Tampere University

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Seinäjoki, January 2021

Ulla Nivukoski

ABSTRACT

Health problems associated with lifestyle are becoming increasingly common in modern societies. The main lifestyle risk factors which contribute to the incidence of chronic diseases and premature death include alcohol drinking, cigarette smoking, excess body weight, physical inactivity and poor diet. On the other hand, a lack of lifestyle-related risk factors has been shown to be associated with prolonged life expectancy.

The present work explores the associations between lifestyle risk factors and biomarkers of liver function, inflammation and lipid status in a large population-based sample (the National FINRISK Study). The material had been collected from six geographical areas in Finland during the years 1997, 2002 and 2007 and provided an age and gender-stratified random sample which included 22,327 apparently healthy individuals aged 25–74 years. Data on health status, alcohol consumption, smoking, physical activity and coffee drinking were collected from structured interviews and questionnaires, and, weight, height and waist circumference were ascertained by means of physical measurements. Self-reported alcohol consumption data for the past 12 months were used to classify the participants into subgroups of abstainers and World Health Organization (WHO) risk drinking categories representing low, moderate, high and very high risk drinkers. The participants were also classified into subgroups according to their frequencies of binge drinking. Serum liver enzymes (gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT), C-reactive protein (CRP) and lipid profiles were measured using standard laboratory procedures. Risk scores for the lifestyle factors (alcohol consumption, cigarette smoking, physical inactivity and excess body weight) were established on a 0–8 scale and used to classify the population into lifestyle-related risk categories, which also allowed estimation of the joint effects of the various lifestyle factors.

The WHO risk drinking category was fairly well linearly related to the occurrence of elevated GGT, ALT and CRP values, and alcohol drinking was also a significant determinant of serum lipid abnormalities. Significantly higher odds for abnormal GGT, ALT and lipid profiles were found in the alcohol drinkers after adjustment for age, waist circumference, physical activity, smoking and coffee intake, while the frequency of binge-type drinking showed a significant association with GGT levels

in both men ($p < 0.0005$) and women ($p < 0.0005$) and with ALT in men ($p < 0.0005$). Even among the individuals with low risk total alcohol consumption, higher GGT ($p < 0.0005$) and ALT ($p < 0.0005$) activities were observed in those with binge drinking episodes more than once a month than in those with no such episodes.

Distinct dose-response associations were found between the total number of lifestyle-related risk factors and serum ALT, GGT, CRP, cholesterol, high-density lipoprotein, low-density lipoprotein and triglycerides ($p < 0.0005$ for a linear trend in all comparisons). When compared with the subjects without any risk factors, the multivariable-adjusted odds ratios for abnormalities in all biomarkers were significantly higher in those with a risk score of two or more. The most notable increases in ORs in the subjects with high numbers of risk factors were observed among men with respect to serum GGT: 26.6 (12.4–57.0), ALT: 40.3 (5.3–307.8), CRP: 16.2 (7.8–33.7) and serum triglycerides: 14.4 (8.6–24.0). The occurrence of a fatty liver index (FLI) ≥ 60 indicating the presence of fatty liver, increased from 2.4% in men with zero risk factors to 81.9% in those with a risk score of 7–8 ($p < 0.0005$ for a linear trend) and from 0% to 73.5% in women ($p < 0.0005$). The most striking impacts on the likelihood of FLI ≥ 60 were observed for physical inactivity ($p < 0.0005$ for both genders) and alcohol consumption ($p < 0.0005$ for men).

The data indicate that systematic use of laboratory tests may improve the assessment of health risks related to lifestyle and behaviour. These results also emphasize the adverse effects of binge-type alcohol drinking on hepatic function even in individuals with low-risk overall alcohol consumption. Combinations of several unfavourable lifestyle factors are associated with distinct abnormalities in laboratory tests for liver function, inflammation and lipid status and a high likelihood of hepatic steatosis. The use of biomarkers could also benefit the assessment of interventions aimed at maintaining a healthy lifestyle.

TIIVISTELMÄ

Elintapoihin liittyvät terveysongelmat yleistyvät nyky-yhteiskunnissa. Lukuisat elintapojen riskitekijät – kuten haitallinen alkoholinkäyttö, tupakointi, ylipaino, fyysinen passiivisuus ja puutteellinen ruokavalio – voivat aiheuttaa kroonisia sairauksia ja eliniän lyhentymistä. Sen sijaan terveellisten elintapojen omaksumisen on osoitettu liittyvän pidentyneeseen eliniänodotteeseen.

Tässä tutkimuksessa selvitettiin erilaisten elintapatekijöiden yhteyttä maksan toimintaa kuvaaviin maksaentsyymiaktiivisuuksiin, tulehdustilojen biomarkkeriin sekä seerumin lipidiprofiiliin käyttäen laajaa kansallista väestötutkimusaineistoa (FINRISKI). Tutkimusaineisto on kerätty kuudelta eri alueelta Suomessa vuosina 1997, 2002 ja 2007. Tutkimuksen otos, 22 327 perustervettä henkilöä iältään 25–74 vuotta, poimittiin satunnaistettuna otoksena ikä- ja sukupuoliryhmittäin. Tiedot terveydentilasta, alkoholinkäytöstä, tupakoinnista, fyysisestä aktiivisuudesta ja kahvinkulutuksesta kerättiin haastatteluin ja kyselylomakkein. Fyysisiä mittauksia käyttäen kerättiin tiedot painosta ja pituudesta sekä vyötärönympäryksestä. Tutkimukseen osallistujat raportoivat alkoholinkäyttönsä viimeisimmän 12 kuukauden ajalta, ja käyttömäärien mukaan heidät jaettiin absolutisteihin sekä Maailman terveysjärjestön WHO äskettäin määrittelemiin alkoholinkäytön riskikategorioihin; vähäisen, kohtalaisen, korkean sekä erittäin korkean riskin kategoriaan. Lisäksi osallistujat ryhmiteltiin sen mukaan, miten usein juominen oli ollut humalahakuista. Seerumin maksaentsyymit (gamma-glutamyylitransferaasi GT ja alaniiniaminotransferaasi ALAT), C-reaktiivinen proteiini (CRP) ja lipidiprofiili määritettiin vakiintuneilla kliiniskemiallisilla menetelmillä. Eri elintapatekijöiden (alkoholinkäyttö, tupakointi, fyysinen passiivisuus sekä ylipaino ja lihavuus) riskipisteet määritettiin asteikolla 0–8. Riskipisteiden mukaan osallistujat jaoteltiin ryhmiin ja näin pystyttiin arvioimaan eri elintapatekijöiden yhteisvaikutuksia.

Alkoholinkäytön mukainen WHO:n riskikategoria oli melko lineaarisesti yhteydessä kohonneisiin GT-, ALAT- ja CRP-arvoihin. Alkoholinkäyttö vaikutti myös merkitsevästi poikkeavien lipidiarvojen esiintymiseen. Nämä havainnot säilyivät merkitsevinä GT-, ALAT- ja lipidiarvojen osalta, vaikka aineisto vakioitiin iällä, vyötärönympärysmitalla, liikunnan määrällä, tupakoinnilla ja kahvin kulutuksella. Humalahakuisen juomisen useus oli merkitsevästi yhteydessä GT-

aktiivisuuksiin sekä miehillä ($p < 0.0005$) että naisilla ($p < 0.0005$) ja ALAT-aktiivisuuksiin miehillä ($p < 0.0005$). Alkoholin kokonaiskulutuksen mukaan vähäisen riskin kategoriaan kuuluvilla osallistujilla, jotka joivat humalahakuisesti useammin kuin kerran kuukaudessa, oli selvästi korkeammat GT- ($p < 0.0005$) ja ALAT-aktiivisuudet ($p < 0.0005$) kuin saman riskikategorian alkoholinkäyttäjillä, joiden juominen ei ollut humalahakuista.

Epäsuotuisien elintapariskitekijöiden kokonaismäärän sekä seerumin GT-, ALAT-, CRP-, kolesteroli-, HDL-, LDL- ja triglyseridiarvojen välillä havaittiin yhdenmukaisia annos-vastesuhteita. Verrattuna niihin, joilla ei ollut riskitekijöitä, kaikkien tutkimuksen kohteena olleiden biomarkkereiden osalta viite- tai tavoitearvoista poikkeavien arvojen monimuuttujakorjatut ristitulosuhteet (OR) olivat merkitsevästi korkeammat niillä, joiden riskipisteiden summa oli kaksi tai enemmän. Tarkasteltaessa ryhmää, jossa oli eniten riskitekijöitä, selvimmät ristitulosuhteiden nousut havaittiin miesten seerumin GT-aktiivisuuksissa: OR 26.6 (12.4–57.0), ALAT-aktiivisuuksissa: OR 40.3 (5.3–307.8), CRP-arvoissa: OR 16.2 (7.8–33.7) ja triglyseridiarvoissa: OR 14.4 (8.6–24.0). Rasvamaksaan viittaavan rasvamaksaindeksin eli FLI-arvon ≥ 60 esiintyminen lisääntyi miehillä 2.4 %:sta 81.9 %:iin, kun kokonaisriskipisteet nousivat 0 pisteestä 7–8 pisteeseen ($p < 0.0005$) ja naisilla vastaavasti 0 %:sta 73.5 %:iin ($p < 0.0005$). Merkittävimmät yksittäiset vaikutukset rasvamaksaan viittaavan FLI-arvon (≥ 60) todennäköisyyteen havaittiin fyysisellä passiivisuudella ($p < 0.0005$ molemmilla sukupuolilla) ja alkoholinkulutuksella, erityisesti miehillä ($p < 0.0005$).

Tutkimus osoittaa, että laboratoriokokeiden systemaattinen käyttö potilaiden seurannassa saattaa parantaa elintapoihin ja käyttäytymiseen liittyvien terveysriskien arviointia. Tutkimuksen tulokset myös korostavat humalahakuisen alkoholinkäytön mahdollisia haitallisia seurauksia maksan toimintaan jopa niillä, joiden alkoholin kokonaiskulutus on vähäisen riskin kulutustasoa. Useiden epäsuotuisien elintapariskitekijöiden samanaikaiseen esiintymiseen osoitettiin liittyvän selviä poikkeavuuksia maksan toimintaa, elimistön tulehdustilaa ja lipidiprofiilia kuvaavissa laboratoriotutkimuksissa sekä suuri maksan rasvoittumisen todennäköisyys. Laboratoriotutkimusten käyttö voi olla hyödyllistä myös interventioissa, joiden päämääränä on ylläpitää terveellisiä elintapoja.

CONTENTS

List of Figures

List of Tables

ABBREVIATIONS

ADH	alcohol dehydrogenase
AH	alcoholic hepatitis
ALD	alcoholic liver disease
ALDH	aldehyde dehydrogenase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
ANOVA	analysis of variance
APRI	aspartate aminotransferase to platelet ratio index
ASH	alcoholic steatohepatitis
AST	aspartate aminotransferase
AUD	alcohol use disorders
AUDIT	Alcohol Use Disorders Identification Test
AUDIT-C	an abbreviated version of AUDIT
BAC	blood alcohol concentration/content
BDE	binge drinking episode
BMI	body mass index
CAGE	Cut down, Annoyed, Guilty, Eye-opener
CD14	cluster of differentiation 14
CDC	Center for Disease Control and Prevention
CI	confidence interval
CRP	C-reactive protein
CDT	carbohydrate-deficient transferrin
CYP2E1	cytochrome P450 2E1 enzyme
DNA	deoxyribonucleic acid
ELF	enhanced liver fibrosis
EMA	European Medicines Agency
EtG	ethyl glucuronide
FAEE	fatty acid ethyl ester
FIB-4	FIB-4 score
FINRISK	national FINRISK study
FLI	fatty liver index
GGT	gamma-glutamyltransferase
GSH	glutathione

HCC	hepatocellular carcinoma
HDL	high-density lipoprotein
HED	heavy episodic drinking
ICD-10	international statistical classification of diseases and related health problems
IDF	International Diabetes Federation
LDL	low-density lipoprotein
LPS	lipopolysaccharide
MAST	Michigan Alcoholism Screening Test
MAST-G	Michigan Alcoholism Screening Test – Geriatric Version
MCV	mean corpuscular volume
MONICA	project monitoring trends and determinants in cardiovascular disease
MRS	magnetic resonance spectroscopy
NAD+	nicotinamide adenine dinucleotide
NADH	reduced form of nicotinamide adenine dinucleotide
NADP+	nicotinamide adenine dinucleotide phosphate
NADPH	reduced form of nicotinamide adenine dinucleotide phosphate
NAFL	non-alcoholic fatty liver
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NFS	NAFLD fibrosis score
NIAAA	National Institute on Alcohol Abuse and Alcoholism
OR	odds ratio
PEth	phosphatidyl ethanol
PNPLA3	patatin-like phospholipase-3
RNS	reactive nitrogen species
ROS	reactive oxygen species
SADD	Short-form Alcohol Dependence Data Questionnaire
SD	standard deviation
SF-12	12-Item Short Form Health Survey
SMAST-G	Short Michigan Alcoholism Screening Test – Geriatric Version
TE	transient elastography
TLFB	Timeline Follow-back Method
TNF-α	tumor necrosis factor-α
U.K.	United Kingdom
ULN	upper limit of normal
US	ultrasound
WHO	World Health Organization

ORIGINAL PUBLICATIONS

Paper I Niemelä O, Nivukoski U, Bloigu A, Bloigu R, Aalto M and Laatikainen T (2019): Laboratory test based assessment of WHO alcohol risk drinking levels. Scand J Clin Lab Invest 79:58-64.

Paper II Nivukoski U, Bloigu A, Bloigu R, Aalto M, Laatikainen T and Niemelä O (2019): Liver enzymes in alcohol consumers with or without binge drinking. Alcohol 78:13-19.

Paper III Nivukoski U, Niemelä M, Bloigu A, Bloigu R, Aalto M, Laatikainen T and Niemelä O (2019): Impacts of unfavourable lifestyle factors on biomarkers of liver function, inflammation and lipid status. PLoS One 14:e0218463.

Paper IV Nivukoski U, Niemelä M, Bloigu A, Bloigu R, Aalto M, Laatikainen T, Niemelä O (2020): Combined effects of lifestyle risk factors on fatty liver index. BMC Gastroenterology 20(1):109.

1 INTRODUCTION

Unfavourable lifestyle-related factors – including excess alcohol drinking, smoking, obesity, poor diet and physical inactivity – are increasing threats to health in modern societies. Alcohol drinking is associated with a broad range of health problems, which may emerge even at relatively low levels of consumption (Lim et al. 2012; Wittchen 2012; Rehm et al. 2013; Spanagel et al. 2013; Connor et al. 2016; GBD 2016 Alcohol and Drug Use Collaborators 2018; Åberg et al. 2020). There may also be different types of manifestations of health problems resulting from different patterns of drinking, such as chronic drinking or repeated episodes of binge drinking when several drinks are consumed within relatively short periods of time. The total consumption of alcoholic beverages among persons aged 15 years or older in Finland in 2019 was 10.0 litres of one hundred percent alcohol per capita. Although total alcohol consumption has declined slightly over the last 10 years, binge drinking remains a relatively common national problem (Åberg et al. 2017; Finnish Institute for Health and Welfare 2018; Terveyden ja hyvinvoinnin laitos 2020).

Health problems due to overweight and obesity have also shown a rapid increase throughout the world over past decades. Current national statistics indicate that over half of our population are overweight (body mass index, BMI $\geq$ 25 and < 30 kg/m^2), and about 20% are obese (BMI $\geq$ 30 kg/m^2) (Männistö et al. 2012; Finnish Institute for Health and Welfare 2018). Statistics further indicate that, at the population level, excess body weight also increases with age (Table 1) (Finnish Institute for Health and Welfare 2018).

Table 1. Proportions of overweight and obese adults in Finland (Finnish Institute for Health and Welfare 2018)

	At least overweight	Obese
Young adults aged 18–29 years		
men	47%	17%
women	35%	19%
Adults aged ≥ 30 years		
men	72%	26%
women	63%	27%

Obesity markedly increases the risk of morbidity and mortality, including diabetes, non-alcoholic fatty liver disease (NAFLD) or cardio- and cerebrovascular diseases (Dixon 2010; Kotronen et al. 2010; Welsh et al. 2013; Danielsson 2014). Smoking and physical inactivity are other common modifiable risk factors of lifestyle, which increase the incidence of chronic diseases (Li et al. 2018; Rutten-Jacobs et al. 2018). There may also be additive effects in the unfavourable lifestyle factors in individuals with clustering of such factors (Ruhl and Everhart 2005b; Puukka et al. 2006; Alatalo et al. 2008; Loomba et al. 2009; Li et al. 2018).

A wide variety of laboratory tests are known to be sensitive to lifestyle factors (Danielsson 2014). Recent studies have indicated that the activities of the common liver enzymes gamma-glutamyltransferase (GGT) and alanine aminotransferase (ALT) in the circulation are readily elevated as a result of excess body weight, especially when co-occurring with regular alcohol consumption, which suggests additive hepatotoxic effects for unfavourable lifestyle determinants (Kim et al. 2008; Ruhl and Everhart 2009; Danielsson 2014; Lau et al. 2015; Niemelä et al. 2017). Smoking and alcohol may also have significant synergistic effects in increasing liver enzyme activities (Breitling et al. 2009; Park et al. 2013). The changes in these biomarkers also appear to be associated with abnormalities in biomarkers of inflammation and lipid status (Ruttmann et al. 2005; Lee et al. 2008).

As yet, however, only a few studies have compared the individual and joint impacts of the various unfavourable lifestyle factors on clinical chemical laboratory indices of health. The purpose of this work was to investigate the effects of alcohol, overweight, smoking and physical inactivity on biomarkers of liver function, inflammation and lipid status in a large series of apparently healthy individuals. The present work also aimed at investigating the individual and combined effects of lifestyle risk factors on the fatty liver index (FLI), an algorithm recently designed for the prediction of fatty liver (Bedogni et al. 2006).

2 REVIEW OF THE LITERATURE

2.1 Common lifestyle factors and health

At the same time as people's life expectancy has increased worldwide, several chronic diseases which can be attributed to an unfavourable lifestyle have become more common. Excessive alcohol drinking, excess caloric intake, smoking, lack of physical activity and poor diet are common lifestyle-related risk factors which may contribute to adiposity, fatty deposition in the internal organs and increased all-cause mortality (Lim et al. 2012; Behrens et al. 2013; Connor et al. 2016; Li et al. 2018; Rutten-Jacobs et al. 2018). Various determinants of an unfavourable lifestyle also frequently occur in combinations in the same individual (McGinnis et al. 2002; Niemelä et al. 2017). Recent studies have indicated that adopting a healthy lifestyle even at the age of 50 could add more than a decade to one's life expectancy (Li et al. 2018), suggesting significant therapeutic possibilities for interventions aimed at maintaining a favourable lifestyle (Tamakoshi et al. 2009; Li et al. 2018; Teeriniemi et al. 2018).

Alcohol use disorders, both acute and chronic, are among the most significant lifestyle-related clinical problems with devastating health impacts and high prevalence throughout the world (Connor et al. 2016; Niemelä 2016). Virtually all tissues in the body can be affected by excessive alcohol consumption (Lieber 1995). It is true, however, that previous studies on the relationships between alcohol consumption and overall mortality have often yielded a J-shaped graph, suggesting that light to moderate alcohol drinkers have the lowest risk of cardiovascular diseases, but this may be due to the inclusion of former heavy drinkers in the subset of abstainers (de Gaetano et al. 2016), and more recent surveys have indeed concluded that the average health risks at any level of alcohol consumption will not be lower than those in abstainers (GBD 2016 Alcohol Collaborators 2018).

Excessive energy intake relative to energy consumption over a long period of time will lead to excessive accumulation of adipose tissue in the internal organs, and certain changes that have taken place in current societies, e.g. the reduced amount of physical work and daily exercise and the availability of energy-rich foods, have led to an increasing prevalence of overweight and obesity (Hill et al. 2012; Vandevijvere et al. 2015; Styne et al. 2017). The presence of obesity significantly increases the risk

of many diseases, such as type 2 diabetes (Guh et al. 2009; Rooney et al. 2015), fatty liver (Loomis et al. 2016), coronary heart disease (Wilson et al. 2002; Guh et al. 2009), osteoarthritis (Zheng and Chen 2015), hypertension (Wilson et al. 2002), dementia (Loef and Walach 2013) and asthma (Beuther and Sutherland 2007).

Smoking is also known to cause a significant increase in the risk of many diseases, especially certain cancers and both respiratory and circulatory diseases (Mason et al. 1985; Christensen et al. 2018; Erzurumluoglu et al. 2019). But in addition to contributing to the development of diseases, smoking also increases the risk of disease complications and may detract from the effectiveness of therapies in individual patients (Rodriguez-Merchan 2018). Thus, smoking has a negative effect on the prognoses for diseases, and it has been estimated that prolonged smoking can shorten one's life expectancy by several years as compared to people who have never smoked (Statistics Canada 2015). It has also been suggested that there is a gender difference in smoking-related morbidity, in that female smokers have a higher risk of lung cancer and cardiovascular diseases than male smokers (Huxley and Woodward 2011; Appelman et al. 2015).

A lack of physical activity has recently been recognized as an increasingly important lifestyle-associated contributor to poor health. The Current Care Guideline concerning physical activity for adults aged 18 to 64 years recommends both moderate-intensity aerobic exercise and exercise that maintains or increases muscle strength and endurance. The recommended time spent on physical activity is dependent on the load, so that brisk walking (64–76% of maximal heart rate) should take place for at least 150 minutes per week and strenuous exercise (77–93% of maximal heart rate), such as running, for 75 minutes per week (Liikunta: Current Care Guidelines 2016; Langhammer et al. 2018). Studies on the relationships between physical activity and health have indicated that sufficient physical activity can provide protection against cardiovascular disease, stroke, diabetes, and some types of cancer. In addition, physical activity has been shown to have a positive effect on mental health, wellbeing and the quality of life, as well as delaying the onset of dementia (Taylor et al. 1985; Langhammer et al. 2018).

Assessments of the health effects induced by combinations of lifestyle-related factors have so far been limited. Breitling et al. (2009) showed that the joint impact of alcohol consumption and smoking on levels of GGT is greater than their individual effects taken together, i.e. the combination of moderate to heavy alcohol consumption and heavy smoking increased GGT levels more than did moderate to heavy consumption by itself. Sánchez-Jiménez et al. (2018) reported that patients with both alcoholic and non-alcoholic steatohepatitis had a higher risk of

cardiovascular disease than patients with alcoholic or non-alcoholic steatohepatitis. The risk of cardiovascular disease seems to be especially high when the alcohol consumption of an overweight person (BMI > 25 kg/m^2) exceeds 140 grams per week.

2.2 Alcohol-related health effects

2.2.1 Main features of alcohol metabolism

Upon ingestion, ethanol is absorbed by passive diffusion through the stomach wall before continuing through the duodenum and small intestine (Lieber 2005; Manzo-Avalos and Saavedra-Molina 2010; Danielsson 2014). It is then freely distributed in the body, particularly to organs with a rich blood supply, including the brain and lungs. Exposure to alcohol is greatest in the liver, however, since this receives blood directly from the stomach and small bowel via the hepatic portal vein (Foster and Marriott 2006). Since ethanol is metabolized mainly in the liver, this organ is also among the major targets for alcohol toxicity (Lieber 1995), since it eliminates the absorbed ethanol primarily through metabolism (95–98%), the remainder being removed in the urine, breathed out through the lungs or excreted in sweat (Manzo-Avalos and Saavedra-Molina 2010; Cederbaum 2012).

