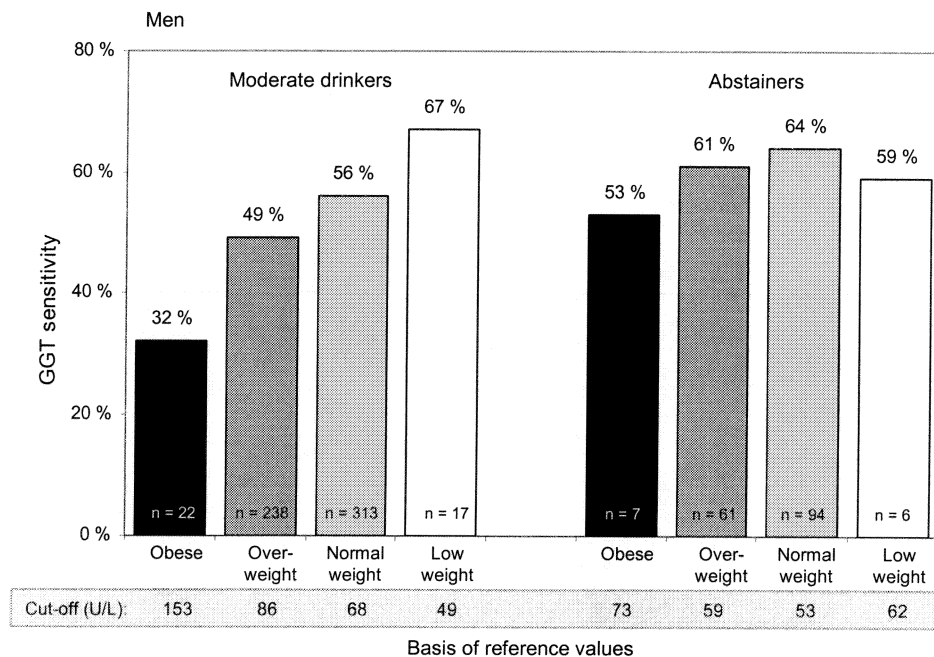
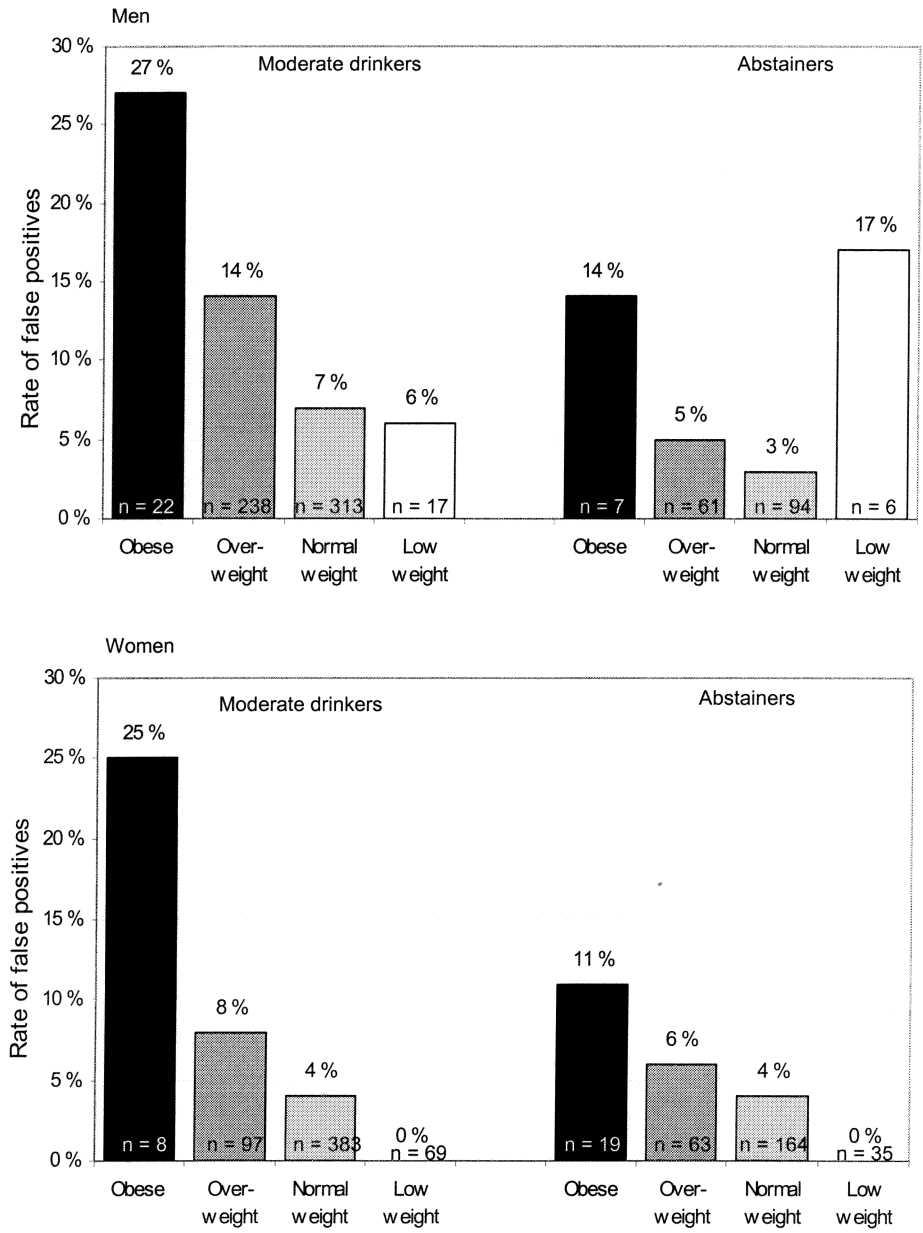


Figure 3



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AGE-RELATED CHANGES ON SERUM GGT ACTIVITY AND THE ASSESSMENT OF ETHANOL INTAKE

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(Received 24 May 2006; first review notified 11 June 2006; in revised form 13 June 2006; accepted 13 June 2006; advance access publication 19 July 2006)

Abstract — **Aims:** Gamma-glutamyl transferase (GGT) is a commonly used marker of ethanol abuse. However, although increasing age has also been suggested to elevate serum GGT activities, the magnitude of such effects on GGT in the assessment of ethanol intake have remained poorly defined. **Methods:** GGT activities from 208 heavy drinkers were compared with those from a reference population including 1330 moderate drinkers and 1160 abstainers, who were further classified to following age intervals: 18–30, 30–50, 50–70, and >70 years. **Results:** GGT activities increased with increasing age until after 70 years decreasing values were noted in male abstainers. The heavy drinkers belonging to age groups 18–30, 30–50, and 50–70 years showed 2.7-, 8.0-, and 6.9-fold higher mean GGT activities than those in the corresponding groups of abstainers, respectively. The values in the group of moderate drinkers also exceeded those of abstainers in all age groups of men, whereas in women the difference was significant only among those aged 18–30 years. **Conclusions:** The data indicate that GGT activities respond to ethanol intake in an age-dependent manner, which should be considered in the clinical use of GGT measurements for detecting alcohol use disorders.

INTRODUCTION

Gamma-glutamyl transferase (GGT) is a membrane-bound glycoprotein enzyme, which catalyses the transfer of the gamma-glutamyl moiety of glutathione to various peptide acceptors. Chronic ethanol consumption is known to induce a rise in serum GGT and therefore it is also a widely used index of excessive ethanol intake (Zein and Discombe, 1970; Reyes and Miller, 1980; Anton *et al.*, 2002; Niemelä, 2002; Conigrave *et al.*, 2003). Although several studies have reported a positive correlation between ethanol intake and serum GGT activity, the sensitivities and specificities observed for GGT as a clinical marker of heavy drinking have, however, shown notable variation (Bagrel *et al.*, 1979; Chick *et al.*, 1981; Papoz *et al.*, 1981; Persson *et al.*, 1990; Hietala *et al.*, 2005). Recent studies by several groups of investigators have also emphasized obesity as an important factor, which can increase serum GGT activities (Daeppen *et al.*, 1998; Peters and Cook, 2002; Lam and Mobarhan, 2004; Colicchio *et al.*, 2005; Lawlor *et al.*, 2005; Puukka *et al.*, 2006). In addition, age has been suggested to affect GGT activities (Daeppen *et al.*, 1998; Sillanaukee *et al.*, 1998; Conigrave *et al.*, 2002; Stromme *et al.*, 2004; Lee *et al.*, 2004b). However, the possible age-related effects on serum GGT activities in the assessment of hazardous drinking practices have remained poorly defined.