Alcohol metabolism takes place through several distinct biochemical pathways. In the principal pathway a cytosolic enzyme, alcohol dehydrogenase (ADH), catalyses the oxidation of alcohol to acetaldehyde in a process called dehydrogenation (Figure 1), in which hydrogen is transferred from ethanol to the cofactor nicotinamide adenine dinucleotide (NAD+), converting it to the reduced form NADH (Weir 1978; Baraona and Lieber 1979; Lieber 1995; Manzo-Avalos and Saavedra-Molina 2010; Wilson and Matschinsky 2020). Acetaldehyde is highly toxic and its generation may lead to a wide range of toxic effects on cells and tissues (Thompson 1986; Lieber 1995; Eriksson 2001; Setshedi et al. 2010). Its electrophilic nature enables it to bind to proteins and macromolecules to form adducts, i.e. covalent chemical addition products, with proteins, lipids and DNA (Freeman et al. 2005; Setshedi et al. 2010), which in turn may play an important pathogenic role in the toxicity of alcohol in tissues (Jokelainen et al. 2000; Niemelä 2001; Seitz and Stickel 2007; Thiele et al. 2008). ADH shows a constant oxidation capacity of

approximately 0.1 grams of alcohol per kilogram of body weight per hour, although the elimination rates may be lower in patients with liver damage (Cederbaum 2012).

The cytochrome P450 2E1 (CYP2E1) and catalase enzymes also metabolize alcohol to acetaldehyde. CYP2E1 is an inducible enzyme and is typically induced after a person has consumed large amounts of alcohol. The oxidation of alcohol by CYP2E1 occurs in the liver microsomes, in a process in which nicotinamide adenine dinucleotide phosphate (NADPH) and oxygen are involved, with CYP2E1 acting as a catalyst. Ethanol is oxidized to acetaldehyde and the oxygen is reduced to form H_2O, while NADPH serves as a donor of hydrogen, forming NADP+. Catalase-mediated metabolism, which accounts for only a small fraction of the body's alcohol metabolism (2%), may take place in the peroxisomes of the brain, where, in an oxidation reaction catalyzed by catalase, ethanol and hydrogen peroxide (H_2O_2) react to form acetaldehyde and water. The limited use made of this pathway is due to the rather low rates of H_2O_2 generation. Minute amounts of alcohol are also removed by interaction with fatty acids and the generation of compounds called fatty acid ethyl esters (FAEEs), which may also play a role in alcohol-induced tissue damage in the liver and pancreas (Lieber 1995; Manzo-Avalos and Saavedra-Molina 2010; Cederbaum 2012; Wilson and Matschinsky 2020).

Acetaldehyde becomes further oxidized to another, less active by-product, acetate, by the enzyme aldehyde dehydrogenase (ALDH), which has both cytosolic and mitochondrial isoforms. Acetate from all pathways is converted to acetyl-CoA by ATP-dependent acetyl-CoA synthetases, whereupon it becomes a substrate for the citric acid cycle, producing cellular energy and releasing water and carbon dioxide (Lieber 1995; Manzo-Avalos and Saavedra-Molina 2010; Cederbaum 2012).

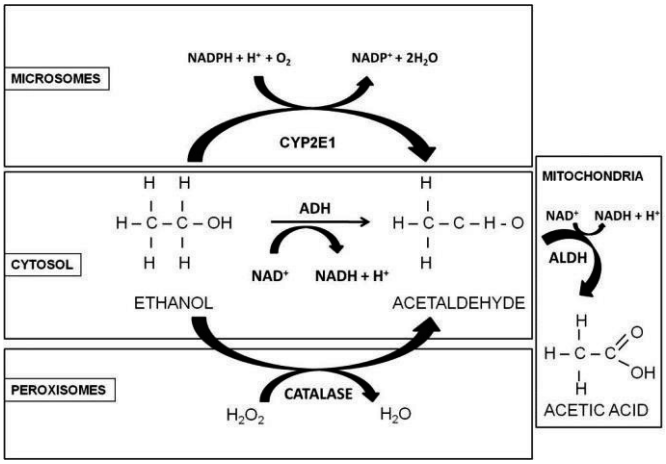

Figure 1. Main pathways of ethanol metabolism. The most common pathway involves the enzymes alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH). ADH metabolizes alcohol to acetaldehyde, which is metabolized in the second step to acetate, which then is metabolized to water and carbon dioxide for elimination (modified from Manzo-Avalos and Saavedra-Molina 2010).

All the above ethanol metabolism pathways induce oxidative stress and the production of reactive oxygen species (ROS), which play a pivotal role in alcohol-induced cellular injuries. These represent highly reactive chemical species containing oxygen, e.g. the superoxide anion radical and hydrogen peroxide (Lieber 1997; Wu et al. 2006; Albano 2008; Zhou et al. 2013). Ethanol metabolism also promotes glutathione depletion, lipid peroxidation and mitochondrial damage in tissues (Setshedi et al. 2010). One major oxidative stress-mediated injury pathway appears to be related to induction of the CYP2E1 enzyme (Lieber 1997; Lucas et al. 2005; Wu et al. 2006), which metabolizes and activates several toxicological substrates to more reactive products and can be induced during a variety of pathophysiological conditions (Ohnishi and Lieber 1977; Lieber 2005). CYP2E1 produces ROS, and also hydroxyl radicals in the presence of iron catalysts (Zakhari 2013), resulting in cellular damage, structural changes in DNA, and the inhibition or activation of genes associated with growth and cell death (Lu and Cederbaum 2008; Walker et al. 2013).

2.2.2 Assessment of alcohol drinking: Levels and patterns

The terms low, moderate, high and very high risk drinking are frequently used when discussing the relationship between alcohol consumption and health risks. The

challenge when using these terms, however, is that their definitions vary greatly from one country to another. In addition, there is variation in the definition of standard drink sizes. In their recent compilation of the definitions used in 37 countries, Kalinowski and Humphreys (2016) found that although the modal definition of one standard drink used by the World Health Organization (WHO) is 10 grams of absolute alcohol, the range is as wide as 8–20 grams. There has also been considerable variation in the limits of low risk drinking, within ranges of 10–42 grams per day for women and 10–56 grams per day for men, or 98–160 grams per week for women and 150–280 grams per week for men.

The Finnish Institute for Health and Welfare categorizes the drinking of alcohol into three classes: low-risk, moderate-risk and high-risk drinking, according to a recommendation from the Finnish Medical Society Duodecim's working group and the Finnish Society of Addiction Medicine (Alkoholiongelmaisen hoito: Current Care Guidelines 2018). In this classification a dose contains 12 grams of absolute ethanol and low-risk consumption encompasses up to one dose (women) and two doses (men) per day on average. By comparison, the limit for moderate-risk drinking is based on data regarding objective signs of tissue toxicity, the current thresholds being more than seven doses but less than 12–16 doses per week continuously for women and more than 14 doses but less than 23–24 doses per week continuously for men. When the weekly doses exceed the above-mentioned figures (≥ 20 grams of absolute ethanol per day for women and ≥ 40 grams for men), alcohol consumption can be linked to a multitude of health problems. In that case we can speak of high-risk consumption (Alkoholiongelmaisen hoito: Current Care Guidelines 2018; Terveyden ja hyvinvoinnin laitos 2020), the Finnish thresholds for which are in line with the daily thresholds of heavy drinking as set out by the European Medicines Agency (EMA) (2010): 60 grams of absolute ethanol or more on average for men, and 40 grams or more for women.

The current WHO classification of risk drinking defines five risk levels: abstinence, low risk, medium risk, high risk and very high risk, specified separately for the genders (Table 2). Low risk drinking refers to 1–40 grams of absolute ethanol per day for men and 1–20 grams for women, while the limits of medium-risk drinking are 41–60 grams per day for men and 21–40 grams for women, those for high risk drinkers 61– 100 grams per day for men and 41–60 grams for women and very high risk drinking implies daily consumption exceeding 100 grams for men and 60 grams for women (Witkiewitz et al. 2017a; 2017b).

Table 2. The WHO classification of risk drinking

Risk level	Absolute ethanol per day	
	Men	Women
Low risk	1–40 g	1–20 g
Medium risk	41–60 g	21–40 g
High risk	61–100 g	41–60 g
Very high risk	> 100 g	> 60 g

The classifications described above nevertheless largely ignore the pattern of alcohol consumption, even though it may be hypothesized that the drinking pattern will also have a major effect on the harm caused to health by the intake of alcohol. The focus in many previous studies of alcohol-related health risks, however, has been on total cumulative alcohol consumption rather than on comparing the adverse health effects of different drinking patterns. Binge drinking is defined as a pattern of drinking that typically consists of occasional alcohol consumption, which may typically exceed 60 grams of alcohol for men and 40 grams for women on a single occasion (World Health Organization 2000a). The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines binge drinking as a pattern of drinking that yields ethanol concentrations in the blood amounting to 0.08 g/dL (= 0.8‰) and above. In real life situations that level of blood ethanol is reached in about two hours with five drinks for men and four drinks for women (National Institute of Alcohol Abuse and Alcoholism 2004).

Since it is quite common to consume much higher amounts of alcohol than the standard binge drinking threshold, the term "high-intensity drinking" has been introduced to distinguish typical binge drinking from alcohol intakes at double the threshold level or more (Patrick et al. 2016; Patrick and Azar 2018; Rosoff et al. 2019). On the other hand, binge drinking can be viewed from the aspect of frequency, with the term heavy episodic drinking (HED) being used for frequent, regular binge drinking. The definition of HED put forward by the World Health Organization (2018a) is the consumption of 60 grams or more of pure alcohol on at least one occasion at least once a month.

Based on two dimensions of drinking mentioned above, amount and frequency, Jastrzębska et al. (2016) have recently classified alcohol consumption into six patterns as follows:

1) Abstinence.

2) Social drinking (moderate drinking): alcohol consumption is usually not more than 2–3 units per day.

3) Hazardous drinking: consumption is more than 1–2 units (women) or 3–4 units (men) per day.

4) Binge drinking (single occasion drinking): consumption is occasionally more than 5 units of alcohol per day. The term refers to heavy drinking over a short period of time, or drinking to intoxication or drunkenness.

5) Heavy drinking (harmful drinking, alcohol abuse): consumption is regularly more than 6 units of alcohol per day. The term refers to a drinking pattern that has already caused some physical or mental health problems but which does not meet the criteria for alcohol dependence.

6) Dependent drinking: chronic drinking of alcohol that fulfils at least three of the following criteria: tolerance of alcohol, withdrawal symptoms after cessation of drinking, impaired control, preoccupation with the acquisition or consumption of alcohol, persistent desire or unsuccessful efforts to quit, sustained social, occupational or recreational disability, and continued consumption despite the adverse consequences.

2.2.3 Typical alcohol-related health risks

Excessive alcohol consumption is associated with a wide variety of public health problems and considerable health care costs. In addition to causing physical and mental illness, alcohol consumption is also a substantial risk factor in traffic accidents, violent incidents, etc. and is often a cause of homicides, drownings, burns, poisonings, falls and other injuries (World Health Organization 2018b). Thus it is a major threat to social well-being both at the individual level and in societies around the world (Lim et al. 2012; Connor et al. 2016; Rehm et al. 2017). Alcohol can affect almost all tissues in the body and has been shown to be a causal factor in more than 200 diseases and in injuries of various types (World Health Organization 2018a).

Typical disease categories in primary health care that may be associated with excessive alcohol consumption include hypertension, insomnia, liver problems, depression and anxiety disorders (Rehm et al. 2016). Alcohol use disorder (AUD) refers to harmful consumption of alcohol and alcohol dependence, often involving a compulsion to use alcohol, a loss of control over alcohol intake, and a negative emotional state when access to alcohol is prevented (ICD-10, International Statistical Classification of Diseases and Related Health Problems; see also Rehm et al. 2005; 2016; Mason 2017). The world-wide prevalence of AUD in 2016 was 5.1% of the adult population (World Health Organization 2018b).

Gender plays an essential role in the development of AUD, as according to the World Health Organization (2018b) its prevalence is 8.6% among adult men but only 1.7% among adult women. Abstinence is a more likely pattern of alcohol consumption among women (Volpato et al. 2004), although they are also more susceptible to the toxic effects of alcohol than men, possibly due to women´s lower body water content and gastric ADH activity (Table 3). The development of many health problems in female drinkers is also different from that in men, so that alcohol dependence, major depressive disorders and anxiety disorders, for instance, develop faster in women (European Medicines Agency 2010). Alcohol-related disorders such as cirrhosis of the liver, neuropsychiatric disorders and cancers are currently five times more common in men than in women, and mortality from these disorders is up to 10 times higher in men, due to differences in drinking habits; men drink more often and consume larger amounts at a time than women do (Brenner et al. 2017).

The impact of ethanol intake on various types of tissue injury seems to be dependent on both the total amount of alcohol consumed and the pattern of drinking. Chronic heavy drinking may lead to different types of health problems from those created by acute (binge-type) drinking. Interestingly, some studies have also concluded that regular low to moderate drinking may even be associated with some beneficial effects on cardiovascular health (Bagnardi et al. 2004; Corrao et al. 2004; Krenz and Korthuis 2012; Walker et al. 2013). In the light of recent studies, however, it appears that no "safe limits" can be set for alcohol consumption (Brenner et al. 2017, Åberg and Färkkilä 2020). Gamma-glutamyltransferase (GGT) enzyme activity may become elevated at much lower alcohol intake levels than those implied in the current limits for heavy drinking in many national guidelines (Niemelä et al. 2017), and the actual alcohol doses leading to possible hepatotoxic effects seem to be markedly lower in persons above 40 years of age (Niemelä et al. 2017). In accordance with this view, Wood et al. (2018) have recently proposed that there is a need to revisit the concept of risk thresholds in many national and international recommendations. According to their large population-based study of nearly 600,000 participants the lowest risk of premature death was among those whose weekly consumption of alcohol did not exceed 100 grams of absolute alcohol. That study did not, however, include any separate assessment for gender, although it is known that men and women are not equal in their susceptibility to alcohol-related injuries (Schenker 1997). A recent meta-analysis (GBD 2016 Alcohol Collaborators 2018) also showed that increasing levels of alcohol consumption are significantly associated with an increasing risk of all-cause mortality and that the safest level for drinking appears to be zero. Thus, in order to minimize adverse health consequences, one

should refrain from drinking alcohol. These authors also recommended revising alcohol policy with the aim of markedly reducing population-level consumption. Although some authors (Foerster et al. 2009; de Gaetano et al. 2016) have promoted alcohol consumption at low to moderate levels as one component of a healthy diet, it appears that any regular alcohol intake will lead to an increase in health problems and should therefore be avoided (Connor and Hall 2018; Wood et al. 2018). The World Cancer Research Fund (2020) also recommends a total refusal of alcohol in order to reduce the risk of cancer.

Although the goal in the treatment of alcohol use disorders has usually been complete abstinence, this may be an overly restrictive end point in routine clinical practice (Knox et al. 2018). Recent evidence has indicated that any reduction in alcohol consumption could yield notable health benefits (Hasin et al. 2017; Witkiewitz et al. 2017a; 2017b; Knox et al. 2018). Studies on alcohol-dependent patients have shown that any reduction in the WHO risk drinking level would predict significantly fewer alcohol-related adverse consequences and improved functioning (Kline-Simon et al. 2013; Laramée et al. 2015; Hasin et al. 2017; Witkiewitz et al. 2017a; 2017b), while greater decreases in the WHO risk levels would predict fewer alcohol-related consequences and improved mental health. A decrease of at least 1 level would predict a significant decrease in adverse alcohol-related consequences relative to participants without any change in WHO risk level, even during a 1-year follow-up, and a similar decrease of at least 1 level would also predict a significant improvement in mental health as measured by the 12-item Short Form Health Survey (SF-12 (Ware et al. 1996), although the improvement after 1 year of follow-up was more clearly noted in those participants who achieved a decrease of at least 2 levels (Hasin et al. 2017; Witkiewitz et al. 2017a; 2017b).

Table 3. Factors contributing to alcohol metabolism and the risk of associated tissue toxicity

Risk factor	Characteristics
Gender	Women have a higher percentage of body fat and a lower water content (66%) than men (75%). Alcohol is distributed to body fluids but not to fat, so that the same amount of alcohol typically results in 15–20% higher blood alcohol levels in women than in men with the same body weight. Women also show lower gastric activity of ADH, the enzyme responsible for the first step in metabolizing alcohol (Schenker 1997; Cederbaum 2012).
	Since alcohol passes easily through the placenta to the foetus, the only safe choice for a pregnant woman is abstinence, due to the very poor ability of the fetal liver to eliminate alcohol (van Faassen and Niemelä 2011; Warren et al. 2011; Cederbaum 2012).
	Estrogen may increase the expression of CD14 (cluster of differentiation 14, lipopolysaccharide-binding protein) in the liver, which may result in hepatic sensitization to endotoxin and thus increase ethanol-induced liver damage (Sato et al. 2001).
Interactions	The synergistic effect of adiposity and alcohol abuse may induce endoplasmic reticulum cell stress, activation of inflammatory cells and changes in adiponectin (Gao and Bataller 2011; Xu et al. 2011) and also liver fibrogenesis (Naveau et al. 1997; Raynard et al. 2002; Zakhari 2013). According to animal experiments, the use of alcohol in combination with a high-fat diet and high iron intake can lead to more serious manifestations of the disease (Tsukamoto et al. 1995). Synergistic effects have also been reported between alcohol use and smoking (Breitling et al. 2009).
Age	Starting alcohol consumption during adolescence elevates the risk of developing high-risk drinking habits, alcohol dependence and associated tissue toxicity (Meier and Seitz 2008; Hatton et al. 2009; Gao and Bataller 2011; Cederbaum 2012; Spear and Swartzwelder 2014).
	The body's water content decreases with age, as does the activity of the enzymes involved in the metabolism of ethanol. As a result, blood ethanol concentrations increase more in older individuals than in younger ones. Diseases and medications can also impair alcohol tolerance (Meier and Seitz 2008; Cederbaum 2012). Age also affects the sensitivity of liver enzyme responses to alcohol intake (Tynjälä et al. 2012).
Drinking habits	The metabolism of alcohol is induced in heavy drinkers prior to the development of significant liver disease (metabolic tolerance) (Cederbaum 2012).
Genetic factors	Genetic factors have an influence on the risk of alcoholic liver disease (ALD). Carriers of the patatin-like phospholipase-3 (PNPLA3) I148M variant are exposed to an elevated risk of liver cirrhosis (Trépo et al. 2011; Chamorro et al. 2014). Other genetic variants which may contribute to individual susceptibility to ALD concern the enzymes involved in alcohol metabolism, oxidative stress and endotoxin-mediated inflammation

	(Stickel and Hampe 2012; Wu et al. 2012). Polymorphisms of the ADH and ALDH genes will influence the rates of acetaldehyde generation and associated toxicity (Setshedi et al. 2010; Stickel et al. 2017).
Ethnic background	Differences in the elimination rate of alcohol have been observed between the isoforms of ADH. Genetic variations of the genes encoding ADH and ALDH are very common among East Asians (Chinese, Japanese, and Korean) and these render the oxidation of acetaldehyde far less efficient and cause acetaldehyde accumulation, which results in facial flushing and an unpleasant physical feeling in association with alcohol consumption (Lee et al. 2014). Some studies have suggested an increased rate of alcohol elimination by native Americans as compared with Caucasians (Cederbaum 2012).
Nutritional status	Alcohol absorption is more rapid when it is ingested in a fasting state. On the other hand, prolonged fasting and protein deficiency reduce the activity of the ADH enzyme, resulting in a slower elimination of alcohol (Cederbaum 2012).
Beverage type	Relative beneficial health effects of beer or wine consumption in particular have been suggested with respect to cardiovascular diseases, but this may be explained by the presence of other ingredients (polyphenols and their metabolites) in the product rather than ethanol (Arranz et al. 2012).
Diseases	A damaged liver will have an impaired ability to eliminate alcohol, leading to a decrease in alcohol metabolism (Cederbaum 2012). Synergistic effects have been observed between alcohol use and liver injury due to hepatitis viruses or human immunodeficiency virus, non-alcoholic fatty liver disease, or iron overload (Szabo et al. 2010; Gao and Bataller 2011; Zakhari 2013; Teschke 2019).
	Regular excessive alcohol consumption increases blood pressure and leads to the exacerbation of various heart problems. People with anxiety and depression symptoms can experience aggravation of their symptoms following heavy alcohol consumption (Ashworth and Gerada 1997; Charlet and Heinz 2017; Iida-Ueno et al. 2017; Day and Rudd 2019).

2.2.3.1 Alcohol and the liver

Alcoholic liver disease (ALD), a well-known condition which is known to result from chronic alcohol drinking (Stickel et al. 2017; Hosseini et al. 2019), currently represents a significant burden on health care, as according to the World Health Organization (2018b), over 40% of liver-related deaths are attributable to alcohol. Morphologically, ALD includes a wide range of abnormalities, from fatty liver (steatosis) to alcoholic hepatitis (steatohepatitis) and progressive fibrosis and cirrhosis. These may subsequently lead to other severe complications such as hepatocellular carcinoma (HCC) and liver failure (Diehl 1998; Lombardi et al. 2015;

Rinella 2015). These injuries usually develop in a sequential manner, but can also be present in various combinations (Diehl 1998; Day 2007). Fatty liver is commonly asymptomatic but occasionally hepatomegaly is found as its primary manifestation (Chrostek and Panasiuk 2014). Patients with alcoholic hepatitis usually have combinations of symptoms and signs: jaundice, loss of appetite, pain or discomfort in the right upper quadrant of the liver, ascites, muscle weakness, confusion, pedal oedema, petechial spots and sometimes fever and hepatomegaly, which may be tender. Cirrhosis in combination with alcoholic hepatitis manifests itself in the following possible symptoms: severe anorexia, weight loss, fatigue, muscle cramps, ascites, palmar erythema and pedal oedema. Jaundice and moderate to tense ascites are present in majority of patients, but liver tenderness does not occur (Sharma and Arora 2020).

Excess deposition of liver fat develops in most heavy drinkers, but such drinking does not always lead to the development of more severe forms of ALD. It is estimated that steatosis is present in up to 90% of heavy drinkers and 30–35% of these develop advanced forms of ALD. Steatosis progresses to cirrhosis in approximately 10–20% of heavy drinkers (Stickel and Hampe 2012; Jokelainen 2013; Lombardi et al. 2015; Moreno et al. 2019). The onset, progression and clinical outcome of liver cirrhosis may all be influenced by genetic and environmental factors, although their interplay has remained unclear (Table 3) (Ramos-Lopez et al. 2015; Stickel et al. 2017).

The clinical symptoms of ALD range from asymptomatic hepatic steatosis to malaise, anorexia, weight loss, abdominal discomfort, tender hepatomegaly and jaundice, all of which characterize the advanced stages of the disease (Diehl 1997; Rehm et al. 2013). Discontinuation of alcohol consumption can allow the steatosis, and even steatohepatitis, to return to normal within a few months, but the cirrhosis that has already occurred is irreversible (Shah et al. 2020).

Due to its key role in the metabolism of ethanol, the liver is an important target for its toxicity. Upon the oxidation of ethanol to acetaldehyde NAD is reduced to NADH, resulting in the promotion of fatty acid synthesis and fat accumulation in the hepatocytes (Lieber 1995; Mahli and Hellerbrand 2016). In fact, ethanol becomes the primary substrate for oxidation in the liver following alcohol intake (Foster and Marriott 2006), whereas the oxidation of fatty acids is inhibited, leading to an accumulation of triglycerides in the liver (Baraona and Lieber 1979; Day 2007). This occurs in more than 90% of heavy drinkers (Gao and Bataller 2011), and in most cases it is devoid of clinical symptoms except for an enlarged liver (hepatomegaly). Excessive alcohol consumption lasting for only a few days may be enough for the

steatosis to appear, whereas recovery from a fatty liver condition usually requires up to 4–6 weeks of abstinence (Lieber 1995; Diehl 1998; O'Shea et al. 2010). When hepatocellular injury and inflammation develop as a consequence of continued heavy alcohol consumption, alcoholic hepatitis (AH) or alcoholic steatohepatitis (ASH) can be diagnosed (Avila et al. 2020). Chronic inflammation and injury will lead to the deposition of collagen and other extracellular matrix proteins in tissue through the activation of hepatic stellate cells and portal fibroblasts. As the imbalance between extracellular matrix production and degradation continues, fibrosis progresses and leads to changes in the structure of the liver, with distortion of the hepatic circulation and tissue architecture, which are characteristics of liver cirrhosis (Lieber 1995; Lombardi et al. 2015; Avila et al. 2020).