This work was initiated to gain further insight on GGT as a marker of alcohol abuse by comparing GGT activities among heavy drinkers with those of either moderate drinkers or abstainers classified into different age cohorts.

METHODS

Study protocol

Serum GGT activities were measured from 208 heavy drinkers (174 men, mean age 43 ± 10 years; 34 women, mean age 42 ± 10 years, range 19–67 years), who had been admitted for detoxification in a consecutive manner. The clinical assessments included detailed personal interviews on the patterns and amounts of ethanol consumption using a time-line follow-back technique. All patients showed a history of chronic ethanol consumption or binge drinking, the mean recent consumption from the period of 4 weeks prior to sampling being 128 g ethanol/day (range 40–540 g).

For comparisons, data from a survey of 2490 apparently healthy individuals (1184 men and 1306 women, mean age 47 ± 18 years, range 18–90 years), which were collected for establishing reference intervals in Nordic countries, were also used as kindly provided by the project coordinator, professor Pål Rustad, Furst Medical Laboratory, Oslo, Norway. These subjects were classified based on self-reports to either moderate drinkers ($n = 1330$: 705 men; 625 women) or abstainers ($n = 1160$: 479 men; 681 women). Those, whose current alcohol consumption had been 0 drinks per week for the past few months were categorized as abstainers. Moderate drinkers were individuals who were devoid of any history of alcohol abuse and who consumed alcohol in amounts, which had ranged from 1 to 21 standard drinks per week corresponding to a range of 1–40 g ethanol/day. The maximum amount during the past 24 h prior to sampling had been 24 g (two standard drinks). The population was further grouped according to age as follows: 18–30 years: 291 men, 318 women; 30–50 years: 343 men, 407 women; 50–70 years: 343 men, 362 women; age >70 years: 207 men, 219 women. The mean body mass index (BMI) was not found to be significantly different between these subgroups (Table 1). The survey excluded individuals who had clinical or laboratory evidence of current or recent illnesses or infections, who were pregnant,

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Table 1. GGT upper and lower normal limits, calculated as mean \pm 2SD either from the population of abstainers or moderate drinkers classified into different age cohorts, as indicated

Age (years)	n	BMI (mean \pm SD)	Lower normal GGT limit (U/l)		Upper normal GGT limit (U/l)	
			Men	Women	Men	Women
Abstainers						
18–30	281	23 \pm 3	9	8	43	35
30–50	301	24 \pm 3	9	7	72	40
50–70	314	25 \pm 3	10	8	87	56
>70	264	25 \pm 3	8	8	69	55
Moderate drinkers						
18–30	328	23 \pm 3	10 (11%)	8 (0%)	51 (19%)	42 (20%)
30–50	449	24 \pm 3	10 (11%)	7 (0%)	86 (19%)	43 (8%)
50–70	391	25 \pm 3	11 (10%)	7 (-13%)	96 (10%)	67 (20%)
>70	162	25 \pm 3	10 (25%)	8 (0%)	98 (42%)	70 (27%)

The percentages in brackets in moderate drinkers indicate the relative change as compared to corresponding level found from abstainers. BMI, body mass index

had donated blood during the past 5 months or had used any prescription drugs during the preceding 1 week. Smoking had not been allowed for 1 h prior to sampling. All GGT measurements were carried out with International Federation of Clinical Chemistry (IFCC) compatible measuring systems and standard clinical chemical methods. The procedure was approved by the institutional review board. Informed consent was obtained from the participants and the study was carried out according to the provisions of the Declaration of Helsinki.

Statistical methods

Values are expressed as mean \pm SD. Comparisons were made with Kruskal–Wallis test and Dunn's multiple comparison test or Mann–Whitney test when comparing two groups. Correlations were calculated with Pearson product-moment correlation coefficients or with the Spearman's rank correlation, as required. Reference intervals were calculated as mean \pm 2 SD after logarithmic transformation of the GGT raw data to obtain symmetrical distributions (Horn and Pesce, 2003). A *P*-value <0.05 was considered statistically significant.

RESULTS

Serum GGT activity (177 \pm 309 U/l, mean \pm SD) in the heavy drinkers significantly exceeded the levels of both moderate drinkers (29 \pm 23 U/l) (*P* < 0.001) and abstainers (24 \pm 17 U/l) (*P* < 0.001). The difference between the latter two groups was also significant (*P* < 0.001). Figure 1 demonstrates the GGT activities in the heavy drinkers, moderate drinkers, and abstainers as further grouped according to age. The heavy drinkers (aged 18–70 years) showed significantly higher values than the reference population, the mean values in age groups 18–30, 30–50, and 50–70 years being 2.7-, 8.0-, and 6.9-fold higher than those of abstainers, respectively. The GGT activities among moderate drinkers also exceeded those of abstainers in all age groups. While in the alcohol-consuming individuals, there was a continuing increase in GGT activities with increasing age, the abstainers were found to show decreased GGT activities in those >70 years.

When the reference population was subsequently divided according to both age and gender, moderate drinkers were found to show higher GGT activities than the abstainers in all

age categories among men, whereas in women a statistically significant difference occurred only in those aged 18–30 years (Fig. 2). In men, the youngest age group (18–30 years) showed significantly lower GGT activities than the other age groups both in moderate drinkers and abstainers (*P* < 0.001). In women, the GGT activities remained essentially similar between 18 and 50 years and significantly lower than the values in those >50 years (*P* < 0.001) (Fig. 2).

Table 1 summarizes the lower and upper normal limits for GGT activities, defined as mean \pm 2SD, in the different age groups and their relative changes as a function of moderate drinking. The upper normal limits (mean + 2SD) were found to be up to 10–42% (men) and 8–27% (women) higher, when the normal ranges were based on the data of moderate drinkers instead of abstainers. The percentages of elevated GGT activities among heavy and moderate drinkers in these comparisons are shown in Table 2. The GGT sensitivities in correctly classifying heavy drinkers in age-matched comparisons were found to vary between 49 and 74% (men), and 41 and 56% (women), the highest sensitivities being found in the youngest age group. In contrast, in comparisons without age-matching, the youngest age group of heavy drinkers was detected with poor sensitivity. Moderate drinkers were found to show a 5–9% incidence of elevated values without age-matching, whereas in age-matched comparisons, the incidence of elevated GGT activities was found to be up to 21% in men and 10% in women (Table 2).

In the analysis of the data controlling for the effect of BMI, no significant interaction was observed. Correlation between GGT activities and age between 18 and 70 years was positive and significant for all study subgroups, as follows: heavy drinkers (*r* = 0.21, *P* < 0.01), moderate drinkers (*r* = 0.22, *P* < 0.0001), and abstainers (*r* = 0.24, *P* < 0.0001). However, in those >70 years, the correlation between GGT and age turned negative both in moderate drinkers (*r* = -0.18, *P* < 0.01) and abstainers (*r* = -0.16, *P* < 0.001).

DISCUSSION

The present data in a large population of individuals representing a wide range of ethanol consumption indicate distinct age- and gender-related effects on GGT activities, which could create significant population variability in studies on GGT