Activation of Kupffer cells and the release of pro-inflammatory cytokines appear to play a key role in the development of ALD (Basista et al. 1993). Tumor necrosis factor-α (TNF-α) is a major proinflammatory cytokine which also seems to be an important mediator of tissue damage in ALD (Kitazawa et al. 2003; Marcos et al. 2009), although the exact mechanism by which it aggravates alcohol toxicity has remained unclear, but it is known that the protein CD14 can bind lipopolysaccharides (LPS), which are endotoxins present in the acute-phase immune system, and that CD14 promoter polymorphisms may lead to an increase in susceptibility to advanced ALD (Järveläinen et al. 2001; Campos et al. 2005; Danielsson 2014).

Recent experimental findings have also shown an association between ALD, CYP2E1 action and the degradative autophagy pathway (Wu et al. 2012). The regulation and function of this autophagy pathway and of lipid metabolism are related, in that the inhibition of autophagy causes lipid accumulation (Amir and Czaja 2011; Dong and Czaja 2011). CYP2E1-derived ROS may play a role in inhibiting autophagy and thereby promoting ethanol-induced hepatotoxicity, steatosis and oxidative stress (Wu et al. 2012).

2.2.3.2 Extrahepatic health effects of alcohol consumption

Relationships between alcohol consumption and a wide spectrum of extrahepatic health effects have been established in numerous studies. Alcohol consumption has been shown to increase the risk of cardiovascular diseases, including hypertensive heart disease, cardiomyopathy, atrial fibrillation, flutter and haemorrhagic and other non-ischaemic strokes (Ashworth and Gerada 1997; Lim et al. 2012; Wood et al. 2018). Alcohol consumption increases the risk of carcinogenesis in the upper

gastrointestinal tract, liver, colon, rectum and the female breast, which may occur even at low levels of alcohol intake (Lieber et al. 1979; Bagnardi et al. 2013; Rehm et al. 2017). According to a statement issued by the American Society of Clinical Oncology, more than 5% of new cases of cancer are caused by alcohol consumption. The carcinogenic property of alcohol is partly explained by its reactive metabolite, acetaldehyde, which, as a group 1 carcinogen, stimulates cell proliferation and induces DNA damage. Increased sex hormone levels in the circulation as a result of alcohol consumption may contribute to an elevated breast cancer risk (Zaitsu et al. 2020). Several studies have also indicated that heavy drinking and alcoholism constitute a major cause of depression and other mental disorders (Tucker et al. 1982; Ashworth and Gerada 1997; Rehm et al. 2016).

Alcohol consumption increases the risk of infectious diseases (Hurley 1977). Excessive alcohol consumption has been shown to impair the functions of phagocytes such as polymorphonuclear leucocytes (especially neutrophils) and macrophages, one of which is to engulf dead cells, and they are thus considered to be the first responders to inflammation of the immune system (Szabo 1998; Rehm et al. 2017). Stimulation of inflammation due to excessive alcohol consumption may play a pivotal role in the sequence of events leading to tissue injury. Several proinflammatory and anti-inflammatory cytokines have been shown previously to be increased in the circulation as a result of heavy alcohol consumption (Latvala et al. 2005). Alcohol also has an effect on the function and number of T-cells (Pasala et al. 2015), leading to changes in the immune system that may occur in an alcohol dose-dependent manner (Sureshchandra et al. 2019).

Alcohol consumption can also lead to acute intoxication, poisoning or injuries due to a loss of judgment (Ashworth and Gerada 1997). O'Dwyer et al. (2019), who recently examined the characteristics of alcohol-related hazards among 4,338 drinkers with different drinking patterns: low-risk drinking, occasional heavy episodic drinking, monthly heavy episodic drinking and alcohol dependence, found a linear association between drinking pattern and the experiencing of hazards, with the proportion of hazards being lowest among the low-risk drinkers and highest among cases of alcohol dependence.

2.2.4 Alcohol consumption and dietary factors

A significant role for alcohol in the aggravation of inflammation and oxidative stress has been found when it is consumed together with a high-fat diet (Tsukamoto et al.

1995; Caro and Cederbaum 2004; Zheng et al. 2017). Diets rich in carbohydrates and processed or red meat but low in vegetables, fruits or vitamins could also have harmful metabolic and hepatic consequences in association with alcohol consumption (Halsted et al. 2002; Li et al. 2014; Manuel et al. 2016; Romero-Gómez et al. 2017; Zheng et al. 2017).

Regular alcohol intake as part of one's diet may also contribute to the development of overweight and obesity (Traversy and Chaput 2015). Alcohol is a rich source of energy, providing 7 kcal (29 kJ) per gram of alcohol, whereas corresponding amounts of carbohydrates and protein produce 4 kcal of energy, and fat produces 9 kcal (Cederbaum 2012). Men typically consume alcohol in larger quantities than women (Nielsen et al. 2012) and are also more likely to consume beer, which is more energy-intensive, containing more carbohydrates than wine, which is often preferred by women (Yeomans 2010). Drinkers who continuously consume large amounts of alcohol can replace almost 60% of their daily energy intake with alcohol, which can lead to malnutrition and nutrient deficiencies. In addition to alcohol consumption, changes in the metabolism of nutrients can also be a significant co-factor in the pathogenesis of alcohol-induced tissue injury (Fawehinmi et al. 2012).

2.3 Assessment of alcohol consumption

2.3.1 Self-reports

Various self-report and questionnaire-based techniques have been developed to identifying alcohol abuse, examples of which include AUDIT (Alcohol Use Disorders Identification/Inventory Test), CAGE (Cut down, Annoyed, Guilty, Eye-opener), MAST (Michigan Alcoholism Screening Test), SADD (Short-form Alcohol Dependence Data Questionnaire) and TLFB (Time-Line Follow-Back). The first four are tools for screening alcohol consumption and related problems and addiction, while TLFB is used to assist in estimating the actual amounts of alcohol consumed. The definition of an alcohol problem in the above tests is based primarily on the patient's self-assessment and honesty in answering the questions, and therefore their results often need to be supplemented in real-life situations with laboratory tests and medical examinations. The risk involved in self-assessments arises from the patient's need to conceal the quantities of alcohol consumed, to

remember them incorrectly and to minimize the stigma attached to them (Livingston and Callinan 2015; Niemelä 2016; Sánchez-Jiménez et al. 2018). According to Livingston and Callinan (2015), underestimation of alcohol consumption is most typical among young men and middle-aged and elderly women. In addition to intentional underestimation, reporting can sometimes be impaired by forgetfulness induced directly by the consumption of highly intoxicating doses of alcohol (Patrick 2016).

The AUDIT questionnaire consists of ten questions, three of which concern quantitative estimates of alcohol consumption (drinking frequency, volume consumed and frequency of binge drinking), three questions explore symptoms of alcohol dependence and four questions are used to collect information on alcohol-related hazards and problems (Saunders et al. 1993). AUDIT is generally considered the most reliable questionnaire for use in clinical work (Avila et al. 2020). Given a maximum score of 40, the limit for risky drinking is considered to be a score of 8, although a much lower cut-off score (4–5) is recommended in a review by Kranzler & Soyka (2018). A lower cut-off score has also been recommended when screening for heavy drinking among the elderly (Aalto et al. 2011). The AUDIT-C questionnaire is an abbreviated version of AUDIT, including only three questions concerning the frequency of alcohol consumption, the number of drinks on one occasion and the frequency of binge drinking during the past year. The limit for risky drinking is considered to be a score of 3 for women and 4 for men, given a maximum score of 12 (Torruellas et al. 2014). Aalto et al. (2011) have reported that AUDIT and AUDIT-C are accurate when screening for hazardous alcohol drinking among the elderly (65–74 years), provided that the cut-off points are properly set for this age group. They suggested cut-off scores of at least 5 points for AUDIT and at least 4 points for AUDIT-C.

CAGE is a short, simple questionnaire that includes four items regarding the reduction or cessation of alcohol drinking, others' concern about the patient's alcohol drinking, the sense of guilt caused by drinking, and the return to alcohol as an eye-opener in the morning. The questions are answered "yes" or "no", with two affirmative answers pointing to a possible alcohol abuse problem which requires further investigation (Bush et al. 1987; Choe et al. 2019). The advantages of CAGE are its brevity compared with AUDIT and its high sensitivity for the severe stages of alcohol abuse. Its weakness lies in the insufficiency of the information given on the actual amounts of alcohol consumed (Choe et al. 2019).

MAST is a test developed to screen for alcohol problems by means of 25 "yes/no" questions related to the patient's self-appraisal of the social, vocational and

family problems possibly associated with heavy drinking. The 24-item MAST-G (Michigan Alcoholism Screening Test – Geriatric version) and its 10-item abbreviated version, SMAST-G, have been developed to detect alcohol misuse primarily among elderly people (Selzer 1971; Westermeyer et al. 2004; Johnson-Greene et al. 2009; Knightly et al. 2016).

The SADD questionnaire, which screens for the severity of alcohol dependence, looks at this phenomenon on a scale ranging from mild to severe, i.e. from minor drinking problems to severe alcohol dependence. The 15-item self-report questionnaire emphasizes the subjective and behavioural aspects of alcohol dependence (Davidson and Raistrick 1986; Rosa-Oliveira et al. 2011).

TLFB is a tool for the retrospective collection of data concerning alcohol consumption as the number of standard drinks consumed over a particular period of time ranging from one day to one year (Kuteesa et al. 2019; Martin-Willett et al. 2020). Vakili et al. (2008) have shown that a three-month data collection period can be recommended in most cases, and that these data can also be used to estimate annual consumption. This saves time and resources with little or no loss in accuracy. TLFB data can also be used to form an estimate of drinking patterns representing the most common styles: daily drinking, weekend or holiday drinking, drinking on special occasions, or binge vs. non-binge drinking (Allen et al. 1992).

2.3.2 Biomarker-based assessment of alcohol consumption

A variety of laboratory tests have been made available to assess alcohol consumption or alcohol-related organ damage and the outcomes of therapy, and to aid in many specific applications, such as forensic medicine. The laboratory tests can be divided into measurements of direct and indirect biomarkers. The former refer to analyses of ethanol itself and its specific metabolites, whereas the latter biomarkers are compounds released as a result of organ damage caused by harmful alcohol use, particularly that caused by liver diseases (Hastedt et al. 2013). The biomarker is selected based on what one is looking for; the object of interest may be the monitoring of abstinence during detoxification, for instance, or assessment of the level of chronic excessive drinking (Andresen-Streichert et al. 2018) (Table 4.). Since factors other than alcohol can also cause similar organ damage, laboratory tests can give false positive values. Indirect biomarkers thus have limited specificities, which vary widely depending on the population to be studied (Hastedt et al. 2013). In addition to direct biomarkers, the most widely used laboratory tests for assessing

alcohol consumption are serum gamma-glutamyltransferase (GGT) activity, carbohydrate-deficient transferrin (CDT) and mean corpuscular volume (MCV), or their combinations, such as GGT-CDT (Niemelä 2016; Andresen-Streichert et al. 2018).

Analysis of ethanol or its metabolites in serum or urine can reliably detect acute alcohol intake, and in combination with clinical observations such measurements can also give information on long-term drinking habits (Niemelä 2016). Methods have also been made available for detecting specific metabolites of ethanol, including phosphatidyl ethanol (PEth) or ethyl glucuronide (EtG). These assays have the advantage of detecting alcohol consumption for several days or even weeks after blood ethanol levels have reached zero (Andresen-Streichert et al. 2018).

Phosphatidylethanol (PEth) is a cellular membrane phospholipid produced in a transphosphatidylation reaction catalyzed by phospholipase D. This reaction takes place exclusively in the presence of ethanol (Aradottir et al. 2006; Viel et al. 2012). PEth is a group of glycerophospholipids with fatty acid groups of varying lengths and varying degrees of saturation. Out of the 50 or so PEth homologues, it is homologue 16:0/18:1 that is typically determined in analyses (Andresen-Streichert et al. 2018). PEth has been shown to be a promising biomarker for detecting chronic excessive alcohol consumption due to its high diagnostic sensitivity and specificity (Viel et al. 2012). PEth can be detected in blood as early as 1–2 hours after alcohol intake (Andresen-Streichert et al. 2018), and its mean half-life ranges from 3 to 12 days. Excessive alcohol consumption can be identified in PEth measurements from red cells even after two weeks of abstinence. The normalization rate of PEth shows interindividual variation, but seems to be independent of gender, age and body mass index (Viel et al. 2012). Helander et al. (2012) found that PEth is more sensitive for detecting relapses in alcoholic patients than is serum carbohydrate-deficient transferrin (CDT).

Ethyl glucuronide (EtG) is a specific metabolite issuing from the non-oxidative metabolism of ethanol (Wurst et al. 1999). Although only 0.1% of ingested ethanol is degraded through this pathway, EtG can be detected in biological fluids even after the consumption of relatively small amounts of ethanol (Mackus et al. 2017). EtG can be detected in urine for up to about 24 hours even after consumption of small quantities of ethanol. After excessive use EtG may be detectable for up to 130 hours (Andresen-Streichert et al. 2018). EtG assays are performed mainly on serum and urine samples but to some extent on other materials, too, such as meconium and hair. Like PEth, EtG has been increasingly used to monitor abstinence in outpatient treatment settings (Torruellas et al. 2014; Andresen-Streichert et al. 2018).

Gamma-glutamyltransferase (GGT) is a glycoprotein enzyme which is found on the cell membrane in several tissues with high secretory or absorptive activities, mainly liver, kidney and pancreas. Small amounts are present in other tissues such as brain, spleen and heart (Conigrave et al. 2003; Kazemi-Shirazi et al. 2007). GGT plays a central role in the metabolism of cysteine and glutathione and thus regulates the oxidation-reduction state of cells (Lee et al. 2004; Danielsson 2014; Hanigan 2014). The serum GGT level rises as a result of chronic alcohol consumption and remains elevated for 2–4 weeks after the cessation of alcohol abuse (Andresen-Streichert et al. 2018). In the assessment of excessive alcohol drinking it is useful both for identifying chronic alcohol abuse and for monitoring sobriety (Whelan 1992; Niemelä 2016). On the other hand, GGT is not specific to alcohol abuse, as many other factors influence its activity, including obesity, advanced age, multiple types of liver disease and some medications (Torruellas et al. 2014). It has been found previously that being overweight alone, without the effect of alcohol, may raise GGT activity by an average of 20% from normal levels and obesity may do so by 30-40% (Alatalo et al. 2008; Danielsson et al. 2014). GGT levels correlate only moderately with actual self-reported alcohol intake; r = 0.30–0.40 in men and 0.15–0.30 in women (Conigrave et al. 2003). The specificity of serum GGT for detecting alcohol abuse in various populations has shown large variations due to a variety of other factors that can lead to elevations in its expression (Jastrzębska et al. 2016). Alterations in serum GGT concentrations can arise in hospitalized patients for various reasons, including pancreatic problems, myocardial disease, renal injury, chronic obstructive pulmonary disease, diabetes or the use of medications (Kazemi-Shirazi et al. 2007), but it has nevertheless been considered a highly useful parameter for detecting individuals with heavy alcohol consumption in health screening programmes (Jastrzębska et al. 2016). A single episode of alcohol drinking does not usually increase the serum GGT level unless the person has a history of excessive alcohol consumption. Drinking patterns have an effect on serum GGT levels but its magnitude has remained unclear. Some studies have suggested that regular drinking is more likely to elevate GGT levels than binge drinking (Conigrave et al. 2003; Jastrzębska et al. 2016), whereas other groups of investigators have found notable increases in GGT levels in frequent binge drinkers (Åberg et al. 2017).

Carbohydrate-deficient transferrin (CDT) is generated as a result of heavy drinking through a decrease in the transferrin isoform with four sialic acid chains and a corresponding increase in the proportion of transferrin with 0–2 sialic acid chains (Stibler and Hultcrantz 1987; Mikkelsen et al. 1998; Niemelä 2016). CDT levels may remain elevated for a further 2–3 weeks after the cessation of alcohol consumption

(Andresen-Streichert et al. 2018). This marker is especially suitable for monitoring treatment in alcoholic patients as it rises in a quite sensitive manner in cases of relapse. Unlike the liver enzymes, increased CDT levels in the circulation are rarely caused by anything other than excess alcohol consumption (Niemelä 2016).

The combination marker GGT-CDT has been found to be a still more sensitive and specific indicator of excessive alcohol consumption than either of its individual components alone. GGT-CDT is calculated by means of the mathematical formula GGT-CDT = 0.8 × ln(GGT) + 1.3 × ln(%CDT) (Hietala et al. 2006). GGT-CDT returns to normal an average of 2–3 weeks after the cessation of alcohol drinking and is well suited for monitoring sobriety. Interpretations must, however, also take account of the fact that GGT levels may increase for reasons unrelated to alcohol, e.g. liver disease or the use of drugs (Hietala et al. 2006; Niemelä 2016).

Alanine aminotransferase (ALT) or *aspartate aminotransferase* (AST) assays are normally used as markers to assess the presence or absence of liver disease (see section 2.8.1.2). In addition to heavy alcohol drinking, the activities of these transaminases may be affected by many other factors such as physical exercise, gender, body mass index and hepatotoxic medicines (Fallatah 2014).

Increased *mean corpuscular volume* (MCV) (macrocytosis) is common among subjects with chronic heavy alcohol drinking (Unger and Johnson 1974; Wu et al. 1974; Morgan et al. 1981; Niemelä 2007). Increased MCV in subjects without anaemia is often due to excessive ethanol consumption. Although the analytical sensitivity of MCV for detecting harmful drinking is usually less than that of CDT or GGT, it is commonly used in combination with these markers. Due to the long life span of erythrocytes (120 days), elevated MCV is a useful indicator of chronic alcohol consumption, whereas it cannot be used to detect acute ethanol intake or relapse (Tavakoli et al. 2011). Macrocytosis may also occur for reasons other than alcohol abuse, such as vitamin B_{12} or folate deficiency and hypothyroidism (Kaferle and Strzoda 2009).

Table 4. Characteristics of common biomarkers of alcohol consumption (modified from Torruellas et al. 2014; Shukla et al. 2017; Andresen-Streichert et al. 2018)

Biomarker (abbreviation)	Sensitivity	Specificity	Searching for
Phosphatidylethanol (PEth)	88–100%	48–89%	Abstinence monitoring
Ethyl glucuronide (urine) (EtG)	89%	99%	Abstinence monitoring
Ethyl glucuronide (serum) (EtG)	85%	89%	Abstinence monitoring
Gamma-glutamyltransferase (GGT)	37–95%	18–93%	Chronic excessive drinking
Carbohydrate-deficient transferrin (CDT)	46–90%	70–100%	Chronic excessive drinking
GGT-CDT combination (GGT-CDT)	83–90%	95–98%	Harmful or heavy alcohol consumption
Mean corpuscular volume of erythrocytes (MCV)	40–50%	80–90%	Chronic excessive drinking
Alanine aminotransferase (ALT)	15–40%	50–57%	Chronic excessive drinking
Aspartate aminotransferase (AST)	25–60%	47–68%	Chronic excessive drinking

2.4 Obesity and health

2.4.1 Indices of obesity

2.4.1.1 Body mass index

The body mass index (BMI) is widely used for categorizing underweight, normal weight, overweight and obese adults. BMI is calculated as a person's weight in kilograms divided by the square of that person's height in metres (kg/m^2). The World Health Organization classifies weight status based on BMI as follows: BMI < 18.50 kg/m^2 (underweight), BMI 18.50–24.99 kg/m^2 (normal weight) and BMI ≥ 25 kg/m^2 (overweight). Overweight can be divided into four subgroups: BMI 25–29.99

kg/m² preobese, BMI 30–34.99 kg/m² obese class I, BMI 35–39.99 kg/m² obese class II and BMI ≥ 40 kg/m² obese class III (World Health Organization 2000b).

BMI has been shown to be a rather specific (0.90) but not very sensitive (0.50) indicator of excessive body fat (Okorodudu et al. 2010). The interpretations may be influenced by variations in muscle mass, so that a higher than average muscle mass, for example, will give a higher than average body mass index (Abramowitz et al. 2018).

BMI correlates with total fat in the body, whereas it does not distinguish between visceral fat, i.e. excess fat in the abdominal and visceral areas, and subcutaneous fat, which is less harmful to health. Previous studies have also indicated that BMI correlates fairly well with body fat in young and middle-aged individuals, whereas it might not be an ideal measure of adiposity in the elderly (Mathus-Vliegen 2012). To get a better understanding of the amount and location of fat, it is necessary to supplement the BMI with other measurements such as waist circumference (Bigaard et al. 2005).

2.4.1.2 Waist circumference

While the BMI is most often chosen as an indicator of adiposity in clinical work, waist circumference is another useful measure which correlates well with total and intra-abdominal fat. The measurement of waist circumference is considered one of the most reliable ways of detecting metabolic syndrome, which comprises a cluster of metabolic abnormalities such as central obesity, hypertension, a high level of triglyserides together with a low level of high-density lipoprotein cholesterol, and glucose intolerance (Rinella 2015; Owolabi et al. 2018).

According to the International Diabetes Federation (IDF) definition, a person will be classified as having the metabolic syndrome, if the waist circumference is at least 94 cm (men) or at least 80 cm (women) and if at least two of the following additional criteria are met:

1) blood glucose ≥ 5.6 mmol/L or previously diagnosed type 2 diabetes
2) reduced high-density lipoprotein (HDL) cholesterol; < 1.0 mmol/L (men) or < 1.3 mmol/L (women), or specific treatment for low HDL
3) blood triglyserides > 1.7 mmol/L or specific treatment for elevated triglyserides
4) elevated blood pressure (≥ 130 mmHg/85 mmHg) or treatment for previously diagnosed hypertension (Saklayen 2018).

By contrast, the WHO criteria for metabolic syndrome do not include waist circumference, but do include the waist-hip ratio (> 0.9 for men and > 0.85 for women) or BMI (> 30 kg/m^2) as an alternative criterion. Other WHO criteria for metabolic syndrome are:

1) reduced high-density lipoprotein (HDL) cholesterol; < 0.9 mmol/L (men) or < 1.0 mmol/L (women)
2) blood triglyserides > 1.7 mmol/L
3) elevated blood pressure (≥ 140 mmHg/90 mmHg) (Saklayen 2018).

2.4.2 Obesity-related health problems

The increasing prevalence of overweight has caused an increasing burden on health care during the past decades (Männistö et al. 2012; Finnish Institute for Health and Welfare 2018). The prevailing Western lifestyles and dietary overconsumption have been suggested as the likely explanations for this phenomenon. Current statistics for adult populations show that the rates of obesity have doubled in the past 20 years and tripled among children within a single generation. Recent reports further suggest that by 2040 as many as half of the adult population in the United States may be obese (Brill 2013; Ogden et al. 2014; Preston et al. 2014). In Finland, 72% of men who are at least 30 years old are overweight, the corresponding percentage for women being 63% (Finnish Institute for Health and Welfare 2018).

Obesity is a consequence of excess calories from food in the long term relative to the body's energy consumption. Over recent decades energy consumption has declined, while the consumption of energy-dense foods has led to a much more intense calorie intake. The excess energy is stored as adipose tissue, mainly under the skin but also in the abdominal cavity. The reduction in energy consumption is primarily due to a more sedentary lifestyle and the reduced need for manual labour. Factors contributing to obesity also include the stress associated with a busy life, various mental disorders, and impaired sleeping habits. Indeed, increased obesity and its associated clinical conditions are currently among the most serious burdens on public health throughout the world (Hill et al. 2012; Vandevijvere et al. 2015).

Individual susceptibility to obesity varies widely and is largely genetically controlled, but the amount and quality of food consumed is also influenced by acquired habits (Hasselbalch 2010). The cause of genetic obesity is suspected to be the impairment of appetite control, although some genetic factors also affect the accumulation of adipose tissue in various parts of the body (Loos 2018). It has also

been shown that deficiencies in the regulation of emotions may also cause problems with eating (Goldschmidt et al. 2017; van Strien 2018).

Obesity is associated with an increased risk of type 2 diabetes, fatty liver, elevated blood pressure, myocardial disease, stroke, osteoarthritis, obstructive sleep apnoea, musculo-skeletal disorders and multiple types of cancer (breast, ovarian, prostate, liver, kidney and colon cancers, for example) (Finnish Institute for Health and Welfare 2018; Blüher 2019). In addition, obesity has been associated with dementia, Alzheimer's disease and a number of psychiatric disorders (Kivipelto et al. 2005; Avila et al. 2015; Blüher 2019). Due to the greater risks involved, obesity is also associated with a significant decrease in life expectancy (Finnish Institute for Health and Welfare 2018; Blüher 2019).