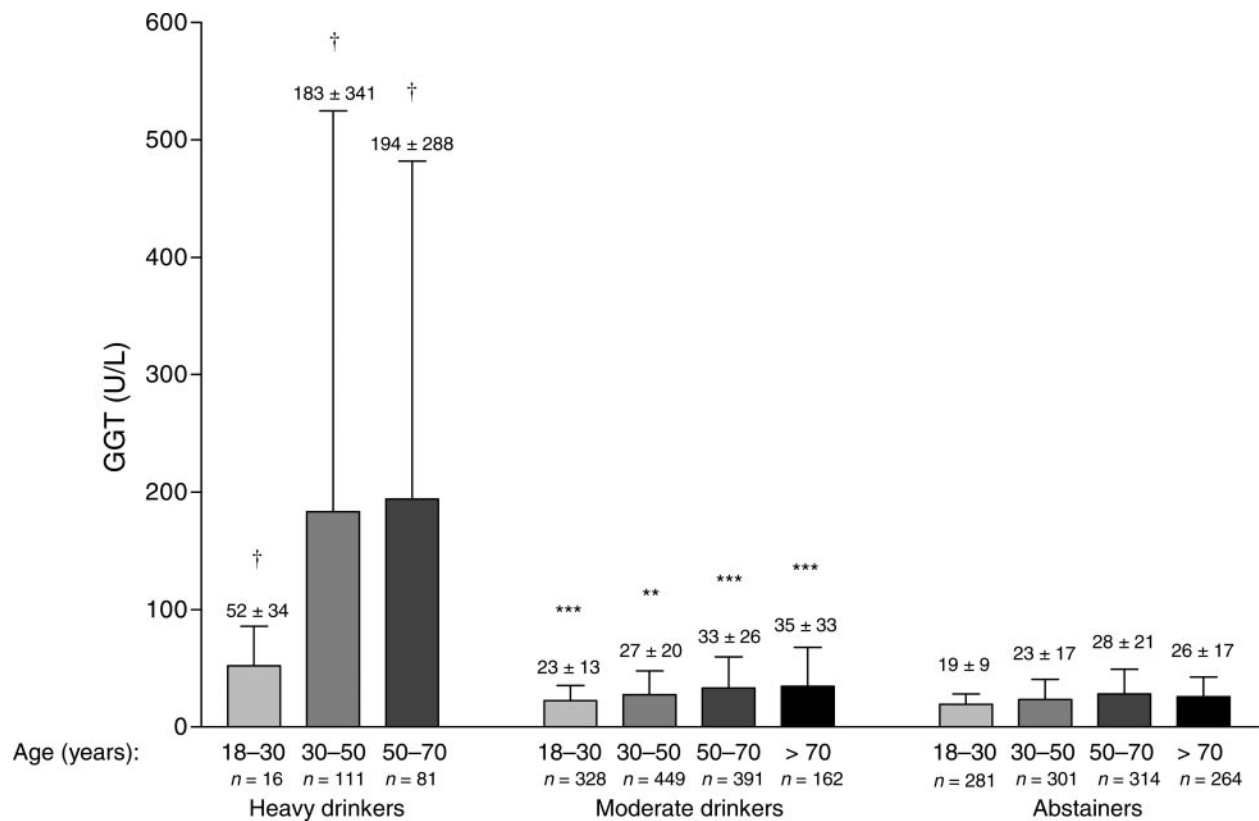


Fig. 1. Serum GGT activities (mean \pm SD) in heavy drinkers, moderate drinkers, and abstainers divided into subgroups according to age as follows: 18–30, 30–50, 50–70, and >70 years. The GGT activities increased with increasing age in all study groups until after 70 years decreasing activities were noted in abstainers. †Group of heavy drinkers significantly different from the corresponding group of moderate drinkers or abstainers ($P < 0.001$). Group of moderate drinkers significantly different from the corresponding group of abstainers, *** $P < 0.001$, ** $P < 0.01$.

activities in the assessment of excessive ethanol consumption and liver induction. The activities increased with increasing age until after 70 years decreasing activities were noted in men who did not consume alcohol. While previous studies have also reported increased GGT activities with increasing age (Daepfen *et al.*, 1998; Sillanaukee *et al.*, 1998; Conigrave *et al.*, 2002; Stromme *et al.*, 2004; Lee *et al.*, 2004b) the decreasing activities specifically among male abstainers in old age have not been previously acknowledged. The correlation between age and GGT values appears to in fact turn negative in those >70 years. The incidence of heavy alcohol consumption is also known to naturally decline with increasing age (Karlmanngla *et al.*, 2006), as also reflected in the present material containing no heavy drinkers above the age 70 among over 200 consecutive admissions for detoxification.

Interestingly, even moderate drinking seems to increase GGT activities to higher levels than those in abstainers, most strikingly in men and in women <30 years, suggesting that these age groups may also show more sensitive liver induction. While the biological mechanisms underlying these observations remain obscure at this time, it may be assumed that the enhanced activities could be regarded as signs of metabolic induction and activation of body's defence mechanisms towards the ethanol-induced metabolic burden (Speisky *et al.*, 1990; Nakanishi *et al.*, 2000b; Kevil *et al.*, 2004). There may also be both age- and gender-dependent susceptibility to

ethanol-induced hepatotoxicity. Young adults may be more resistant to the damaging effects of alcohol (Chan *et al.* 1989). On the other hand, women are known to be more vulnerable to the development of alcoholic liver disease (Ashley *et al.*, 1977; Schenker, 1997). It is possible that GGT enzyme induction could play a hepatoprotective role in the early phase of the toxic stimuli due to the fact that the enzyme, which occurs on the sinusoidal side of the hepatocytes, may break glutathione into cysteine, which plays a key role in the regulation of the cellular redox status (Speisky *et al.*, 1990; Shoveller *et al.*, 2005). Recently, serum GGT has also been shown to readily respond to overweight and obesity, especially in the individuals with moderate drinking practices (Puukka *et al.*, 2006). Thus, increasing age together with ethanol and/or obesity could create an interaction triad, which synergistically increases the metabolic burden and the risk of liver injury. GGT enzyme induction has also been recently linked with the generation of reactive oxygen species possibly serving also as a marker of oxidative stress (Browning and Horton, 2004; Furukawa *et al.*, 2004; Lee *et al.*, 2004a; Lim *et al.*, 2004). It is also of interest to note that recent studies have suggested that coffee consumption may be inversely related to serum GGT and that coffee could inhibit the inducing effects of aging on serum GGT activities (Nakanishi *et al.*, 2000a).

It appears that the clinical value of serum GGT measurements in the assessment of excessive ethanol intake could be further improved if long-term biological influences of