Studies of the pathogenesis of obesity-related disorders have indicated that, in addition to lipid storage, adipose tissue also plays an active role as an endocrine organ (Kaur 2014). Adipose tissue contains adipocytes, preadipocytes, and cells with active immunological functions, such as macrophages, leucocytes and lymphocytes (Halberg et al. 2008). Under conditions of hypoxia due to a reduced blood supply to enlarged adipocytes there may be adipocyte cell death and macrophage infiltration into the adipose tissue. This may result in the production of proinflammatory mediators, adipocytokines and other biologically active mediators of inflammation (Trayhurn and Wood 2004; Cinti et al. 2005). The effect on adipose tissue is not limited to local inflammation, however, but causes systemic inflammation, which in turn can cause dysfunction in various tissues such as the liver (Brunt 2011; Yki-Järvinen 2014).

2.4.3 Obesity and the liver

Obesity is currently one of the most common causes of liver disease throughout the world. While there may be notable differences in the prevalence of obesity-induced fatty liver disease in various populations, the global prevalence is estimated to be around 25% and relatively similar in the U.S. and the European countries (Younossi et al. 2016).

Overweight and obesity are associated with the accumulation of fat (triglycerides) in the liver and adipose tissue. Non-alcoholic fatty liver disease (NAFLD) is defined by the manifestation of at least 5% hepatic fat content in individuals with no history of hazardous drinking (exceeding 30 grams per day in men or 20 grams per day in women) and the absence of competing liver disease aetiologies such as chronic viral

hepatitis, drug-induced injury or autoimmune hepatitis (Yki-Järvinen 2014; Younossi et al. 2016). The clinical presentation of NAFLD ranges from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) and cirrhosis (Yki-Järvinen 2014). NAFLD is often associated with an elevated risk of cardiovascular disease, diabetes or metabolic syndrome (Musso et al. 2012). The prevalence of NAFLD and the associated disease burden appear to be continuously increasing (Ofosu et al. 2018), so that it is essential to introduce more means of disease prevention.

The most effective treatment for NAFLD is weight loss, since as much as a few kilograms of weight loss can markedly reduce the amount of fat in the liver (Musso et al. 2012). Physical exercise may also reduce liver fat, even without any notable loss of body weight (Yki-Järvinen 2014). It remains unclear, however, as to whether patients with NAFLD should be counselled for total alcohol abstinence in order to prevent disease progression. Petroni et al. (2019) have recently reviewed the 15 original studies and three review articles making up the literature concerning NAFLD and alcohol intake and concluded that there is a potential for disease progression even with low to moderate alcohol consumption. Their work also emphasized the association between alcohol intake and an increased risk of cancer, particularly in women.

2.5 Smoking and health

The situation in Finland is that 16% of men and 11% of women aged 30 years or more are smoking daily. Although successful interventions have reduced the prevalence of smoking over the past decades, it continues to be one of the most significant individual lifestyle risk factors related to mortality, affecting virtually all tissues in the body (Finnish Institute for Health and Welfare 2018). Smoking has been shown to lead to many adverse health consequences, including chronic bronchitis, emphysema and lung fibrosis, myocardial infarction, stroke and carcinogenesis in the lungs, upper gastrointestinal tract, pancreas and colon (Altamirano and Bataller 2010). Smoking has been estimated to be responsible for approximately 4,000 deaths annually in Finland, (Finnish Institute for Health and Welfare 2018).

Cigarette smoking has also been shown to correlate with alcohol consumption (Bien and Burge 1990; Volpato et al. 2004), and smoking and alcohol drinking may also exert synergistic effects in creating and exacerbating health problems, including liver diseases (Altamirano and Bataller 2010; Li et al. 2014). Smoking either alone or

especially in conjunction with alcohol consumption elevates the risk of abnormal GGT activity (Breitling et al. 2009; Niemelä et al. 2017), and heavy smoking has also been shown to lead to a significant loss in life expectancy, which can be accentuated further in the presence of other lifestyle-related risk factors (Jha and Peto 2014; Li et al. 2014).

Accordingly, the cessation of smoking may yield significant health benefits: persons who stop smoking before 40 years of age having started in early adulthood avoid over 90% of the excess risk during their next few decades of life as compared with those who continue to smoke, and the corresponding percentage for those who quit smoking at the age of 50 is still more than 50% (Jha and Peto 2014).

2.6 Physical activity and health

Physical inactivity has been widely recognized as a major lifestyle-related contributor to poor health (Sundberg 2016; Warburton and Bredin 2016), and it is evident that prolonged sitting time has become an increasingly common habit in our current societies. Sedentary behaviour may be associated with a number of adverse health outcomes, including cardiovascular problems, diabetes and carcinogenesis. Thus the lack of physical exercise increases the risk of premature mortality at the individual level and may also lead to a substantial economic burden on society (Chomistek et al. 2013; Kyu et al. 2016; Smith et al. 2016; Romero-Gómez et al. 2017; Li et al. 2018; Ekelund et al. 2019). On the other hand, regular physical activity yields notable long-term health benefits, e.g. in reducing hepatic steatosis and improving symptoms of insulin resistance (Lawlor et al. 2005; St George et al. 2009; Oh et al. 2015; Kyu et al. 2016; Perreault et al. 2017). Moderate to vigorous physical activity has recently been shown to improve hepatic steatosis in fatty liver disease through the reduction of inflammation and oxidative stress even in those without any notable changes in BMI status (Oh et al. 2015). Thus, there may be significant health benefits of exercise even before any loss of body weight occurs (Johnson et al. 2012; Sullivan et al. 2012; Oh et al. 2015). A recent U.K. biobank-based study concluded that physically active individuals have longer life expectancies regardless of the level of adiposity than those with low activity levels (Zaccardi et al. 2019). Interestingly, subjects taking physical fitness walks at a brisk pace also seem to have longer life expectancies independently of the various levels and indices of adiposity than do subjects reporting a slow pace of walking (Zaccardi et al. 2019). In addition to physical well-

being, regular activity appears to improve mental health, emotional, psychological and social well-being and also cognitive function (Langhammer et al. 2018).

2.7 Coffee consumption and health

Coffee is one of the most widely consumed beverages all over the world. It is known to be a rich source antioxidative bioactive compounds, which may be related to protection from the hazardous effects of free radicals (Butt and Sultan 2011, Morisco et al. 2014; Saab et al. 2014).

Several studies have suggested hepatoprotective effects of coffee drinking. Experimental results have demonstrated that coffee drinking reduces fat accumulation and collagen deposition in the liver, and elsewhere it has been reported to reduce the fat content of the liver and the severity of fibrosis (Anty et al. 2012; Molloy et al. 2012; Morisco et al. 2014). Coffee drinking may also reduce the risks of developing liver cirrhosis and hepatocellular carcinoma (Klatsky and Armstrong 1992; Gallus et al. 2002; Tverdal and Skurtveit 2003; Klatsky et al. 2006; Catalano et al. 2010; Birerdinc et al. 2012; Gutiérrez-Grobe et al. 2012; Chen et al. 2014; Saab et al. 2014).

The histologically assessed benefits of a daily coffee intake may, however, be dose-dependent (Corrao et al. 2001; Modi et al. 2010), as subjects who consumed at least two cups of coffee daily are reported to have shown only half the rate of chronic liver disease recorded in those who drank less than one cup of coffee daily (Ruhl and Everhart 2005a).

There have also been reports of an inverse association between coffee consumption and serum GGT (Arnesen et al. 1986; Nilssen et al. 1990; Casiglia et al. 1993; Nilssen and Førde 1994; Tanaka et al. 1998; Danielsson et al. 2013) and ALT activities (Ikeda et al. 2010; Saab et al. 2014). In addition, coffee consumption has been associated with lower ALT levels among individuals at risk of developing liver injury due to factors such as excess body weight, impaired glucose metabolism or alcohol abuse (Ruhl and Everhart 2005b).

Case-control studies have pointed to an inverse association with insulin resistance among coffee drinkers and thus a reduced incidence of type 2 diabetes and cardiovascular diseases (Catalano et al. 2010; Gutiérrez-Grobe et al. 2012; Morisco et al. 2014; Saab et al. 2014). Moreover, coffee consumption is associated with a reduced risk of other chronic diseases such as Parkinson's disease and neurodegenerative diseases (Morisco et al. 2014; Saab et al. 2014). Despite the

healthy effects of coffee, its consumption is also associated with a risk of side effects such as gastric irritation, anxiety or sleep problems (Nawrot et al. 2003; Freedman et al. 2012; Bhatti et al. 2013; Torres and Harrison 2013).

2.8 Use of biomarkers to assess lifestyle-related health effects

2.8.1 Liver enzymes

The common liver enzymes are known to be readily elevated by factors such as alcohol intake, excess body weight and smoking, these influences apparently being most striking in the case of gamma-glutamyltransferase (GGT) and serum alanine aminotransferase (ALT), the activities of which are also commonly used to screen for abnormal liver function (Niemelä 2016). It has been suggested that GGT is sensitive to alcohol consumption, whereas ALT is frequently elevated in obese individuals. In addition to monitoring liver status, however, it should be remembered that changes in liver enzyme activities may also be associated with extrahepatic conditions, including cardio- and cerebrovascular diseases, tissue deposition of triglycerides and insulin resistance (Ruttmann et al. 2005; Kazemi-Shirazi et al. 2007; Kim et al. 2008; Niemelä 2016).

2.8.1.1 Gamma-glutamyltransferase (GGT)

Serum GGT is traditionally used as an indicator of liver dysfunction and as a marker of excessive alcohol consumption (see also section 2.3.2). All forms of liver disease increase GGT activity, especially biliary obstruction, whereas mild to moderate increases (2–5 times normal) may be seen in patients with fatty liver. Thus GGT can be used to a limited extent to screen alcohol consumption in patients with co-existing liver diseases or in hospitalized patients (Salaspuro 1999; Niemelä 2007).

The elevation in serum GGT level that follows a heavy intake of alcohol occurs within 1–14 days, whereas normalization of serum GGT levels upon abstinence usually takes 2–4 weeks in patients without liver disease. Thus a GGT level that is still elevated after several weeks of abstinence is a sign of liver disease (Conigrave et al. 2003; Hastedt et al. 2013; Jastrzębska et al. 2016; Niemelä 2016).

The activation of GGT is associated with the generation of oxidative stress (Lee et al. 2004; Kazemi-Shirazi et al. 2007; Takigawa et al. 2008). A major biological

function of GGT lies in the maintenance of the intracellular levels of glutathione, which is the major antioxidant in mammalian cells, and the metabolism of glutathione conjugates (Zhang and Forman 2009). GGT may thus be capable of regulating oxidative stress, the development of superoxide ions, hydrogen peroxide and unintended oxidation of low-density lipoprotein within the vascular endothelium (Emdin et al. 2005). Accordingly, the existing evidence strongly suggests that elevated GGT activity may be a biomarker of oxidative stress and proinflammatory status in the body (Lee et al. 2004; Danielsson et al. 2014).

2.8.1.2 Serum aminotransferases (AST, ALT)

Serum ALT is a transaminase enzyme located in the cytosol of the hepatocytes with an essential role in catalyzing the transfer of amino groups to generate products of gluconeogenesis and amino acid metabolism. ALT is also present in kidney, heart and skeletal muscle cells, while both acute and chronic hepatocellular injuries result in increased serum aminotransferase activities. While ALT originates primarily from the hepatocytes, aspartate aminotransferase (AST) is found additionally in the heart, skeletal muscle tissue, kidneys and brain. As a consequence, serum ALT is a more specific marker of liver disease, whereas AST often shows increased activity originating from injury to the heart or skeletal muscle (Pratt and Kaplan 2000).

Increases in serum aminotransferase activity can be traced to a variety of reasons such as heavy alcohol intake, excess body weight, viral or autoimmune hepatitis, iron overload, muscle diseases, or strenuous exercise (Niemelä 2016). Over half of the abnormal aminotransferase findings in Western countries are currently estimated to result from obesity and its comorbidities (Kim et al. 2008). Even mild to moderate weight gain may be associated with an increase in serum liver enzymes (Alatalo et al. 2008; Lee et al. 2001). When alcohol consumption and adiposity occur together, an increased risk of abnormal ALT activity is seen (Alatalo et al. 2008). Activities exceeding the upper normal limits two to three-fold indicate a higher risk of liver injury and are common even in moderate drinkers, if they present with overweight. In cases of obesity, ALT activities mark ectopic fat deposition, and the values tend to decline with weight loss (Luyckx et al. 1998; Tiikkainen et al. 2003; St George et al. 2009). The co-occurrence of increased ALT, the deposition of triglycerides and liver steatosis has been linked with type 2 diabetes, metabolic syndrome and insulin resistance. Like the changes in GGT, ALT levels also seem to be able to predict vascular morbidity (Lee et al. 2008; Danielsson 2014; Niemelä 2016; Karaphillis et al. 2017).

Also in common with GGT activity, ALT activity has been shown to be associated with NAFLD (Clark and Diehl 2003; Kotronen et al. 2007; Schindhelm et al. 2007; Ghouri et al. 2010). Mild elevations in ALT were found in nearly half of a series of NAFLD patients even without any alcohol consumption (Brunt 2004). On the other hand, more than half of all NAFLD patients have activities below the reference values (Browning et al. 2004; Kotronen et al. 2007).

2.8.1.3 The AST/ALT ratio

The combined use of aminotransferases provides a useful clinical tool for examining the nature of liver disease. An increased AST/ALT ratio has been considered to suggest an alcoholic aetiology (Nalpas et al. 1984; Salaspuro 1987; Sheth et al. 1998; Pratt and Kaplan 2000). Alcohol-related deficiency in pyridoxal 5'-phosphate (vitamin B6) in patients with more advanced liver disease may reduce ALT serum activity and contribute to an increase in the AST/ALT ratio (Diehl et al. 1984). Alterations in the relative activities of AST and ALT in alcoholic patients may be due to pronounced hepatic mitochondrial damage and skeletal or cardiac muscle damage (alcoholic myopathy). (Nalpas et al. 1984), and changes in the relative activities of the liver enzymes may also have a predictive value in terms of a disease prognosis (Nakanishi et al. 2004; Goessling et al. 2008; Yun et al. 2009; Wannamethee and Shaper 2010). An AST/ALT ratio greater than 1, which suggests a poor outcome, is a limit that is exceeded in 61% of patients with advanced fibrosis and 24% of patients without fibrosis or with early-stage fibrosis at most (Angulo et al. 1999; Giannini et al. 2005).

In hepatocellular injuries variations in serum ALT activity comparable to those in AST also depend on the plasma half-lives of the two enzymes: 47±10 hours versus 17±5 hours, respectively. In acute hepatocellular injury serum ALT activity rises more slowly than AST activity, but because of its longer half-life, it may be higher after 24–48 hours. Chronic hepatocellular injury usually leads to higher ALT than AST activity at the initial stages, until fibrosis progresses. After that the ALT activity typically decreases and the ratio of AST to ALT is elevated (Kim et al. 2008).

2.8.2 Interactions between multiple lifestyle factors and liver enzymes

It has been shown that smoking and alcohol consumption together have synergistic effects on liver enzyme activities (Breitling et al. 2009; Park et al. 2013), and similarly,

a relatively low alcohol intake in obese individuals can increase the relative risk of hepatotoxicity, fatty changes in the liver and cirrhosis-related mortality (Lau et al. 2015; Niemelä et al. 2017; Tapper and Parikh 2018; Åberg et al. 2020). Serum aminotransferase activities which exceed the reference limits two-fold indicate an increased risk of liver injury and are common in moderate drinkers who present with overweight (Alatalo et al. 2008). When several risk factors co-occur, they may also be expected to lead to more striking elevations in liver enzyme levels (Daeppen et al. 1998; Lam and Mobarhan 2004; Lawlor et al. 2005; Puukka et al. 2006; Adams et al. 2008; Alatalo et al. 2008).

Oxidative stress is a key pathogenic feature involved in health problems that are due to both excess alcohol consumption and obesity (Lieber 2004; Wu et al. 2006; Alatalo et al. 2008; Bondia-Pons et al. 2012). In this condition there is excessive formation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) relative to the antioxidant defence capacity (McCord 2000). Under normal conditions ROS and RNS are generated constantly through normal oxidative metabolic reactions (Miller and Britigan 1997; McCord 2000; Tohyama et al. 2004; Naviaux 2012), whereas excessive formation of these free radicals is associated with certain disease states (Willcox et al. 2004; Roberts and Sindhu 2009; Chen et al. 2012; Zhao and Zhao 2013). Antioxidants (e.g. glutathione, GSH), some vitamins and certain enzymes (e.g. catalase, superoxide dismutase and various peroxidases) have the function of delaying, preventing or eliminating such noxious events (Willcox et al. 2004).

The composition of the diet may significantly contribute to alcohol-induced liver toxicity (Ruhl and Everhart 2005b; Alatalo et al. 2008; Ioannou et al. 2009), and diet also plays an important role in the body's antioxidant defence status (Lindsay and Astley 2002; Nordic Nutrition Recommendations 2012 2014). Therefore, a diet including berries, fruits and vegetables together with other sources of antioxidants has been recommended (Lindsay and Astley 2002). An additional possible dietary source of antioxidants is coffee (see section 2.7) (Butt and Sultan 2011, Morisco et al. 2014; Saab et al. 2014).

2.8.3 C-reactive protein (CRP) and lifestyle

The concentration of C-reactive protein (CRP), an acute-phase inflammatory protein synthesized by the liver, rises in a sensitive manner in response to infection, inflammation and tissue injury (Stewart et al. 2002), and also in association with many

age-related diseases, incident frailty and alcohol consumption (Shah and Paulson 2016). In addition, other lifestyle habits and conditions that stimulate low-grade inflammation in the body are likely to play a role in changes in the circulating levels of CRP (Volpato et al. 2004). CRP has also been shown to predict cardiovascular events, even in subjects without any atherosclerotic manifestations or conventional risk factors (Koenig 2017; Sproston and Ashworth 2018).

Some studies involving middle-aged or older participants have shown that light to moderate alcohol consumption may be associated with lower levels of CRP than in individuals with full abstinence or heavy alcohol consumption (Sierksma et al. 2002; Volpato et al. 2004; Shah and Paulson 2016). According to Volpato et al. (2004), the weekly alcohol consumption that resulted in the lowest levels of CRP was 1-7 drinks, suggesting a J-shaped association between alcohol and CRP levels.

2.8.4 Lipid status and lifestyle

Analyses of lipid profiles have been widely used for assessing cardiovascular health risks and genetic abnormalities in lipid metabolism. Together with the increasing prevalence of obesity and the metabolic syndrome, increasing attention has also been given recently to changes in serum lipids as determinants of the metabolic burden created by lifestyle factors and excess body weight. The typical components of a lipid profile include total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol and triglyseride levels (Szczygielska et al. 2003; Cugnetto et al. 2008; Kawamoto et al. 2011).

Mean total cholesterol and triglyceride concentrations have been demonstrated to be higher in obese individuals than in subjects of normal weight, while their HDL cholesterol levels were lower than in both normal weight and overweight individuals and no significant difference was observed in the mean concentrations of LDL cholesterol (Szczygielska et al. 2003). An effective dietary therapy has been observed to change the values of all the components of a lipid profile towards their target ranges (Yu-Poth et al. 1999).

Previous findings have indicated that there is an association between heavy alcohol consumption and increased triglyseride levels (de Gaetano et al. 2016), and it has also been suggested that this finding may play a role in the increased risk of cardiovascular disease in alcohol consumers, alcoholic fatty liver disease and the development of pancreatic disorders. By contrast, low to moderate alcohol consumption of wine has been thought to lower plasma triglycerides. So far,

however, results regarding the relationships and dose-responses between alcohol consumption and triglyseride levels have remained inconclusive (Klop et al. 2013).

2.8.5 Diagnostic assessment of liver disease

The detection of liver disease and the categorization of its severity can be crucial in clinical work in order to choose the optimal treatment, determine the prognosis for the patient and monitor the activity of the disease. A liver biopsy has been considered the gold standard in such diagnostic assessments and has served as a reference method for grading hepatic inflammation and staging hepatic fibrosis. Various scoring systems, such as Metavir, have been developed for determining the stage of liver fibrosis from liver biopsy samples (Poynard et al. 1997). This is customarily based on a five-point scale from F0 to F4 (Goodman 2007):

F0: no fibrosis
F1: portal fibrosis without bridges or septa
F2: few bridges or septa, excludes significant fibrosis
F3: numerous bridges or septa without cirrhosis, advanced fibrosis
F4: cirrhosis.

There are nevertheless some weaknesses associated with liver biopsy sampling and its interpretation, such as low specimen representativity, the possibility of errors in sampling, inter-observer variation and, since this is an invasive method, the risk of complications (Afdhal 2004; Mumtaz et al. 2019). It has been reported that up to 5% of liver biopsies lead to complications, and in any case, due to its invasive nature this method is poorly suited for repeated monitoring (Chrostek and Panasiuk 2014). Consequently, novel non-invasive tools and diagnostic algorithms have been made available to allow more frequent patient monitoring and more dynamic assessments of the disease prognosis (Kotronen et al. 2009; Niemelä and Alatalo 2010; Poynard et al. 2012; Chrostek et al. 2019). Such approaches include both imaging techniques and combinations of clinical and laboratory markers which are able to reflect fatty changes in the liver or the development of fibrosis in a more specific manner (Moreno et al. 2019; Avila et al. 2020). Possible scanning techniques include ultrasound (US) transient elastography (TE), acoustic radiation force impulse imaging, and magnetic resonance spectroscopy (MRS) (De Robertis et al. 2014; Moreno et al. 2019). Biological markers reflect the extracellular matrix turnover, the products generated during the fibrogenic process or the consequences of liver damage or inflammation (Papastergiou et al. 2012; Lombardi et al. 2015; Niemelä

2016). The proposed biomarkers also include fibrogenic cytokines, specific extracellular matrix components and degradation products and enzymes involved in connective tissue metabolism (Lombardi et al. 2015; Niemelä 2016). Such approaches have been proposed as being simple, cost-effective and accurate non-invasive predictors of both alcoholic and non-alcoholic liver diseases. Clinical studies to define their feasibility, advantages and limitations have so far been limited, however (Avila et al. 2020).

2.8.5.1 Laboratory indices of fatty liver

The fatty liver index (FLI) is a non-invasive tool for assessing fatty liver (hepatic steatosis) based on an algorithm derived from the body mass index, waist circumference and serum GGT and triglyceride levels. The index is calculated as follows:

$$FLI = (e^{0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745}) / (1 + e^{0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745}) \times 100$$

Comparison of the data resulting from FLI and conventional biomarkers of liver function with ultrasonography data has shown FLI to be more accurate for the identification of fatty liver than imaging or any single conventional biomarker (Bedogni et al. 2006; Koehler et al. 2013). The numerical value given by FLI varies from 0 to 100, so that fatty liver can be ruled out in cases of FLI < 30, whereas FLI ≥ 60 indicates that fatty liver is present. Values between these thresholds remain inconclusive (Bedogni et al. 2006).

The liver fat content and associated metabolic aberrations in patients with diabetes or metabolic syndrome have been assessed in a non-invasive manner using a formula that requires information on metabolic syndrome and diabetes together with fasting insulin, AST and the ratio of AST to ALT (Kotronen et al. 2009). When using this formula a result of more than 5.6% refers to fatty liver:

$$\text{Liver fat (\%)} = 10^{(-0.805 + 0.282 \times \text{metabolic syndrome (yes = 1, no = 0)} + 0.078 \times \text{type 2 diabetes (yes = 2, no = 0)} + 0.525 \times \log(\text{fS-insulin[mU/L]}) + 0.521 \times \log(\text{fS-AST [U/L]}) - 0.454 \times \log(\text{AST/ALT}))}$$

2.8.5.2 Fibrosis scores

Monitoring the development of fibrosis is clinically important because the presence of fibrosis is a major determinant of the prognosis in patients with liver diseases. In the early phase of fibrogenesis the changes can be reversible (Sun and Kisseleva 2015; Lackner and Tiniakos 2019), and several predictive models are available for assessing the risk of hepatic fibrosis in liver diseases of various types.

For patients with non-alcoholic liver disease the NAFLD fibrosis score (NFS) has been recommended. This is calculated using readily available clinical data: age, body mass index, presence or absence of hyperglycaemia, platelet count, albumin level, and the ratio of AST to ALT. The result provides an estimate of the stage of fibrosis and thus helps to identify patients with a more severe disease (Angulo et al. 2007; Rinella 2015). The formula for calculating NFS is:

$$\text{NFS} = -1.675 + 0.037 \times \text{age (years)} + 0.094 \times \text{BMI (kg/m}^2\text{)} + 1.13 \times \text{impaired fasting glucose level or diabetes (yes = 1, no = 0)} + 0.99 \times \text{(AST/ALT)} - 0.013 \times \text{platelet count (10}^9\text{/L)} - \text{albumin level (g/dL)}$$

A score that is less than –1.455 denotes fibrosis stages 0–2 and a score greater than 0.675 denotes stages 3–4. Results lying between these limits cannot be classified into either of the above groups (Angulo et al. 2007).

The FIB-4 score is also a widely used surrogate marker for liver fibrosis, especially for patients with obesity-induced liver disease or viral hepatitis. It may also be useful for patients with alcohol-induced liver disease (Chrostek et al. 2019). This algorithm is based on serum AST and ALT activities, platelet count and the patient's age.

$$\text{FIB-4} = \text{(age (years)} \times \text{AST [IU/L])} \,/\, \text{(PLT [10}^9\text{/L]} \times \sqrt{\text{(ALT [IU/L])}}$$

The AST/ALT ratio is typically high in cases of alcoholic liver injury, because AST often shows a more striking elevation (Vonghia et al. 2014) (see section 2.8.1.3), while alcoholic hepatitis normally entails a ratio between 1.5 and 2.0 or greater (Thiele et al. 2018; Moreno et al. 2019).

Heavy alcohol consumption can lead to a reduction in blood platelet counts, as alcohol interferes with the production of platelets in the bone marrow. The simultaneously occurring decrease in platelet counts and increase in serum AST may be used to define the AST to platelet ratio index (APRI) (Wai et al. 2003; Fallatah 2014; Niemelä 2016), which has been proposed as a useful and cost-effective tool for predicting fibrosis in alcoholics.

$$\text{APRI} = ((\text{AST [IU/L]} / \text{ULN}) / \text{PLT [10}^9\text{/L]}) \times 100$$

ULN = upper limit of normal

Chrostek et al. (2019) recently found that APRI is suitable for distinguishing mild fibrosis (F0–F1 and F1) from status F0 and cirrhosis ((F3–F4, F4) from status ≤ F3.

Enhanced liver fibrosis (ELF) is defined in terms of a combination of three serum biomarkers: hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP) and tissue inhibitor of metalloproteinase 1 (TIMP-1) (Parkes et al. 2010; Miele et al. 2017). The ELF score, calculated using the equation

$$\text{ELF score} = 2.494 + 0.846 \ln(C_{HA}) + 0{,}735 \ln(C_{PIIINP}) + 0.391 \ln(C_{TIMP\text{-}1}),$$

has been recommended for detecting advanced fibrosis (≥ F3) and for excluding significant fibrosis (≥ F2) (Thiele et al. 2018).

Fibrotest is a commercially available algorithm that uses the results of several laboratory tests (α2-macroglobulin, haptoglobin, γ-glutamyltransferase, bilirubin and apolipoprotein A1) together with the patient's age and gender to generate a score which correlates with the severity of fibrosis (Poynard et al. 2004; Chrostek et al. 2019). Fibrotest has been widely used in Europe and is thought to achieve a high diagnostic accuracy in identifying advanced fibrosis and cirrhosis (Chrostek and Panasiuk 2014).

The Forns index is based on three routine blood tests and age, as follows (Forns et al. 2002):

$$\text{Forns index} = 7.811 - 3.131 \times \ln(\text{PLT [10}^9\text{/L]}) + 0{,}781 \times \ln(\text{GGT [IU/L]}) + 3.467 \times \ln(\text{age}) - 0.014 \times \text{CHOL [mg/dL]}.$$

It has been maintained that this index achieves higher diagnostic accuracies than either APRI or the FIB-4 index when monitoring fibrogenesis in alcoholics (Chrostek et al. 2019).

3 AIMS OF THE RESEARCH

Health problems which can be attributed to unhealthy lifestyle are common in modern societies. Alcohol drinking, excess body weight, smoking and physical inactivity are typical such factors and are known to have harmful effects on health, leading to abnormal liver function and increased oxidative stress. The early-phase interactions between the various risk factors and their dose responses and health outcomes have remained poorly known, however. The aims of this research were the following:

1) to examine the levels of biomarkers of liver status, inflammation and lipid profiles in a large population-based sample of individuals classified into alcohol drinking risk categories,
2) to compare the joint and individual effects of binge-type drinking and regular alcohol consumption on biomarkers of liver status,
3) to investigate the combined effects of alcohol, smoking, physical inactivity and obesity on the biomarkers of liver status (ALT, GGT), inflammation (C-reactive protein) and lipid metabolism (cholesterol, HDL cholesterol, LDL cholesterol, triglycerides), and
4) to investigate the individual and combined effects of the above lifestyle risk factors on the fatty liver index (FLI), a proxy for fatty liver disease.

4 MATERIALS AND METHODS

4.1 Study design, data sources and participants

The Finnish National Institute for Health and Welfare has been carrying out a cross-sectional population health survey (The National FINRISK Study) every five years since 1972, and the work reported on here is based on data from the 1997, 2002 and 2007 surveys. The participants in these FINRISK studies involved were selected randomly from the population register following an international protocol (the World Health Organization MONICA project) with the intention of providing a nationally representative age and gender-stratified sample of persons aged 25–74 years (World Health Organization 1988). The clinical examinations included physical measurements (body weight, height and waist circumference) and laboratory tests (GGT, ALT, CRP, cholesterol, HDL cholesterol, LDL cholesterol and triglycerides), and the survey also included specifically designed and validated questionnaires gathering information on current health status, alcohol intake during the past 12 months, diet, smoking, physical activity, medical history and socioeconomic factors. The participants had no apparent clinical signs of liver disease, ischaemic heart disease or brain disease or active infection at the time of the survey. Data on alcohol intake included information on the types of beverages consumed, the frequencies of intake, and the amounts of alcoholic drinks consumed. Ethanol intakes in grams were quantified based on the amounts of absolute alcohol (ethanol) contained in the various beverages as follows: regular beer 12 grams (1/3 L), strong beer 15.5 grams (1/3 L), long drinks 15.5 grams (1/3 L), spirits 12 grams (4 cL), wine 12 grams (12 cL) and cider 12 grams (1/3 L).

Smoking was assessed in terms of the number of cigarettes smoked per day and daily coffee consumption as the number of cups drunk, both based on a set of standardized questions. The frequencies of physical activity and the total time spent on this were similarly ascertained using structured questionnaires, so that the participants could be classified into the three subgroups:

1) moderate or vigorous activity (more than 4 hours a week)
2) light activity (0.5–4 hours a week)
3) sedentary activity (less than 0.5 hours of physical activity a week).

For calculation of the body mass index (BMI, kg/m^2), body weights were measured in kilograms to one decimal place and body heights in metres to three decimal places. Waist circumference was measured between the lowest rib and the iliac crest to the nearest 0.5 cm while the participant was exhaling.

The numbers of participants in Papers I–IV varied from 12,368 to 22,327 depending on the data available.

Based on self-reported total alcohol consumption in grams of absolute ethanol per day during the past 12 months, the participants considered in Paper I were categorized into five risk levels according to the current World Health Organization criteria as follows:

1) abstinence, i.e. to non-drinkers,
2) low risk level, consumption from 1 to 40 grams of absolute alcohol (men) or from 1 to 20 grams (women),
3) medium risk level, consumption from 41 to 60 grams (men) or from 21 to 40 grams (women),
4) high risk level, consumption from 61 to 100 grams (men) or from 41 to 60 grams (women), and
5) very high risk level, consumption more than 100 grams (men) or more than 60 grams (women).

In Paper II the participants were categorized into subgroups according to both the amount of regular drinking (low, medium and high risk) and the frequency of binge drinking, as follows:

1) low-risk drinking (1–40 grams of absolute alcohol for men, 1–20 grams for women) with no binge drinking,
2) low-risk drinking with binge drinking once a month or less,
3) low-risk drinking with binge drinking more than once a month,
4) medium-risk drinking (41–60 grams for men, 21–40 grams for women) with binge drinking once a month or less,
5) medium-risk drinking (41–60 grams for men, 21–40 grams of for women) with binge drinking more than once a month
6) high-risk drinking (61–100 grams for men, 41–60 grams for women) with binge drinking once a month or less
7) high-risk drinking (61–100 grams for men, 41–60 grams for women) with binge drinking more than once a month.

Binge drinking was specified as a drinking pattern which consists of consuming large quantities of alcohol in a single session, the lower limits being defined as 60 grams of absolute ethanol for men and more than 40 grams for women within a

relatively short period of time. Such consumption typically results in a blood alcohol concentration (BAC) of 0.08 g/dL (= 0.8‰) or higher. The data on the frequency of binge episodes were used to divide the participants to subgroups of those without binge drinking and those with given intensities of binge episodes.

In Papers III–IV the data on the various determinants of lifestyle, including alcohol consumption, smoking, BMI status and physical activity, were scored for low risk (= 0), medium risk (= 1) and high risk (= 2) as shown in Table 5:

Table 5. Categorization of subjects according to lifestyle-related risk factors

Lifestyle factor	0 (low risk)	1 (medium risk)	2 (high risk)
Alcohol consumption	no consumption	1–14 standard drinks/week (men) 1–7 standard drinks/week (women)	> 14 standard drinks/week (men) > 7 standard drinks/week (women)
Smoking	no smoking	1–19 cigarettes/day	≥ 20 cigarettes/day
BMI status	< 25 kg/m^2	≥ 25 and < 30 kg/m^2	≥ 30 kg/m^2
Physical activity	> 4 hours/week	0.5–4 hours/week	< 0.5 hours/week

The total risk factor score was the sum of the above scores (maximum = 8, indicating an extremely unhealthy lifestyle).

4.2 Ethical aspects

All the research described as having taken place within the present work was conducted in accordance with the Declaration of Helsinki and the ethical rules of the National Public Health Institute of Finland. Approval for the work was received from the Coordinating Ethics Committee of the Helsinki Hospital District.

4.3 Blood sampling and laboratory measurements

Venous blood samples were taken into vacuum blood collection tubes after at least four hours of fasting. The samples were centrifuged at the survey site 20–30 minutes after sample collection. In the 1997 and 2002 surveys total serum cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, GGT, and C-reactive protein were determined from fresh serum samples. The transport of these fresh samples was possible as the parameters concerned are known to be stable for prolonged

periods even at room temperature (Cuhadar et al. 2012; Hedayati et al. 2020). The ALT determinations were performed on frozen samples. In 2007 the sera separated from centrifuged samples were frozen immediately at the survey site and transported on dry ice to the laboratory for analysis. Parts of some of the samples were used for analytical comparisons to ensure repeatability of the data between the assays carried out using fresh and frozen samples.

The biochemical parameters were measured in the laboratory of the National Public Health Institute of Finland in Helsinki using standard methods approved for clinical chemistry. The principles of the analytical methods remained the same throughout the research. The serum liver enzymes ALT and GGT were measured by standard kinetic methods using an Optima analyser (Thermo Electron Corporation, Waltham, Massachusetts, USA) in 1997 and 2002, and an Abbott Architect c8000 clinical chemistry analyser (Abbott Laboratories, Abbott Park, IL, USA) in 2007. High-sensitivity CRP (hs-CRP) was determined using a latex immunoturbidimetric assay (Orion Diagnostica, Espoo, Finland, in 1997 and 2002, and Sentinel Diagnostics, Milan, Italy, in 2007). The detection limit for the high-sensitivity CRP (hs-CRP) used as a biomarker of inflammation was 0.2 mg/L and the mean intra-and inter-assay coefficients of variation for CRP were 1.6% and 1.7%, respectively. A systematic comparison of the analytical data between the samples from the different years was performed in each of the later years to ensure the repeatability of the results. The lipid profiles included assays for serum total cholesterol, high-density lipoprotein-cholesterol (HDL) and total triglycerides, which were measured by standard enzymatic methods: the CHOD-PAP method for total cholesterol, the direct enzymatic method for HDL cholesterol and the GPO method for triglycerides (Thermo Electron Corporation, Waltham, Massachusetts, USA, in 1997 and 2002, and Abbott Laboratories, Abbott Park, IL, USA, in 2007). The low-density lipoprotein cholesterol (LDL) results were calculated using Friedewald's formula: LDL = Chol – HDL – 0.45×trigly. The requirement for using this formula is that the triglyceride value should not exceed 4.5 mmol/l (Friedewald et al. 1972).

All the laboratory tests were subject to continuous external quality control programmes as organized by Labquality, Finland, and the CDC (Center for Disease Control and Prevention) quality assurance and standardization programme for serum lipids. The previously established national cut-offs for the biomarkers were as follows: ALT (50 U/L men; 35 U/L women), GGT (60 U/L men; 40 U/L women) (Danielsson et al. 2014; Niemelä and Danielsson 2015; Eskelinen 2016a; Eskelinen 2016b), CRP (3.0 mg/L) (Eskelinen 2016c), cholesterol (5 mmol/L), HDL

cholesterol (1.0 mmol/L men, 1.2 mmol/L women), LDL cholesterol (3.0 mmol/L), triglycerides (1.7 mmol/L) (Dyslipidemiat: Current Care Guidelines, 2020).

The fatty liver index (FLI) algorithm based on BMI, waist circumference, triglycerides and GGT, was calculated according to the following formula (Bedogni et al. 2006):

$$FLI = (e^{0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745}) : (1 + e^{0.953 \times \log_e(\text{triglycerides}) + 0.139 \times \text{BMI} + 0.718 \times \log_e(\text{GGT}) + 0.053 \times \text{waist circumference} - 15.745}) \times 100.$$

FLI scores below 30 rule out fatty liver, scores between 30 and 60 remain inconclusive, whereas scores of 60 and above indicate that fatty liver is present.

4.4 Statistical methods

The main characteristics of the groups under investigation are shown in terms of a mean ± standard deviation (SD) and were compared using the analysis of variance (ANOVA). Due to the skewed distribution of the laboratory values, a logarithmic transformation was performed prior to analysis (II, IV). The potential linear or quadratic trend in mean values across ordered groups was evaluated using the trend test available in ANOVA (I–IV). The analysis of covariance (ANCOVA) was used to control for confounders (II). The presence of a linear trend in proportions was evaluated by means of the Chi-square test for a trend (I, III, IV). The Breslow–Day test was used to assess whether the effect of binge drinking was homogenous across the various BMI categories (II). Binary logistic regression was used to estimate the relative risk of dichotomous outcomes associated with the variable of interest and the covariates (I–III), and multinomial logistic regression was used in the case of a three-class outcome (IV). Potential multicollinearity among the covariates was examined by calculating the Variance Inflation Factor (VIF). The estimated risks are presented as odds ratios (OR) with 95% confidence intervals (95% CI). A likelihood ratio test was performed between the multivariate logistic regression models to evaluate the individual impact of the lifestyle risk factors studied here as predictors of an abnormal FLI (IV). Spearman's rank correlation coefficient was used for the correlation analyses (II, III, IV). All the analyses were carried out with IBM SPSS Statistics 22.0 and 24.0 (Armonk, NY: IBM Corp.).

5 RESULTS

5.1 Biomarkers and risk drinking levels (Paper I)

The series discussed in this publication included a total of 22,327 participants (10,724 men and 11,603 women), of whom 9.3% of the men were abstainers, 80.6% low risk drinkers, 5.4% moderate risk drinkers, 3.1% high risk drinkers and 1.6% very high risk drinkers. The corresponding percentages among the women were 14.7%, 79.0%, 4.6%, 1.0% and 0.7%. For both genders the prevalence of cigarette smoking was found to vary in parallel alcohol consumption (Figure 2) ($p < 0.001$ for both genders). Thus, while the male abstainers smoked an average of 2.5 cigarettes a day, their very high risk drinking counterparts smoked an average of 12.9 cigarettes, the corresponding figures for the women being 1.0 and 10.3 cigarettes.

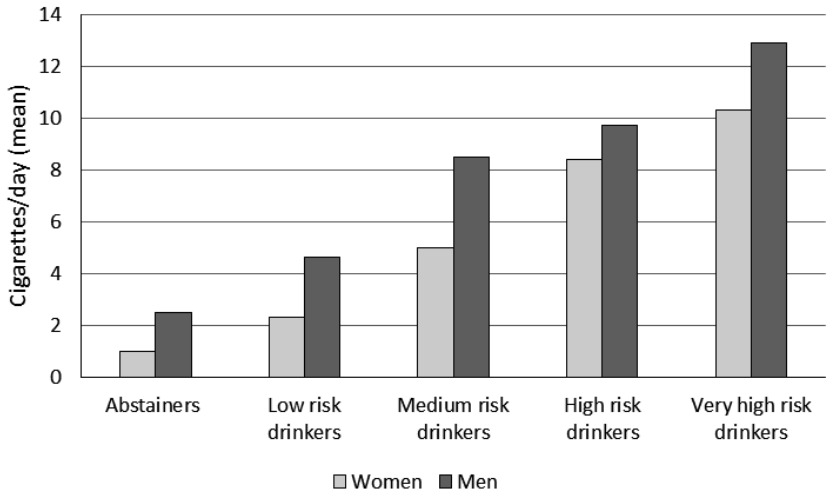

Figure 2. Smoking (cigarettes per day) in subjects with different levels of alcohol drinking

The distributions of abnormal GGT, ALT, cholesterol and HDL levels were found to be significantly associated with drinking status. In both genders the occurrence of abnormal GGT and ALT findings was found to increase in a more or less linear

manner as a function of alcohol drinking status, and a significant association was also found in the rates of abnormal CRP values in men.

The data on the odds ratios (OR) of abnormal biomarker findings across the different drinking categories after adjustment for age, waist circumference, physical activity, smoking and coffee consumption indicated that the low risk drinkers ($p < 0.0005$ for men; $p < 0.01$ for women), moderate risk drinkers ($p < 0.0005$ for both genders), high risk drinkers ($p < 0.0005$ for both genders) and very high risk drinkers ($p < 0.0005$ for both genders) all showed significantly higher ORs for abnormal GGT activities as compared with the abstainers. In the case of ALT the relative risks of abnormal activities were significantly increased in all the alcohol consumption groups except for the low risk drinkers among the men and the low to medium risk drinkers among the women, while with regard to the serum lipid profiles, alcohol drinking was associated with lower odds for HDL values lying outside the target range, whereas increased risks were observed in serum cholesterol and LDL in men, although not in women.

5.2 Biomarkers and drinking patterns (Paper II)

For insights into possible links between liver enzyme activities and drinking patterns we analysed data from 19,225 subjects (9,492 men, 9,733 women) classified according to their drinking status and numbers of binge drinking episodes. Of the men in this series, 90.6% were low-risk drinkers, 6.0% moderate-risk drinkers, and 3.4% high-risk drinkers, while the corresponding percentages among the women were 93.8%, 5.1%, and 1.1%. The participants were further divided into subgroups according to the frequencies of their binge drinking episodes, again considering the genders separately. Smoking status ($p < 0.001$ for both genders) and heavy drinking episodes ($p < 0.001$ for both genders) were associated with higher levels of total alcohol intake (Figure 3), while age was found to be inversely correlated with the number of heavy drinking episodes ($p < 0.001$ for both genders). The activities of the liver enzymes were found to increase in a fairly linear manner as a function of total regular alcohol consumption, with the individuals regularly consuming the highest quantities of alcohol showing the highest enzyme activities.

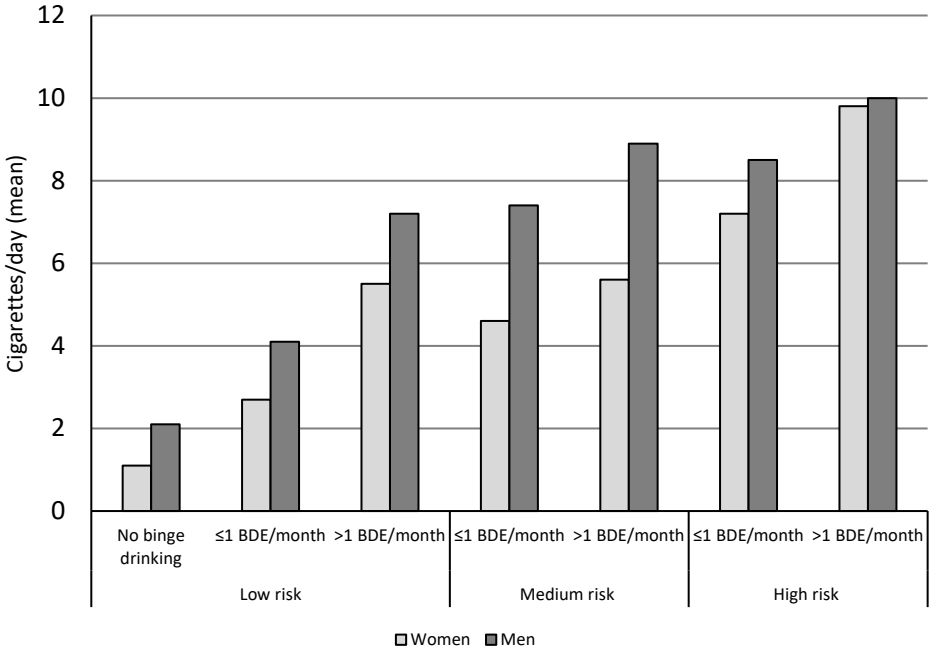

Figure 3. Distributions of findings in subjects classified according to their alcohol consumption, binge drinking (BDE = binge drinking episode) and smoking

The findings regarding GGT activities across the subgroups indicated significant increases in association with increasing frequencies of binge drinking episodes to be seen in both genders ($p < 0.0005$), while for ALT the same observation was made among the men but not the women. Separate comparisons between the groups with binge episodes and those not reporting any such episodes pointed to a significant increase in GGT and ALT already in the men reporting heavy drinking episodes 2–3 times per year ($p < 0.05$ for both comparisons) and a more notable increase in those with such episodes at least once a month ($p < 0.0005$ for both GGT and ALT). Among the women a significant increase in GGT values was noted in the subgroups reporting heavy drinking episodes once a week and those who had these episodes more often ($p < 0.0005$).

When the data was examined according to both total alcohol consumption and the frequency of heavy drinking episodes the activities of the liver enzymes were found to increase in a more or less linear manner as a function of total regular alcohol consumption, the individuals with the highest total ethanol intake showing the highest enzyme activities. Furthermore, drinking patterns were found to influence the liver enzyme activities in the sense that the subjects with low-risk total alcohol

consumption who reported heavy drinking episodes more than once a month showed elevated GGT ($p < 0.0005$) and ALT ($p < 0.0005$) activities significantly more often than did those who did not report any binge episodes. Episodes of heavy drinking once a month or less were also associated with GGT ($p < 0.0005$) and ALT ($p < 0.05$) values that were significantly higher than in those subjects without any such episodes. No such differences were evident in the subgroups representing medium or high risk drinkers.

When the odds ratios among the low risk drinkers were compared between those reporting no episodes of binge drinking and those reporting binge drinking episodes, GGT ($p < 0.0005$ for both genders) and ALT ($p < 0.02$ for men) indicated significantly higher odds on exceeding the upper thresholds in these enzyme activities if one had a history of binge drinking. The comparison of CRP levels showed slightly higher figures in low risk drinkers with binge drinking (1.36; 1.29–1.44 mg/L) than in those without (1.25; 1.17–1.34 mg/L) ($p < 0.05$). No similar effect on CRP levels was observed in women.