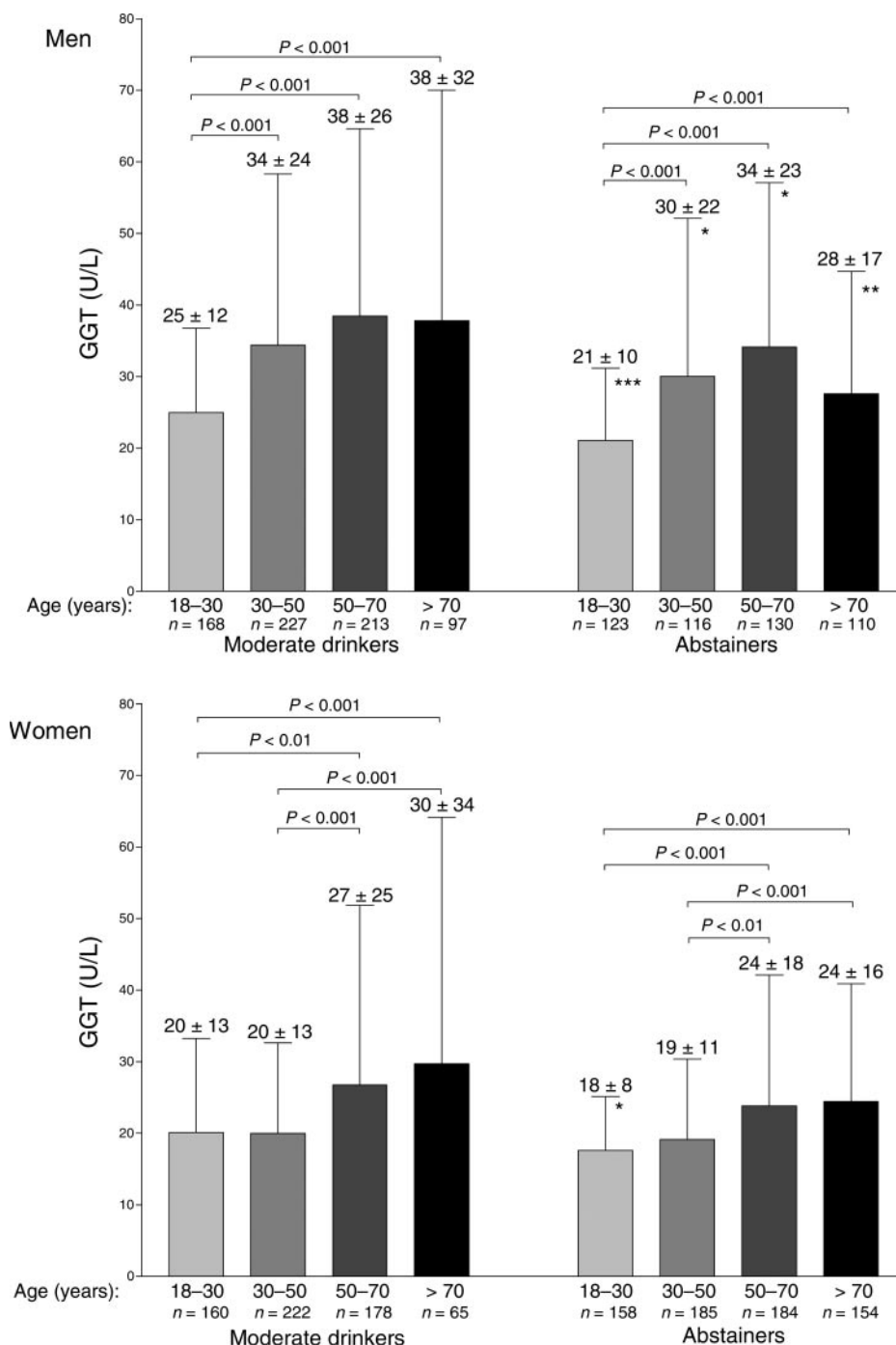


Fig. 2. Serum GGT activities (mean \pm SD) in male and female abstainers and moderate drinkers as further classified according to age. In men, all age groups of moderate drinkers showed higher values than abstainers, whereas in women, only those aged 18-30 years show a statistically significant difference between moderate drinkers and abstainers. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$, when compared to the values obtained from the corresponding group of abstainers.

moderate drinking (Hietala *et al.*, 2005) and factors unrelated to alcohol yet affecting GGT levels could be more efficiently controlled when defining assay normal ranges. Here, depending on the age group, GGT upper normal limits would be up to 40% higher if moderate drinkers would be included into the reference population. Since the mean alcohol consumption is continuously increasing in our society (Room *et al.*, 2005), there may also be a trend towards increases in mean GGT

activities at population level. Consequently, an increasing percentage of alcoholics may escape detection in clinical settings. In accordance with this view the recent NORIP survey on enzyme determinations in Nordic countries concluded that in middle-aged (>40 years) men GGT activities up to 110 U/l might be considered normal (Stromme *et al.*, 2004). In previous studies, the diagnostic sensitivity of GGT in detecting alcohol use disorders has usually been shown to be lower for

Table 2. The incidence of elevated GGT values (%) in heavy drinkers and moderate drinkers

Age (years)	Heavy drinkers		Moderate drinkers	
	Men (n = 174) (%)	Women (n = 34) (%)	Men (n = 705) (%)	Women (n = 625) (%)
A. Age-matched comparisons				
18–30	74	56	21	10
30–50	54	50	6	7
50–70	49	41	4	4
>70	55	41	7	4
B. Comparisons without age-matching				
18–30	41	50	8	9
30–50	54	52	7	5
50–70	51	56	6	7
>70	—	—	7	8

Data when compared with the reference data obtained from abstainers with or without age-matching.

women than for men (Anton and Moak, 1994; Yersin *et al.*, 1995; Mundle *et al.*, 2000). The sensitivity of GGT as a clinical marker of alcohol abuse has also been shown to be especially disappointing in studies dealing with young adults (<30 years) (Chan *et al.*, 1989; Nyström *et al.*, 1993; Sillanaukee *et al.*, 1998; Conigrave *et al.*, 2002), even when they have alcohol dependence (Bisson and Milford-Ward, 1994). It is therefore noteworthy that in the present work the reference populations with or without age-matching provided markedly different views on GGT sensitivities.

Taken together, the present data supports the concept that the diagnostic potential of GGT measurements could be improved by establishing specific age-categorized reference intervals based on healthy individuals who abstain from ethanol. These findings should also be considered in studies on the pathogenesis of ethanol-induced oxidative stress and liver induction.

Acknowledgements — The studies were supported in part by a grant from the Finnish Foundation for Alcohol Studies. The help of Professor Pål Rustad, Først Medical Laboratory, Oslo, Norway, for providing data on GGT measurements in the Nordic NORIP Survey for establishing reference intervals is gratefully acknowledged.

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