5.3 Biomarkers and lifestyle risk factors (Paper III)

The series included 22,273 participants (10,561 men, 11,712 women) aged 25–74 years from the National FINRISK Study. These data on alcohol consumption, smoking, body weight, and physical activity had been gathered from structured interviews and were used here to establish risk scores for the various life style factors on a scale of 0–8. Higher levels of alcohol consumption, adiposity, smoking and physical inactivity were found to characterize the individuals with high risk scores. The highest mean ages were noted in the middle portion of the risk score categories ($p < 0.0005$ for both genders). Interestingly, coffee consumption was also found to increase with increasing risk factor scores in both men and women ($p < 0.0005$ for a linear trend in both genders).

Consistent dose-response relationships were observed between the number of unfavourable risk factors and GGT, ALT, CRP and lipid status (Figures 4 and 5). The occurrence of abnormal findings for each laboratory parameter was found to increase as a function of risk score status in a more or less linear and significant manner ($p < 0.0005$ for all comparisons).

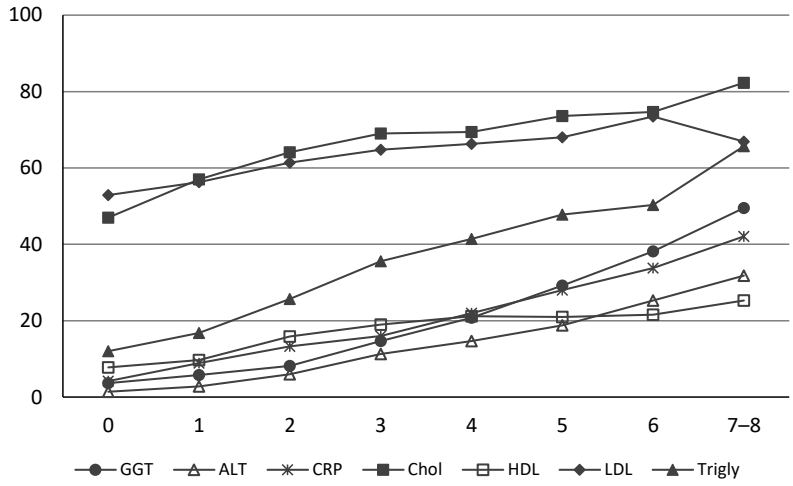

Figure 4. Proportions (%) of biomarker findings exceeding the reference values or target ranges in males, classified according to the number of lifestyle-associated risk factor scores (x-axis)

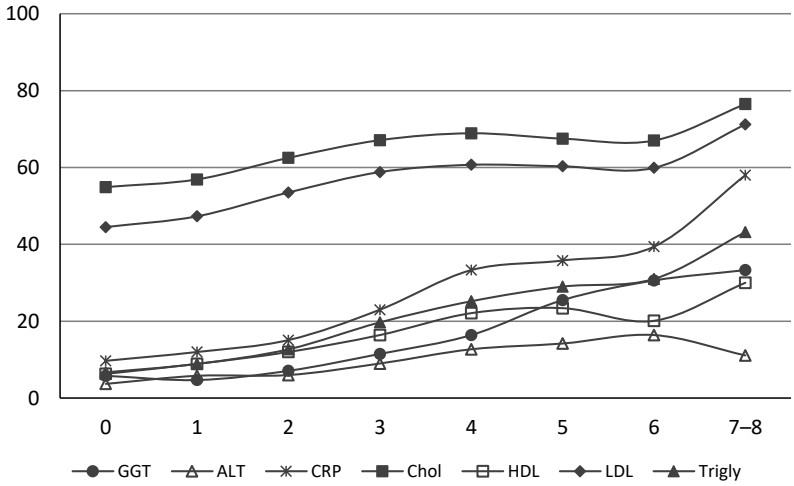

Figure 5. Proportions (%) of biomarker findings exceeding the reference values or target ranges in women, classified according to the number of lifestyle-associated risk factor scores (x-axis)

The multivariable analyses of the relative risks of abnormal biomarker findings among the risk categories indicated that the biomarkers of liver status, inflammation and lipid profiles were all found to show significant associations with the number of risk scores as compared with the participants having zero risk factors. The most

striking increases in ORs in the group with the highest numbers of risk factors were observed for men in serum GGT: 26.6 (12.4–57.0), ALT: 40.3 (5.3–307.8), CRP: 16.2 (7.8–33.7) and serum triglycerides: 14.4 (8.6–24.0).

The closest correlations between the numbers of unfavourable risk factors and the laboratory tests were observed for serum GGT (r_s = 0.381 for men; r_s = 0.311 for women); ALT (r_s = 0.252 for men; r_s = 0.166 for women), CRP (r_s = 0.308 for men; r_s = 0.293 for women) and serum triglycerides (r_s = 0.274 for men, r_s = 0.258 for women) ($p < 0.0001$ for all comparisons).

5.4 Lifestyle risk factors and the fatty liver index (FLI) (Paper IV)

The association between lifestyle risk factors and FLI was studied in a population including 12,368 participants aged 25–74 years classified according to each of the lifestyle risk factors (alcohol consumption, smoking, adiposity and physical activity), as explained in Paper III.

The proportions of individuals with FLI ≥ 60 (indicating that fatty liver was present) in the subgroups with different levels of lifestyle risk factor scores are summarized in Table 2 of Paper IV. Distinct dose-response relationships were observed between the number of unfavourable risk factors and FLI levels in all the comparisons. In those with zero risk factors a FLI below 30 (ruling out fatty liver) was observed in 87.5% of men and 98.5% of women, whereas an increase in the amount of risk factors was found to lead to a sharp increase in the prevalence of FLI 60 or above, suggesting fatty liver, in both genders. The FLI changes, when adjusted for BMI, appeared to be more striking among the men (Table 6).

Table 6. Percentages of observations among subjects classified according to FLI and lifestyle risk factor scores adjusted for body mass index (BMI)

	FLI	0	1	2	3	4	5	6	7–8
Men	≥ 60	2.5	6.3	20.1	41.0	51.3	63.9	68.1	84.4
	30–60	10.7	27.9	38.6	34.9	29.7	22.7	22.6	11.1
	< 30	86.8	65.8	41.2	24.2	19.0	13.4	9.3	4.5
Women	≥ 60	0.0	0.3	4.5	16.8	33.2	40.8	49.8	76.8
	30–60	1.6	7.3	15.1	25.6	29.8	27.0	29.5	21.0
	< 30	98.4	92.4	80.5	57.6	37.0	32.2	20.7	2.2

The rates of abnormal results in this population when classified according to their risk factor scores were also compared when alcohol consumption, smoking and physical inactivity were taken as independent components of the risk factor classification (score range 0–6). By comparison with those having zero risk factors, a significant increase in the occurrence of abnormal FLI was found in those with one or more risk factors in both genders ($p < 0.0005$ for all comparisons). The data obtained from the multinomial logistic regression analysis after adjustment for BMI, age and coffee consumption, further showed that risk score status was associated with significant increases in the OR for FLI 60 and above in both genders in the groups with one or more risk factors. The most striking influences on the likelihood of the occurrence of an abnormal FLI were observed in the case of a lack of physical activity ($p < 0.0005$ for both genders) and in that of alcohol consumption in men (14 drinks per week) ($p < 0.0005$).

Among the various parameters studied here, significant correlations with FLI emerged in the case of serum ALT ($r_s = 0.512$ for men; $r_s = 0.322$ for women) and CRP ($r_s = 0.429$ for men; $r_s = 0.479$ for women) ($p < 0.001$ for all comparisons).

6 DISCUSSION

6.1 Biomarkers of liver function, inflammation and lipid status in the WHO risk drinking categories

Distinct relationships between alcohol intake and abnormalities in common laboratory tests were demonstrated in Paper I in individuals classified according to the recently established WHO risk drinking categories. Such data may prove to be useful when using these categories for clinical assessments of alcohol-drinking patients and also when making public health recommendations on these matters.

The results indicated that the risk of abnormal liver enzyme activities, especially in the case of GGT, increase with increasing alcohol consumption in almost a linear manner, even in participants with fairly moderate levels of drinking. Recent reports on patients classified according to the WHO risk drinking levels have indicated that any reduction in risk level may lead to clinical improvement (Hasin et al. 2017; Witkiewitz et al. 2017a; 2017b). This is important because for many alcohol users a reduction in alcohol intake would be preferable to complete abstinence (Hasin et al. 2017). Changes in laboratory biomarkers could then prove useful for detecting shifts in risk drinking categories at the individual level.

The current data also support previous findings based on liver enzyme changes (Niemelä et al. 2017) or all-cause mortality (Wood et al. 2018) indicating that the thresholds for low-risk alcohol consumption in many national guidelines are at too high a level (over 100 grams per week). Previous analyses on patterns of alcohol-attributable health risks have traditionally yielded different types of dose-response curve. Although the relationships between heavy alcohol consumption and a wide variety of chronic diseases have been well established, there has been controversy regarding the health effects of light to moderate alcohol consumption, which has even been thought to have protective effects against cardiovascular disease (Di Castelnuovo et al. 2006). More recent studies, however, have not been able to demonstrate any health benefits when weekly alcohol consumption exceeds 100 grams, which is equivalent to about eight standard drinks per week (Holmes et al. 2014; Klatsky 2015; Sipilä et al. 2016; Stockwell et al. 2016; Niemelä et al. 2017;

Topiwala et al. 2017; Wood et al. 2018). It should also be noted that light to moderate drinking has recently been linked to an increased risk of carcinogenesis in various tissues (Bagnardi et al. 2013; Cao et al. 2015; Choi et al. 2018), cognitive decline (Topiwala et al. 2017; Schwarzinger et al. 2018), heart problems (Catena et al. 2016; McManus et al. 2016) and all-cause mortality (Sipilä et al. 2016; Wood et al. 2018).

The assessment of health risks and appropriate alcohol consumption at the individual level should also take into account possible gender differences in biomarker responses. Liver enzyme activities in women increase in response to smaller amounts of alcohol than those in men, but women appear to be less sensitive than men to changes in inflammation status. The pathogenic mechanisms associated with such divergent disease associations remain unknown (Holmes et al. 2014). It has been suggested previously that the cardiovascular effects of alcohol consumption may be mediated by the diverse effects of alcohol on blood lipid profiles and inflammation status (Libby et al. 2009; Moradi et al. 2017; Ridker et al. 2017), and there may also be a mechanistic link between lipid metabolism, fatty liver and atherosclerosis, since GGT is an enzyme that is able to fuel LDL oxidation in coronary plaques (Kozakova et al. 2012). Interestingly, current data suggest that serum CRP, a marker of inflammation, is increased among men with rather moderate levels of alcohol consumption, especially when occurring together with excess body weight or a sedentary lifestyle. Thus even low to moderate alcohol consumption could increase the risk of triglyceride accumulation in tissues, insulin resistance and adverse vascular events in obese individuals (Ruttmann et al. 2005; Kuulasmaa et al. 2006; Lee et al. 2006; Targher et al. 2007; Lee et al. 2008; Fraser et al. 2009; Ghouri et al. 2010; Kozakova et al. 2012; Tsai et al. 2012; Lau et al. 2015). Since serious alcohol consumption often co-exists with obesity in real life situations, the synergistic health problems occasioned by these two triggers may be expected to occur in an increasing manner, so that separate drinking reduction goals would obviously be justified for such individuals (Åberg and Färkkilä 2020).

6.2 Liver enzymes in alcohol consumers with or without binge drinking

While regular excessive alcohol consumption is known to lead to addiction and substantial health loss, comparisons of the health effects brought about by a combination of repeated episodes of heavy drinking bouts and regular alcohol consumption have been limited. As demonstrated in Paper II, bouts of binge

drinking may lead to increased activities of liver-derived enzymes even in individuals with low risk overall alcohol consumption. This observation should also be considered in health guidelines related to alcohol drinking and in efforts aimed at population-level reductions in alcohol consumption.

Recent findings resulting from large international collaborations have indicated that all-cause mortality increases significantly when regular alcohol consumption exceeds 100 grams (~8 drinks) per week (GBD 2016 Alcohol and Drug Use Collaborators 2018; Wood et al. 2018). In the light of the present data the frequency of binge episodes should also be recorded in a more systematic manner in the follow-up of patients with alcohol problems. Those engaging in heavy drinking bouts appear to show increased activities of both GGT and ALT, and it remains to be established whether these increases could also be related to higher odds ratios for the typical adverse health outcomes described previously in binge-drinking populations (Kazemi-Shirazi et al. 2007; Sundell et al. 2008; Hillbom et al. 2011).

The present results also raise the interesting question of whether previous contradictory research findings on the health effects of low to moderate alcohol consumption could be explained by drinking patterns. The effects of alcohol consumption on health have typically been studied using dose-response curves, without considering the drinking pattern, and interestingly, possible beneficial health effects of low to moderate alcohol consumption have been reported mainly in societies with a low prevalence of binge drinking (Renaud and de Lorgeril 1992; Di Castelnuovo et al. 2006). Several studies of populations following Mediterranean diets, for instance, have assigned cardio-protective properties to light to moderate drinking habits (Di Castelnuovo et al. 2006), whereas a number of studies based on other types of population have found no evidence of such benefits (Holmes et al. 2014; Klatsky 2015; Sipilä et al. 2016; Stockwell et al. 2016; Niemelä et al. 2017; Topiwala et al. 2017; Wood et al. 2018).

The current data underscore the need for more attention to be paid to binge drinking in alcohol control policies. Elevated activities of liver enzymes may require closer examination and evaluation of the reasons for the increases even though the changes may occur within the current reference ranges. In addition to hepatic health risks, the monitoring of liver enzyme activities may also be useful for identifying extrahepatic risks, including risks of cardio- or cerebrovascular events and metabolic syndrome (Ruttmann et al. 2005; Kazemi-Shirazi et al. 2007; Kim et al. 2008; Ruhl and Everhart 2009; Niemelä 2016). A systematic use of liver enzyme measurements in addition to alcohol self-reports may facilitate the monitoring of treatment aimed at the reduction of drinking and the early identification of possible tissue toxicity.

For the patient, laboratory measurements concretize the consequences of reducing alcohol use for health status, and may thus provide an incentive for aiming at this goal.

Current research has indicated that binge drinking seems to increase the activities of liver enzymes in a more pronounced manner in men than in women, and similarly, CRP appears to rise slightly more sensitively in men, although it has previously been suggested that the immune and inflammatory consequences of binge drinking may be more notable among women (Orio et al. 2017; Pascual et al. 2017). While the primary mechanisms underlying such observations remain unknown at this time, it is possible that alcohol stimulates oxidative stress and inflammatory responses in a gender-dependent manner (Finkel and Holbrook 2000; Zhang and Forman 2009). GGT plays a pivotal role in the metabolism of glutathione (GSH), and elevated activities could be related to an attempt to maintain intracellular GSH levels during oxidative stress, which could also be considered a protective mechanism against alcohol toxicity (Speisky et al. 1990; Emdin et al. 2005; Zhang and Forman 2009). Women, however, actually seem to show elevated liver enzyme activities following a smaller total alcohol consumption. Women are also known to be more vulnerable to alcohol addiction, alcohol-induced liver disease and central nervous system effects (Liu et al. 2010; Hillbom et al. 2011; Alfonso-Loeches et al. 2013; Schwarzinger et al. 2018).

The present results also indicate that those who engage frequently in binge drinking are younger than those with a low number of such episodes. Another clear difference between the subgroups examined was smoking, for as the frequency of binge drinking increased, the prevalence of smoking was also found to increase. This result is consistent with previous research showing that binge drinking and smoking co-occur, especially in young adults (Harrison et al. 2008; Woolard et al. 2015). Thus it can be assumed that if a patient consciously aims to reduce the frequency of either binge drinking or smoking, the other unfavourable lifestyle risk factor will also decrease, which could lead to a reduced risk of hepatotoxicity (Breitling et al. 2009; Park et al. 2013).

6.3 Biomarkers and combined lifestyle factors

Paper III pointed to previously unrecognized relationships between the total sum of lifestyle risk factors and certain biomarker abnormalities which may also prove to be useful for planning public health recommendations. The parameters chosen for the

comparisons were conventional and readily available laboratory tests for assessing liver status, inflammation and lipid profiles, and the outcome was that typical pathophysiological features associated with lifestyle and disease risks seem to be those involving chronic inflammation, oxidative stress and altered fatty acid metabolism (Danielsson 2014; Oh et al. 2015; Zheng et al. 2017). The data showed that there was an almost linear relationship between the biomarker abnormalities and the total sum of lifestyle risk factors, supporting the view that profound health benefits could be achieved by adopting a healthy lifestyle. In the light of recent observations this may also be expected to lead to prolonged residual life expectancy (Li et al. 2018) and a reduced disease burden (Li et al. 2014; Manuel et al. 2016; Rutten-Jacobs et al. 2018). Laboratory tests could be used in routine clinical practice as tools for motivating patients to achieve a more favourable lifestyle and to aim at long-term maintenance of the lifestyle modifications suggested by clinicians.

The main individual determinants of a healthy lifestyle include alcohol drinking in moderation, weight control, avoidance of smoking, and regular physical exercise (Romaguera et al. 2011; Li et al. 2014; Manuel et al. 2016; Li et al. 2018). Previous studies on the assessment of alcohol consumption as a lifestyle risk factor have concluded that regular alcohol drinking in amounts exceeding 8 standard drinks per week would lower the residual life expectancy at the age of 40 years by 0.5 years, while 30 drinks per week would leading to a loss of 4–5 years (Li et al. 2014; Manuel et al. 2016; Wood et al. 2018). In individuals with excess body weight even smaller levels of alcohol consumption would increase the relative risk of health problems (Lau et al. 2015; Niemelä et al. 2017; Åberg and Färkkilä 2020).

Synergistic effects of smoking and alcohol consumption in increasing liver enzyme activities have also reported (Breitling et al. 2009; Park et al. 2013), and Li et al. (2014) have shown a significant loss of residual life expectancy associated with smoking, amounting to 9.4 years for men and 7.3 years for women among heavy smokers (over 10 cigarettes per day). In fact the loss of residual life expectancy was even more remarkable when combined impacts of heavy smoking, obesity, heavy alcohol drinking and high consumption of processed or red meat were studied, as these taken together shortened residual life expectancy by 17.0 years in the case of men and 13.9 years in women relative to persons who adopted a healthy lifestyle. Similar conclusions on the smoking-induced reduction in estimated life expectancy have also been reported elsewhere (Tamakoshi et al. 2010; Jha and Peto 2014).

Recent findings have been interpreted as suggesting that lifestyle intervention could be highly effective when treating patients with liver problems (Oh et al. 2015; Romero-Gómez et al. 2017; Teeriniemi et al. 2018), there is a likelihood that a wide

variety of other clinical conditions such as heart diseases, diabetes or cancer may also be driven by lifestyle to a significant extent (Tamakoshi et al. 2009; Li et al. 2014; Manuel et al. 2016; Li et al. 2018). Thus it may be expected that systematic measurements of biomarkers reflecting liver status, inflammation and lipid profiles could also be helpful in the comprehensive assessment of patients presenting with lifestyle risk factors.

Laboratory tests of liver function, inflammation and lipid status could also be useful in elucidating the mechanisms behind the adverse effects of various behavioural phenotypes. It has been suggested previously that hepatic and extrahepatic disease outcomes share certain pathogenic mechanisms, as supported by findings indicating that the enzyme GGT is able to fuel LDL oxidation in coronary plaques (Kozakova et al. 2012), and in accordance with this, alcohol and its reactive metabolites are known to exert toxic effects on virtually all tissues and even relatively low levels of chronic drinking may increase the risk of carcinogenesis (Bagnardi et al. 2013; Cao et al. 2015; Choi et al. 2018), dementia (Topiwala et al. 2017; Schwarzinger et al. 2018), cardiac insufficiency (Klatsky 2015; Catena et al. 2016; McManus et al. 2016) and mortality (Sipilä et al. 2016; Wood et al. 2018), as also may abnormalities in blood lipid profiles and indices of inflammation (Libby et al. 2009; Moradi et al. 2017; Ridker et al. 2017). According to the present data, abnormalities in serum CRP and lipid profiles appear to coincide with a burden of unfavourable risk factors and abnormalities in markers of liver function. Previous research has shown that CRP levels may mark a low-grade inflammation status and predict cardiovascular events even in individuals without any atherosclerotic manifestations or conventional risk factors (Koenig 2017; Sproston and Ashworth 2018), and there is also evidence to suggest an important role for CRP in regulating inflammation (Sproston and Ashworth 2018).

Sedentary behaviour is another typical characteristic of an unhealthy lifestyle and is increasingly becoming a common cause of health problems worldwide (Romaguera et al. 2011; Kyu et al. 2016; Smith et al. 2016; Sundberg 2016; Warburton and Bredin 2016; Romero-Gómez et al. 2017; Li et al. 2018). The present data also emphasize physical activity as an independent component of a favourable lifestyle. Individuals engaged in moderate or vigorous physical activity have significantly lower risks of biomarker abnormalities than those with little activity or a sedentary lifestyle, even in the presence of other risk factors. Thus physical exercise may also be recommended as a therapeutic approach to counteract lifestyle-associated adverse metabolic influences (Lawlor et al. 2005; Kyu et al. 2016; Perreault et al. 2017; Zaccardi et al. 2019).

In addition to the lifestyle factors studied here, there may also be other types of unhealthy behaviour, such as certain particular dietary patterns, which may contribute to adverse health effects (Tamakoshi et al. 2009; Li et al. 2014; Manuel et al. 2016; Li et al. 2018). It should be noted, however, that unfavourable lifestyle factors were found to be associated with an increasing trend towards coffee consumption in the high risk subgroups, which is in accordance with previous observations indicating an association between heavy smoking and coffee intake (Bjørngaard et al. 2017). On the other hand, coffee consumption has been shown previously to be associated with a reduced risk of both all-cause and cause-specific mortality (Gunter et al. 2017). Decreased levels of liver-derived enzymes have been measured previously in alcohol drinkers with high levels of coffee consumption as compared with those who do not drink coffee, suggesting possible hepatoprotective effects of coffee intake (Gunter et al. 2017; Niemelä et al. 2017).

Although it may be difficult in real life situations to pay attention simultaneously to several favourable lifestyle factors, it is essential that national health policies should provide comprehensive guidance on what tools to recommend for patients presenting with an unfavourable lifestyle. The present findings suggest that selected clinical laboratory tests could play a significant role in this endeavour.

6.4 Lifestyle factors and the fatty liver index

The results presented in Paper IV indicate that combinations of unfavourable determinants in terms of lifestyle also markedly increase the risk of fatty liver (steatosis), as assessed by means of a recently developed predictor algorithm, the fatty liver index (FLI). The relatively linear association existing between an abnormal FLI and lifestyle risk factors supports the view that significant benefits for the liver could be gained by behaviour change and adherence to a healthy lifestyle (Teeriniemi et al. 2018). Our data further indicate that FLI, a non-invasive biomarker of steatosis, could be a useful clinical tool for patient guidance and motivation during interventions aimed at achieving a more favourable lifestyle.

Fatty liver is currently an extremely common condition in high income countries, affecting at least 25–30% of adults in the general population and over 70% of those with gross obesity or diabetes (Byrne et al. 2018). Greater awareness of this public health challenge is therefore needed. Excess deposition of liver fat has been regarded as the hepatic manifestation of metabolic syndrome, and is thereby associated with cardio- or cerebrovascular risks and insulin resistance (Ruttmann et al. 2005;

Kazemi-Shirazi et al. 2007; Kim et al. 2008; Niemelä 2016; Byrne et al. 2018). As a consequence, clinical tools for diagnosing fatty liver in an early phase are also needed to prevent possible metabolic, cardiovascular and renal complications. Although the FLI algorithm has recently been shown to improve the identification of fatty liver as compared with other non-invasive methods (Bedogni et al. 2006; Koehler et al. 2013; Jäger et al. 2015), only a few studies have been available as yet on the clinical characteristics of FLI or on the effects of various unfavourable lifestyle factors on the index.

The present findings indicate that the main modifiable high-risk lifestyle determinants (excessive alcohol drinking, cigarette smoking and physical inactivity), and especially their combinations, increase the risk of fatty liver, so that in persons with several triggers of hepatotoxicity even low to moderate levels of alcohol consumption may lead to elevated liver enzyme activities, fatty deposition and increased rates of cirrhosis (Breitling et al. 2009; Park et al. 2013; Lau et al. 2015; Niemelä et al. 2017; Tapper and Parikh 2018; Wood et al. 2018; Åberg et al. 2020).

Previous studies have indicated that there may be shared mechanisms involved in the hepatotoxic effects of an unfavourable lifestyle, including low-grade inflammation, oxidative stress and altered fatty acid metabolism (Danielsson 2014; Oh et al. 2015), and recently a GGT-mediated mechanistic link between hepatic and extrahepatic diseases have been proposed based on findings indicating that GGT is able to fuel LDL oxidation in coronary plaques (Kozakova et al. 2012). The medical consequences of unfavourable lifestyle factors also appear to be mediated by an interplay between oxidative stress and inflammation (Libby et al. 2009; Koenig 2017; Moradi et al. 2017; Ridker et al. 2017). Interestingly, the present data suggest that abnormalities in serum CRP, a marker of inflammation, and lipid profiles also correlate with FLI and the burden of unhealthy behavioural traits.

The spending of more time on sedentary activities is known to be associated with a wide variety of adverse health effects, such as an increased risk of cardiovascular diseases, diabetes or breast cancer (Chomistek et al. 2013; Kyu et al. 2016; Smith et al. 2016; Romero-Gómez et al. 2017; Li et al. 2018), while the present findings indicate that physical inactivity can be a major independent contributor to an abnormal fatty liver index. Individuals engaged in moderate or vigorous physical activity show a lower risk of fatty liver than the corresponding groups of those with low activity or a sedentary lifestyle. Adequate levels of physical exercise may also be assumed to counteract possible adverse metabolic effects resulting from unfavourable exposure to lifestyle-associated risk factors and to yield long-term

benefits with respect to the lifestyle-associated liver disease burden in general (Lawlor et al. 2005; Kyu et al. 2016; Perreault et al. 2017).

In addition to the effects of the risk factors reported here, there may also be other factors, such as dietary composition, which may significantly influence the status of fatty changes in the liver. Unfortunately, with the exception of coffee intake, no detailed dietary information was available for use here. Previous studies of non-alcoholic fatty liver disease have indicated that a vegetarian diet may be inversely associated with fatty liver (Chiu et al. 2018). In addition, vitamin D deficiency may be associated with NAFLD (Pacifico et al. 2019). Vitamin D supplementation may in turn have a protective effect on the progression of fibrosis in patients with chronic liver disease (Chen et al. 2020). Deficiencies in vitamin D, vitamin A and zinc are common in cases of cirrhosis and have also been shown to correlate with survival (Koop et al. 2018). In the present work the number of unfavourable lifestyle factors was found to be associated with an increase in coffee consumption, the highest levels of which were observed in high risk subgroups. Interestingly, previous studies have suggested possible hepatoprotective effects of coffee consumption, so that alcohol drinkers whose coffee consumption exceeds 4 cups per day have shown lower GGT levels than those without who do not drink coffee (Danielsson et al. 2013; Niemelä et al. 2017). A poor diet including high fat and carbohydrate intake together with insufficient vitamin intake may also provide additional triggers for hepatotoxicity (Tsukamoto et al. 1995; Day and James 1998; Fraser et al. 2009; Ruhl and Everhart 2009; Ghouri et al. 2010; Liu et al. 2010; Tsai et al. 2012).

The findings reported in paper IV further suggest that measurements of FLI could be used as a tool to support adherence to a low-risk lifestyle on the part of patients with suspected liver problems, and these findings should definitely be taken into account in national health policies. Novel biomarkers providing feedback on possible health risks may also prove to be of value in individualized medicine to motivate patients towards adopting more favourable lifestyles and reducing their health risks.

6.5 Strengths and limitations of the present research

The strengths of this work include the large number of subjects and the comprehensive assessment made of both the clinical factors involved and the set of biomarkers. Regular alcohol consumption was evaluated on the basis of data from the past year, allowing an estimate to be made of each subject's long-term alcohol

consumption and frequencies of heavy drinking episodes. A questionnaire for the collection of data on other risk factors was included in the statistical models to reduce the effect of confounding factors. Finally, separate assessments were made for men and women throughout.

The work also has some potential limitations. Self-reports are prone to the shortcomings of this memory-dependent aspect of day to day activity and it is possible that the subjects' alcohol recall techniques may have led to the overestimation of the proportion of those not drinking alcohol at all. The cross-sectional setting and lack of follow-up data for derive causal relationships can also be regarded as a limitation, as does the lack of information on the quality and composition of the diet, hampers the making of a more comprehensive evaluation of the various combinations of lifestyle-related factors. In addition, although the total number of participants was very large, there were relatively small numbers belonging to the high and very high risk lifestyle categories. On the other hand, the present work underscores the importance of uniform international guidelines and risk classification criteria when assessing lifestyle-associated disadvantages by means of biomarker data.

6.6 Future considerations

The present data provide novel information on the separate and joint effects of various lifestyle factors on certain biomarkers of liver function, inflammation and lipid status, biomarkers that may prove to be of value in the assessment of interventions aimed at reducing unfavourable risk factors and helping individuals to persevere in the long-term maintenance of the lifestyle modifications suggested for them. The present findings should also be considered when assessing possible mechanistic links between hepatic and extrahepatic disease manifestations resulting from an unfavourable lifestyle. There is also a need for future follow-up surveys to analyse the causal relationships between biomarker changes and lifestyle factors.

7 CONCLUSIONS

The following conclusions can be derived from the present work:

1) A more systematic use of laboratory tests may improve the assessment of alcohol-related health risks. A follow-up of biomarker responses may also be useful in health interventions aimed at reducing alcohol consumption.
2) Research into the relationships between biomarker responses and the patterns of alcohol drinking shows that there may be adverse consequences of binge drinking for hepatic function even in subjects with low-risk overall consumption. The pattern of drinking should be taken into account more systematically when making clinical recommendations on the reduction of drinking.
3) The presence of unfavourable lifestyle risk factors is associated with distinct abnormalities in laboratory tests for liver function, inflammation and lipid status. Such changes occur in an additive manner and may prove to be of value for the assessment of interventions aimed at reducing unfavourable risk factors and for helping individuals to maintain their lifestyle modifications in the long term.
4) The data also indicate that unfavourable lifestyle risk factors, and especially their combinations, can lead to a high likelihood of hepatic steatosis. The fatty liver index (FLI) may prove to be a useful non-invasive tool for assessing the risk of hepatotoxicity.

8 REFERENCES

Aalto M, Alho H, Halme JT and Seppä K (2011): The alcohol use disorders identification test (AUDIT) and its derivatives in screening for heavy drinking among the elderly. Int J Geriatr Psychiatry 26:881-885.

Abramowitz MK, Hall CB, Amodu A, Sharma D, Androga L and Hawkins M (2018): Muscle mass, BMI, and mortality among adults in the United States: A population-based cohort study. PLoS One 13:e0194697.

Adams LA, Knuiman MW, Divitini ML and Olynyk JK (2008): Body mass index is a stronger predictor of alanine aminotransaminase levels than alcohol consumption. J Gastroenterol Hepatol 23:1089-1093.

Afdhal NH (2004): Biopsy or biomarkers: is there a gold standard for diagnosis of liver fibrosis? Clin Chem 50:1299-1300.

Alatalo PI, Koivisto HM, Hietala JP, Puukka KS, Bloigu R and Niemelä OJ (2008): Effect of moderate alcohol consumption on liver enzymes increases with increasing body mass index. Am J Clin Nutr 88:1097-1103.

Albano E (2008): Oxidative mechanisms in the pathogenesis of alcoholic liver disease. Mol Aspects Med 29:9-16.

Alfonso-Loeches S, Pascual M and Guerri C (2013): Gender differences in alcohol-induced neurotoxicity and brain damage. Toxicology 311:27-34.

Alkoholiongelmaisen hoito: Current Care Guidelines (2018): Working group set up by the Finnish Medical Society Duodecim and the Finnish Society of Addiction Medicine. The Finnish Medical Society Duodecim, 2018, Helsinki. Available online at: https://www.kaypahoito.fi/hoi50028 (Last accessed January 24, 2020).

Allen JP, Litten RZ and Anton R (1992): Measures of alcohol consumption in perspective. In: Measuring Alcohol Consumption. Psychosocial and Biochemical Methods, pp. 205-226. Eds. RZ Litten and JP Allen, The Humana Press Ins., Totowa, New Jersey.

Altamirano J and Bataller R (2010): Cigarette smoking and chronic liver diseases. Gut 59:1159-1162.

Amir M and Czaja MJ (2011): Autophagy in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol 5:159-166.

Andresen-Streichert H, Müller A, Glahn A, Skopp G and Sterneck M (2018): Alcohol biomarkers in clinical and forensic contexts. Dtsch Arztebl Int 115:309-315.

Angulo P, Keach JC, Batts KP and Lindor KD (1999): Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 30:1356-1362.

Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM and Day CP (2007): The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 45:846-854.

Anty R, Marjoux S, Iannelli A, Patouraux S, Schneck AS, Bonnafous S, Gire C, Amzolini A, Ben-Amor I, Saint-Paul MC, Mariné-Barjoan E, Pariente A, Gugenheim J, Gual P and Tran A (2012): Regular coffee but not espresso drinking is protective against

fibrosis in a cohort mainly composed of morbidly obese European women with NAFLD undergoing bariatric surgery. J Hepatol 57:1090-1096.
Appelman Y, van Rijn BB, Ten Haaf ME, Boersma E and Peters SA (2015): Sex differences in cardiovascular risk factors and disease prevention. Atherosclerosis 241:211-218.
Aradottir S, Asanovska G, Gjerss S, Hansson P and Alling C (2006): PHosphatidylethanol (PEth) concentrations in blood are correlated to reported alcohol intake in alcohol-dependent patients. Alcohol Alcohol 41:431-437.
Arnesen E, Huseby NE, Brenn T and Try K (1986): The Tromsø Heart Study: distribution of, and determinants for, gamma-glutamyltransferase in a free-living population. Scand J Clin Lab Invest 46:63-70.
Arranz S, Chiva-Blanch G, Valderas-Martínez P, Medina-Remón A, Lamuela-Raventós RM and Estruch R (2012): Wine, beer, alcohol and polyphenols on cardiovascular disease and cancer. Nutrients 4:759-781.
Ashworth M and Gerada C (1997): ABC of mental health. Addiction and dependence--II: Alcohol. BMJ 315:358-360.
Avila C, Holloway AC, Hahn MK, Morrison KM, Restivo M, Anglin R and Taylor VH (2015): An overview of links between obesity and mental health. Curr Obes Rep 4:303-310.
Avila MA, Dufour JF, Gerbes AL, Zoulim F, Bataller R, Burra P, Cortez-Pinto H, Gao B, Gilmore I, Mathurin P, Moreno C, Poznyak V, Schnabl B, Szabo G, Thiele M and Thursz MR (2020): Recent advances in alcohol-related liver disease (ALD): summary of a Gut round table meeting. Gut 69:764-780.
Åberg F and Färkkilä M (2020): Drinking and obesity: alcoholic liver disease/nonalcoholic fatty liver disease interactions. Semin Liver Dis. 40(2):154-162.
Åberg F, Helenius-Hietala J, Puukka P and Jula A (2017): Binge drinking and the risk of liver events: A population-based cohort study. Liver Int 37:1373-1381.
Åberg F, Puukka P, Salomaa V, Männistö S, Lundqvist A, Valsta L, Perola M, Färkkilä M and Jula A (2020): Risks of light and moderate alcohol use in fatty liver disease: follow-up of population cohorts. Hepatology. 71(3):835-848.
Bagnardi V, Zambon A, Quatto P and Corrao G (2004): Flexible meta-regression functions for modeling aggregate dose-response data, with an application to alcohol and mortality. Am J Epidemiol 159:1077-1086.
Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V, Scotti L, Jenab M, Turati F, Pasquali E, Pelucchi C, Bellocco R, Negri E, Corrao G, Rehm J, Boffetta P and La Vecchia C (2013): Light alcohol drinking and cancer: a meta-analysis. Ann Oncol 24:301-308.
Baraona E and Lieber CS (1979): Effects of ethanol on lipid metabolism. J Lipid Res 20:289-315.
Basista MH, Gavaler J, Stieffenhofer A, Love K, Rosenblum E and Dindzans VJ (1993): Effect of ethanol on Kupffer cell function. Alcohol Clin Exp Res 17:556-560.
Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A and Tiribelli C (2006): The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol 6:33.
Behrens G, Fischer B, Kohler S, Park Y, Hollenbeck AR and Leitzmann MF (2013): Healthy lifestyle behaviors and decreased risk of mortality in a large prospective study of U.S. women and men. Eur J Epidemiol 28:361-372.

Beuther DA and Sutherland ER (2007): Overweight, obesity, and incident asthma: a meta-analysis of prospective epidemiologic studies. Am J Respir Crit Care Med 175:661-666.

Bhatti SK, O'Keefe JH and Lavie CJ (2013): Coffee and tea: perks for health and longevity? Curr Opin Clin Nutr Metab Care 16:688-697.

Bien TH and Burge R (1990): Smoking and drinking: a review of the literature. Int J Addict 25:1429-1454.

Bigaard J, Frederiksen K, Tjønneland A, Thomsen BL, Overvad K, Heitmann BL and Sørensen TI (2005): Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. Int J Obes (Lond) 29:778-784.

Birerdinc A, Stepanova M, Pawloski L and Younossi ZM (2012): Caffeine is protective in patients with non-alcoholic fatty liver disease. Aliment Pharmacol Ther 35:76-82.

Bjørngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafò MR, Nordestgaard BG, Åsvold BO, Romundstad P and Davey Smith G (2017): Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. Int J Epidemiol 46:1958-1967.

Blüher M (2019): Obesity: global epidemiology and pathogenesis. Nat Rev Endocrinol 15:288-298.

Bondia-Pons I, Ryan L and Martinez JA (2012): Oxidative stress and inflammation interactions in human obesity. J Physiol Biochem 68:701-711.

Breitling LP, Raum E, Müller H, Rothenbacher D and Brenner H (2009): Synergism between smoking and alcohol consumption with respect to serum gamma-glutamyltransferase. Hepatology 49:802-808.

Brenner DR, Haig TR, Poirier AE, Akawung A, Friedenreich CM and Robson PJ (2017): Alcohol consumption and low-risk drinking guidelines among adults: a cross-sectional analysis from Alberta's Tomorrow Project. Health Promot Chronic Dis Prev Can 37:413-424.

Brill A (2013): The long-term returns of obesity prevention policies. Robert Wood Johnson Foundation, Princeton, NJ. www.rwjf.org/content/dam/farm/reports/reports/2013/rwjf405694 (Last accessed April 25, 2020).

Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM and Hobbs HH (2004): Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. Hepatology 40:1387-1395.

Brunt EM (2004): Nonalcoholic steatohepatitis. Semin Liver Dis 24:3-20.

Brunt EM (2011): Non-alcoholic fatty liver disease: what's new under the microscope? Gut 60:1152-1158.

Bush B, Shaw S, Cleary P, Delbanco TL and Aronson MD (1987): Screening for alcohol abuse using the CAGE questionnaire. Am J Med 82:231-235.

Butt MS and Sultan MT (2011): Coffee and its consumption: benefits and risks. Crit Rev Food Sci Nutr 51:363-373.

Byrne CD, Patel J, Scorletti E and Targher G (2018): Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults. BMJ 362:k2734.

Campos J, Gonzalez-Quintela A, Quinteiro C, Gude F, Perez LF, Torre JA and Vidal C (2005): The -159C/T polymorphism in the promoter region of the CD14 gene is associated with advanced liver disease and higher serum levels of acute-phase proteins in heavy drinkers. Alcohol Clin Exp Res 29:1206-1213.

Cao Y, Willett WC, Rimm EB, Stampfer MJ and Giovannucci EL (2015): Light to moderate intake of alcohol, drinking patterns, and risk of cancer: results from two prospective US cohort studies. BMJ 351:h4238.

Caro AA and Cederbaum AI (2004): Oxidative stress, toxicology, and pharmacology of CYP2E1. Annu Rev Pharmacol Toxicol 44:27-42.

Casiglia E, Spolaore P, Ginocchio G and Ambrosio GB (1993): Unexpected effects of coffee consumption on liver enzymes. Eur J Epidemiol 9:293-297.

Catalano D, Martines GF, Tonzuso A, Pirri C, Trovato FM and Trovato GM (2010): Protective role of coffee in non-alcoholic fatty liver disease (NAFLD). Dig Dis Sci 55:3200-3206.

Catena C, Colussi G, Verheyen ND, Novello M, Fagotto V, Soardo G and Sechi LA (2016): Moderate alcohol consumption is associated with left ventricular diastolic dysfunction in nonalcoholic hypertensive patients. Hypertension 68:1208-1216.

Cederbaum AI (2012): Alcohol metabolism. Clin Liver Dis 16:667-685.

Chamorro AJ, Torres JL, Mirón-Canelo JA, González-Sarmiento R, Laso FJ and Marcos M (2014): Systematic review with meta-analysis: the I148M variant of patatin-like phospholipase domain-containing 3 gene (PNPLA3) is significantly associated with alcoholic liver cirrhosis. Aliment Pharmacol Ther 40:571-581.

Charlet K and Heinz A (2017): Harm reduction-a systematic review on effects of alcohol reduction on physical and mental symptoms. Addict Biol 22:1119-1159.

Chen AF, Chen DD, Daiber A, Faraci FM, Li H, Rembold CM and Laher I (2012): Free radical biology of the cardiovascular system. Clin Sci (Lond) 123:73-91.

Chen S, Teoh NC, Chitturi S and Farrell GC (2014): Coffee and non-alcoholic fatty liver disease: brewing evidence for hepatoprotection? J Gastroenterol Hepatol 29:435-441.

Chen T, Zuo X, Wang S, Yu P, Yuan J, Wei S, Chen J, Sun Y, Gao Y and Li X (2020): The effect of vitamin D supplementation on the progression of fibrosis in patients with chronic liver disease: A protocol for a systematic review and meta-analysis. Medicine (Baltimore) 99:e20296.

Chiu TH, Lin MN, Pan WH, Chen YC and Lin CL (2018): Vegetarian diet, food substitution, and nonalcoholic fatty liver. Ci Ji Yi Xue Za Zhi 30:102-109.

Choe YM, Lee BC, Choi IG, Suh GH, Lee DY and Kim JW (2019): Combination of the CAGE and serum gamma-glutamyl transferase: an effective screening tool for alcohol use disorder and alcohol dependence. Neuropsychiatr Dis Treat 15:1507-1515.

Choi YJ, Myung SK and Lee JH (2018): Light alcohol drinking and risk of cancer: a meta-analysis of cohort studies. Cancer Res Treat 50:474-487.

Chomistek AK, Manson JE, Stefanick ML, Lu B, Sands-Lincoln M, Going SB, Garcia L, Allison MA, Sims ST, LaMonte MJ, Johnson KC and Eaton CB (2013): Relationship of sedentary behavior and physical activity to incident cardiovascular disease: results from the Women's Health Initiative. J Am Coll Cardiol 61:2346-2354.

Christensen CH, Rostron B, Cosgrove C, Altekruse SF, Hartman AM, Gibson JT, Apelberg B, Inoue-Choi M and Freedman ND (2018): Association of cigarette, cigar, and pipe use with mortality risk in the US population. JAMA Intern Med 178:469-476.

Chrostek L and Panasiuk A (2014): Liver fibrosis markers in alcoholic liver disease. World J Gastroenterol 20:8018-8023.

Chrostek L, Przekop D, Gruszewska E, Gudowska-Sawczuk M and Cylwik B (2019): Noninvasive indirect markers of liver fibrosis in alcoholics. Biomed Res Int 2019:3646975.

Cinti S, Mitchell G, Barbatelli G, Murano I, Ceresi E, Faloia E, Wang S, Fortier M, Greenberg AS and Obin MS (2005): Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. J Lipid Res 46:2347-2355.

Clark JM and Diehl AM (2003): Nonalcoholic fatty liver disease: an underrecognized cause of cryptogenic cirrhosis. JAMA 289:3000-3004.

Conigrave KM, Davies P, Haber P and Whitfield JB (2003): Traditional markers of excessive alcohol use. Addiction 98 Suppl 2:31-43.

Connor J and Hall W (2018): Thresholds for safer alcohol use might need lowering. Lancet 391:1460-1461.

Connor JP, Haber PS and Hall WD (2016): Alcohol use disorders. Lancet 387:988-998.

Corrao G, Zambon A, Bagnardi V, D'Amicis A and Klatsky A (2001): Coffee, caffeine, and the risk of liver cirrhosis. Ann Epidemiol 11:458-465.

Corrao G, Bagnardi V, Zambon A and La Vecchia C (2004): A meta-analysis of alcohol consumption and the risk of 15 diseases. Prev Med 38:613-619.

Cugnetto ML, Saab PG, Llabre MM, Goldberg R, McCalla JR and Schneiderman N (2008): Lifestyle factors, body mass index, and lipid profile in adolescents. J Pediatr Psychol 33:761-771.

Cuhadar S, Atay A, Koseoglu M, Dirican A and Hur A (2012): Stability studies of common biochemical analytes in serum separator tubes with or without gel barrier subjected to various storage conditions. Biochem Med (Zagreb) 22:202-214.

Daeppen JB, Smith TL and Schuckit MA (1998): Influence of age and body mass index on gamma-glutamyltransferase activity: a 15-year follow-up evaluation in a community sample. Alcohol Clin Exp Res 22:941-944.

Danielsson J (2014): Liver enzymes and lifestyle. Academic dissertation. University of Tampere, Tampere.

Danielsson J, Kangastupa P, Laatikainen T, Aalto M and Niemelä O (2013): Dose- and gender-dependent interactions between coffee consumption and serum GGT activity in alcohol consumers. Alcohol Alcohol 48:303-307.

Danielsson J, Kangastupa P, Laatikainen T, Aalto M and Niemelä O (2014): Impacts of common factors of life style on serum liver enzymes. World J Gastroenterol 20:11743-11752.

Davidson R and Raistrick D (1986): The validity of the Short Alcohol Dependence Data (SADD) Questionnaire: a short self-report questionnaire for the assessment of alcohol dependence. Br J Addict 81:217-222.

Day CP (2007): Alcohol and the liver. Medicine 35:22-25.

Day CP and James OF (1998): Steatohepatitis: a tale of two "hits"? Gastroenterology 114:842-845.

Day E and Rudd JHF (2019): Alcohol use disorders and the heart. Addiction 114:1670-1678.

de Gaetano G, Costanzo S, Di CA, Badimon L, Bejko D, Alkerwi A, Chiva-Blanch G, Estruch R, La Vecchia C, Panico S, Pounis G, Sofi F, Stranges S, Trevisan M, Ursini F, Cerletti C, Donati MB and Iacoviello L (2016): Effects of moderate beer consumption on health and disease: a consensus document. Nutr Metab Cardiovasc Dis 26:443-467.

De Robertis R, D'Onofrio M, Demozzi E, Crosara S, Canestrini S and Pozzi Mucelli R (2014): Noninvasive diagnosis of cirrhosis: a review of different imaging modalities. World J Gastroenterol 20:7231-7241.

Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L and de Gaetano G (2006): Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med 166:2437-2445.

Diehl AM (1997): Alcoholic liver disease: natural history. Liver Transpl Surg 3:206-211.

Diehl AM (1998): Alcoholic liver disease. Clin Liver Dis 2:103-118.

Diehl AM, Potter J, Boitnott J, Van Duyn MA, Herlong HF and Mezey E (1984): Relationship between pyridoxal 5'-phosphate deficiency and aminotransferase levels in alcoholic hepatitis. Gastroenterology 86:632-636.

Dixon JB (2010): The effect of obesity on health outcomes. Mol Cell Endocrinol 316:104-108.

Dong H and Czaja MJ (2011): Regulation of lipid droplets by autophagy. Trends Endocrinol Metab 22:234-240.

Dyslipidemiat: Current Care Guidelines (2020): Working group set up by the Finnish Medical Society Duodecim and the Finnish Society of Internal Medicine. The Finnish Medical Society Duodecim, Helsinki. Available online at: https://www.kaypahoito.fi/hoi50025 (Last accessed December 15, 2020).

Ekelund U, Tarp J, Steene-Johannessen J, Hansen BH, Jefferis B, Fagerland MW, Whincup P, Diaz KM, Hooker SP, Chernofsky A, Larson MG, Spartano N, Vasan RS, Dohrn IM, Hagströmer M, Edwardson C, Yates T, Shiroma E, Anderssen SA and Lee IM (2019): Dose-response associations between accelerometry measured physical activity and sedentary time and all cause mortality: systematic review and harmonised meta-analysis. BMJ 366:l4570.

Emdin M, Pompella A and Paolicchi A (2005): Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: triggering oxidative stress within the plaque. Circulation 112:2078-2080.

Eriksson CJ (2001): The role of acetaldehyde in the actions of alcohol (update 2000). Alcohol Clin Exp Res 25:15S-32S.

Erzurumluoglu AM, Liu M, Jackson VE, Barnes DR, Datta G, Melbourne CA, Young R, Batini C, Surendran P, Jiang T, Adnan SD, Afaq S, Agrawal A, Altmaier E, Antoniou AC, Asselbergs FW, Baumbach C, Bierut L, Bertelsen S, Boehnke M, Bots ML, Brazel DM, Chambers JC, Chang-Claude J, Chen C, Corley J, Chou YL, David SP, de Boer RA, de Leeuw CA, Dennis JG, Dominiczak AF, Dunning AM, Easton DF, Eaton C, Elliott P, Evangelou E, Faul JD, Foroud T, Goate A, Gong J, Grabe HJ, Haessler J, Haiman C, Hallmans G, Hammerschlag AR, Harris SE, Hattersley A, Heath A, Hsu C, Iacono WG, Kanoni S, Kapoor M, Kaprio J, Kardia SL, Karpe F, Kontto J, Kooner JS, Kooperberg C, Kuulasmaa K, Laakso M, Lai D, Langenberg C, Le N, Lettre G, Loukola A, Luan J, Madden PAF, Mangino M, Marioni RE, Marouli E, Marten J, Martin NG, McGue M, Michailidou K, Mihailov E, Moayyeri A, Moitry M, Muller-Nurasyid M, Naheed A, Nauck M, Neville MJ, Nielsen SF, North K, Perola M, Pharoah PDP, Pistis G, Polderman TJ, Posthuma D, Poulter N, Qaiser B, Rasheed A, Reiner A, Renstrom F, Rice J, Rohde R, Rolandsson O, Samani NJ, Samuel M, Schlessinger D, Scholte SH, Scott RA, Sever P, Shao Y, Shrine N, Smith JA, Starr JM, Stirrups K, Stram D, Stringham HM, Tachmazidou I, Tardif JC, Thompson DJ, Tindle HA, Tragante V, Trompet S, Turcot V, Tyrrell J, Vaartjes I, van der Leij AR, van der Meer P, Varga TV, Verweij N, Volzke H, Wareham NJ, Warren HR, Weir DR, Weiss S, Wetherill L, Yaghootkar H, Yavas E, Jiang Y, Chen F, Zhan X, Zhang W, Zhao W, Zhao W, Zhou K, Amouyel P, Blankenberg S, Caulfield MJ, Chowdhury R, Cucca F, Deary IJ, Deloukas P, Di Angelantonio E, Ferrario M, Ferrieres J, Franks

PW, Frayling TM, Frossard P, Hall IP, Hayward C, Jansson JH, Jukema JW, Kee F, Männistö S, Metspalu A, Munroe PB, Nordestgaard BG, Palmer CNA, Salomaa V, Sattar N, Spector T, Strachan DP, van der Harst P, Zeggini E, Saleheen D, Butterworth AS, Wain LV, Abecasis GR, Danesh J, Tobin MD, Vrieze S, Liu DJ and Howson JMM (2019): Meta-analysis of up to 622,409 individuals identifies 40 novel smoking behaviour associated genetic loci. Mol Psychiatry. Online ahead of print: https://doi.org/10.1038/s41380-018-0313-0.

Eskelinen S (2016a): Alaniiniaminotransferaasi (P-ALAT). Kustannus Oy Duodecim 1.7.2016, Helsinki. Available online at: https://www.terveyskirjasto.fi/ terveyskirjasto/tk.koti?p_artikkeli=snk03071 (Last accessed December 15, 2020).

Eskelinen S (2016b): Glutamyylitransferaasi (P-GT). Kustannus Oy Duodecim 1.7.2016, Helsinki. Available online at: https://www.terveyskirjasto.fi/ terveyskirjasto/ tk.koti?p_artikkeli=snk03073 (Last accessed December 15, 2020).

Eskelinen S (2016c): CRP (P-CRP). Kustannus Oy Duodecim 30.6.2016, Helsinki. Available online at: https://www.terveyskirjasto.fi/terveyskirjasto/ tk.koti?p_artikkeli=snk03052 (Last accessed December 15, 2020).

European Medicines Agency (2010): Guideline on the development of medicinal products for the treatment of alcohol dependence. London, UK. https://www.ema.europa.eu/en/search/search?search_api_views_fulltext=%22guideline+on+the+development+of+medical+products+for+the+treatment+of+alcohol+dependence%22&=Refine+results (Last accessed January 24, 2020).

Fallatah HI (2014): Noninvasive biomarkers of liver fibrosis: an overview. Advances in Hepatology 2014:Article ID 357287.

Fawehinmi TO, Ilomäki J, Voutilainen S and Kauhanen J (2012): Alcohol consumption and dietary patterns: the FinDrink study. PLoS One 7:e38607.

Finkel T and Holbrook NJ (2000): Oxidants, oxidative stress and the biology of ageing. Nature 408:239-247.

Finnish Institute for Health and Welfare (2018): National FinHealth Study. Finnish Institute for Health and Welfare (THL), Helsinki. Available online at: https://thl.fi/en/web/thlfi-en/research-and-expertwork/population-studies/ national-finhealth-study (Last accessed May 15, 2020).

Foerster M, Marques-Vidal P, Gmel G, Daeppen JB, Cornuz J, Hayoz D, Pécoud A, Mooser V, Waeber G, Vollenweider P, Paccaud F and Rodondi N (2009): Alcohol drinking and cardiovascular risk in a population with high mean alcohol consumption. Am J Cardiol 103:361-368.

Forns X, Ampurdanès S, Llovet JM, Aponte J, Quintó L, Martínez-Bauer E, Bruguera M, Sánchez-Tapias JM and Rodés J (2002): Identification of chronic hepatitis C patients without hepatic fibrosis by a simple predictive model. Hepatology 36:986-992.

Foster RK and Marriott HE (2006): Alcohol consumption in the new millennium – weighing up the risks and benefits for our health. Nutr Bull 31:286-331.

Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G and Lawlor DA (2009): Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care 32:741-750.

Freedman ND, Park Y, Abnet CC, Hollenbeck AR and Sinha R (2012): Association of coffee drinking with total and cause-specific mortality. N Engl J Med 366:1891-1904.

Freeman TL, Tuma DJ, Thiele GM, Klassen LW, Worrall S, Niemelä O, Parkkila S, Emery PW and Preedy VR (2005): Recent advances in alcohol-induced adduct formation. Alcohol Clin Exp Res 29:1310-1316.

Friedewald WT, Levy RI and Fredrickson DS (1972): Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18:499-502.

Gallus S, Tavani A, Negri E and La Vecchia C (2002): Does coffee protect against liver cirrhosis? Ann Epidemiol 12:202-205.

Gao B and Bataller R (2011): Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 141:1572-1585.

GBD 2016 Alcohol and Drug Use Collaborators (2018): The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Psychiatry 5:987-1012.

GBD 2016 Alcohol Collaborators (2018): Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 392:1015-1035.

Ghouri N, Preiss D and Sattar N (2010): Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology 52:1156-1161.

Giannini EG, Testa R and Savarino V (2005): Liver enzyme alteration: a guide for clinicians. CMAJ 172:367-379.

Goessling W, Massaro JM, Vasan RS, D'Agostino RB, Sr., Ellison RC and Fox CS (2008): Aminotransferase levels and 20-year risk of metabolic syndrome, diabetes, and cardiovascular disease. Gastroenterology 135:1935-1944.e1.

Goldschmidt AB, Lavender JM, Hipwell AE, Stepp SD and Keenan K (2017): Emotion regulation and loss of control eating in community-based adolescents. J Abnorm Child Psychol 45:183-191.

Goodman ZD (2007): Grading and staging systems for inflammation and fibrosis in chronic liver diseases. J Hepatol 47:598-607.

Guh DP, Zhang W, Bansback N, Amarsi Z, Birmingham CL and Anis AH (2009): The incidence of co-morbidities related to obesity and overweight: a systematic review and meta-analysis. BMC Public Health 9:88.

Gunter MJ, Murphy N, Cross AJ, Dossus L, Dartois L, Fagherazzi G, Kaaks R, Kühn T, Boeing H, Aleksandrova K, Tjønneland A, Olsen A, Overvad K, Larsen SC, Redondo Cornejo ML, Agudo A, Sánchez Pérez MJ, Altzibar JM, Navarro C, Ardanaz E, Khaw KT, Butterworth A, Bradbury KE, Trichopoulou A, Lagiou P, Trichopoulos D, Palli D, Grioni S, Vineis P, Panico S, Tumino R, Bueno-de-Mesquita B, Siersema P, Leenders M, Beulens JWJ, Uiterwaal CU, Wallström P, Nilsson LM, Landberg R, Weiderpass E, Skeie G, Braaten T, Brennan P, Licaj I, Muller DC, Sinha R, Wareham N and Riboli E (2017): Coffee drinking and mortality in 10 European countries: a multinational cohort study. Ann Intern Med 167:236-247.

Gutiérrez-Grobe Y, Chávez-Tapia N, Sánchez-Valle V, Gavilanes-Espinar JG, Ponciano-Rodríguez G, Uribe M and Méndez-Sánchez N (2012): High coffee intake is associated with lower grade nonalcoholic fatty liver disease: the role of peripheral antioxidant activity. Ann Hepatol 11:350-355.

Halberg N, Wernstedt-Asterholm I and Scherer PE (2008): The adipocyte as an endocrine cell. Endocrinol Metab Clin North Am 37:753-768.

Halsted CH, Villanueva JA, Devlin AM, Niemelä O, Parkkila S, Garrow TA, Wallock LM, Shigenaga MK, Melnyk S and James SJ (2002): Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci U S A 99:10072-10077.

Hanigan MH (2014): Gamma-glutamyl transpeptidase: redox regulation and drug resistance. Adv Cancer Res 122:103-141.

Harrison EL, Desai RA and McKee SA (2008): Nondaily smoking and alcohol use, hazardous drinking, and alcohol diagnoses among young adults: findings from the NESARC. Alcohol Clin Exp Res 32:2081-2087.

Hasin DS, Wall M, Witkiewitz K, Kranzler HR, Falk D, Litten R, Mann K, O'Malley SS, Scodes J, Robinson RL, Anton R and Alcohol Clinical Trials Initiative (ACTIVE) Workgroup (2017): Change in non-abstinent WHO drinking risk levels and alcohol dependence: a 3 year follow-up study in the US general population. Lancet Psychiatry 4:469-476.

Hasselbalch AL (2010): Genetics of dietary habits and obesity - a twin study. Dan Med Bull 57:B4182.

Hastedt M, Büchner M, Rothe M, Gapert R, Herre S, Krumbiegel F, Tsokos M, Kienast T, Heinz A and Hartwig S (2013): Detecting alcohol abuse: traditional blood alcohol markers compared to ethyl glucuronide (EtG) and fatty acid ethyl esters (FAEEs) measurement in hair. Forensic Sci Med Pathol 9:471-477.

Hatton J, Burton A, Nash H, Munn E, Burgoyne L and Sheron N (2009): Drinking patterns, dependency and life-time drinking history in alcohol-related liver disease. Addiction 104:587-592.

Hedayati M, Razavi SA, Boroomand S and Kheradmand Kia S (2020): The impact of pre-analytical variations on biochemical analytes stability: A systematic review. J Clin Lab Anale23551.

Helander A, Péter O and Zheng Y (2012): Monitoring of the alcohol biomarkers PEth, CDT and EtG/EtS in an outpatient treatment setting. Alcohol Alcohol 47:552-557.

Hietala J, Koivisto H, Anttila P and Niemelä O (2006): Comparison of the combined marker GGT-CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. Alcohol Alcohol 41:528-533.

Hill JO, Wyatt HR and Peters JC (2012): Energy balance and obesity. Circulation 126:126-132.

Hillbom M, Saloheimo P and Juvela S (2011): Alcohol consumption, blood pressure, and the risk of stroke. Curr Hypertens Rep 13:208-213.

Holmes MV, Dale CE, Zuccolo L, Silverwood RJ, Guo Y, Ye Z, Prieto-Merino D, Dehghan A, Trompet S, Wong A, Cavadino A, Drogan D, Padmanabhan S, Li S, Yesupriya A, Leusink M, Sundstrom J, Hubacek JA, Pikhart H, Swerdlow DI, Panayiotou AG, Borinskaya SA, Finan C, Shah S, Kuchenbaecker KB, Shah T, Engmann J, Folkersen L, Eriksson P, Ricceri F, Melander O, Sacerdote C, Gamble DM, Rayaprolu S, Ross OA, McLachlan S, Vikhireva O, Sluijs I, Scott RA, Adamkova V, Flicker L, van Bockxmeer FM, Power C, Marques-Vidal P, Meade T, Marmot MG, Ferro JM, Paulos-Pinheiro S, Humphries SE, Talmud PJ, Mateo L, I, Verweij N, Linneberg A, Skaaby T, Doevendans PA, Cramer MJ, van der Harst P, Klungel OH, Dowling NF, Dominiczak AF, Kumari M, Nicolaides AN, Weikert C, Boeing H, Ebrahim S, Gaunt TR, Price JF, Lannfelt L, Peasey A, Kubinova R, Pajak A, Malyutina S, Voevoda MI, Tamosiunas A, Maitland-van der Zee A, Norman PE, Hankey GJ, Bergmann MM, Hofman A, Franco OH, Cooper J, Palmen J, Spiering W, de Jong PA, Kuh D, Hardy

R, Uitterlinden AG, Ikram MA, Ford I, Hyppönen E, Almeida OP, Wareham NJ, Khaw KT, Hamsten A, Husemoen LL, Tjønneland A, Tolstrup JS, Rimm E, Beulens JW, Verschuren WM, Onland-Moret NC, Hofker MH, Wannamethee SG, Whincup PH, Morris R, Vicente AM, Watkins H, Farrall M, Jukema JW, Meschia J, Cupples LA, Sharp SJ, Fornage M, Kooperberg C, LaCroix AZ, Dai JY, Lanktree MB, Siscovick DS, Jorgenson E, Spring B, Coresh J, Li YR, Buxbaum SG, Schreiner PJ, Ellison RC, Tsai MY, Patel SR, Redline S, Johnson AD, Hoogeveen RC, Hakonarson H, Rotter JI, Boerwinkle E, de Bakker PI, Kivimaki M, Asselbergs FW, Sattar N, Lawlor DA, Whittaker J, Davey SG, Mukamal K, Psaty BM, Wilson JG, Lange LA, Hamidovic A, Hingorani AD, Nordestgaard BG, Bobak M, Leon DA, Langenberg C, Palmer TM, Reiner AP, Keating BJ, Dudbridge F, Casas JP and InterAct Consortium (2014): Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 349:g4164.

Hosseini N, Shor J and Szabo G (2019): Alcoholic hepatitis: a review. Alcohol Alcohol 54:408-416.

Hurley DL (1977): Infectious complications of alcoholism. Postgrad Med 61:160-162.

Huxley RR and Woodward M (2011): Cigarette smoking as a risk factor for coronary heart disease in women compared with men: a systematic review and meta-analysis of prospective cohort studies. Lancet 378:1297-1305.

Iida-Ueno A, Enomoto M, Tamori A and Kawada N (2017): Hepatitis B virus infection and alcohol consumption. World J Gastroenterol 23:2651-2659.

Ikeda M, Maki T, Yin G, Kawate H, Adachi M, Ohnaka K, Takayanagi R and Kono S (2010): Relation of coffee consumption and serum liver enzymes in Japanese men and women with reference to effect modification of alcohol use and body mass index. Scand J Clin Lab Invest 70:171-179.

Ioannou GN, Morrow OB, Connole ML and Lee SP (2009): Association between dietary nutrient composition and the incidence of cirrhosis or liver cancer in the United States population. Hepatology 50:175-184.

Jastrzębska I, Zwolak A, Szczyrek M, Wawryniuk A, Skrzydło-Radomańska B and Daniluk J (2016): Biomarkers of alcohol misuse: recent advances and future prospects. Prz Gastroenterol 11:78-89.

Jäger S, Jacobs S, Kröger J, Stefan N, Fritsche A, Weikert C, Boeing H and Schulze MB (2015): Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam study. PLoS One 10:e0124749.

Järveläinen HA, Orpana A, Perola M, Savolainen VT, Karhunen PJ and Lindros KO (2001): Promoter polymorphism of the CD14 endotoxin receptor gene as a risk factor for alcoholic liver disease. Hepatology 33:1148-1153.

Jha P and Peto R (2014): Global effects of smoking, of quitting, and of taxing tobacco. N Engl J Med 370:60-68.

Johnson NA, Keating SE and George J (2012): Exercise and the liver: implications for therapy in fatty liver disorders. Semin Liver Dis 32:65-79.

Johnson-Greene D, McCaul ME and Roger P (2009): Screening for hazardous drinking using the Michigan Alcohol Screening Test-Geriatric Version (MAST-G) in elderly persons with acute cerebrovascular accidents. Alcohol Clin Exp Res 33:1555-1561.

Jokelainen K (2013): Alkoholin kulutuksen kasvu lisää maksasairauksien riskiä. Suom Lääkäril 68:1880-1884.

Jokelainen K, Parkkila S, Salaspuro M and Niemelä O (2000): Covalent adducts of proteins with acetaldehyde in the liver as a result of acetaldehyde administration in drinking water. J Hepatol 33:926-932.

Kaferle J and Strzoda CE (2009): Evaluation of macrocytosis. Am Fam Physician 79:203-208.

Kalinowski A and Humphreys K (2016): Governmental standard drink definitions and low-risk alcohol consumption guidelines in 37 countries. Addiction 111:1293-1298.

Karaphillis E, Goldstein R, Murphy S and Qayyum R (2017): Serum alanine aminotransferase levels and all-cause mortality. Eur J Gastroenterol Hepatol 29:284-288.

Kaur J (2014): A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014:943162.

Kawamoto R, Tabara Y, Kohara K, Miki T, Kusunoki T, Takayama S, Abe M, Katoh T and Ohtsuka N (2011): Relationships between lipid profiles and metabolic syndrome, insulin resistance and serum high molecular adiponectin in Japanese community-dwelling adults. Lipids Health Dis 10:79.

Kazemi-Shirazi L, Endler G, Winkler S, Schickbauer T, Wagner O and Marsik C (2007): Gamma glutamyltransferase and long-term survival: is it just the liver? Clin Chem 53:940-946.

Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC and Public Policy Committee of the American Association for the Study of Liver Disease (2008): Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 47:1363-1370.

Kitazawa T, Nakatani Y, Fujimoto M, Tamura N, Uemura M and Fukui H (2003): The production of tumor necrosis factor-alpha by macrophages in rats with acute alcohol loading. Alcohol Clin Exp Res 27:72S-75S.

Kivipelto M, Ngandu T, Fratiglioni L, Viitanen M, Kåreholt I, Winblad B, Helkala EL, Tuomilehto J, Soininen H and Nissinen A (2005): Obesity and vascular risk factors at midlife and the risk of dementia and Alzheimer disease. Arch Neurol 62:1556-1560.

Klatsky AL (2015): Alcohol and cardiovascular diseases: where do we stand today? J Intern Med 278:238-250.

Klatsky AL and Armstrong MA (1992): Alcohol, smoking, coffee, and cirrhosis. Am J Epidemiol 136:1248-1257.

Klatsky AL, Morton C, Udaltsova N and Friedman GD (2006): Coffee, cirrhosis, and transaminase enzymes. Arch Intern Med 166:1190-1195.

Kline-Simon AH, Falk DE, Litten RZ, Mertens JR, Fertig J, Ryan M and Weisner CM (2013): Posttreatment low-risk drinking as a predictor of future drinking and problem outcomes among individuals with alcohol use disorders. Alcohol Clin Exp Res 37 Suppl 1:E373-E380.

Klop B, do Rego AT and Cabezas MC (2013): Alcohol and plasma triglycerides. Curr Opin Lipidol 24:321-326.

Knightly R, Tadros G, Sharma J, Duffield P, Carnall E, Fisher J and Salman S (2016): Alcohol screening for older adults in an acute general hospital: FAST v. MAST-G assessments. BJPsych Bull 40:72-76.

Knox J, Wall M, Witkiewitz K, Kranzler HR, Falk D, Litten R, Mann K, O'Malley SS, Scodes J, Anton R, Hasin DS and Alcohol Clinical Trials (ACTIVE) Workgroup (2018): Reduction in nonabstinent WHO drinking risk levels and change in risk for liver disease and positive AUDIT-C scores: prospective 3-year follow-up results in the U.S. general population. Alcohol Clin Exp Res 42:2256-2265.

Koehler EM, Schouten JN, Hansen BE, Hofman A, Stricker BH and Janssen HL (2013): External validation of the fatty liver index for identifying nonalcoholic fatty liver disease in a population-based study. Clin Gastroenterol Hepatol 11:1201-1204.

Koenig W (2017): C-reactive protein and cardiovascular risk: will the controversy end after CANTOS? Clin Chem 63:1897-1898.

Koop AH, Mousa OY, Pham LE, Corral-Hurtado JE, Pungpapong S and Keaveny AP (2018): An argument for vitamin D, A, and zinc monitoring in cirrhosis. Ann Hepatol 17:920-932.

Kotronen A, Westerbacka J, Bergholm R, Pietiläinen KH and Yki-Järvinen H (2007): Liver fat in the metabolic syndrome. J Clin Endocrinol Metab 92:3490-3497.

Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melander M and Yki-Järvinen H (2009): Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. Gastroenterology 137:865-872.

Kotronen A, Yki-Järvinen H, Männistö S, Saarikoski L, Korpi-Hyövälti E, Oksa H, Saltevo J, Saaristo T, Sundvall J, Tuomilehto J and Peltonen M (2010): Non-alcoholic and alcoholic fatty liver disease - two diseases of affluence associated with the metabolic syndrome and type 2 diabetes: the FIN-D2D survey. BMC Public Health 10:237.

Kozakova M, Palombo C, Eng MP, Dekker J, Flyvbjerg A, Mitrakou A, Gastaldelli A and Ferrannini E (2012): Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. Hepatology 55:1406-1415.

Kranzler HR and Soyka M (2018): Diagnosis and pharmacotherapy of alcohol use disorder: a review. JAMA 320:815-824.

Krenz M and Korthuis RJ (2012): Moderate ethanol ingestion and cardiovascular protection: from epidemiologic associations to cellular mechanisms. J Mol Cell Cardiol 52:93-104.

Kuteesa MO, Cook S, Weiss HA, Kamali A, Weinmann W, Seeley J, Ssentongo JN, Kiwanuka T, Namyalo F, Nsubuga D and Webb EL (2019): Comparing Alcohol Use Disorders Identification Test (AUDIT) with Timeline Follow Back (TLFB), DSM-5 and Phosphatidylethanol (PEth) for the assessment of alcohol misuse among young people in Ugandan fishing communities. Addict Behav Rep 10:100233.

Kuulasmaa K, Tolonen H, Cepaitis Z, Laatikainen T, Nissinen A, Vartiainen E and Virman-Ojanen T (2006): European health risk monitoring project. Finnish National Public Health Institute (KTL), Helsinki. Available online at: http://www.thl.fi/ ehrm/ (Last accessed April 5, 2020).

Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K, Veerman JL, Delwiche K, Iannarone ML, Moyer ML, Cercy K, Vos T, Murray CJ and Forouzanfar MH (2016): Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. BMJ 354:i3857.

Lackner C and Tiniakos D (2019): Fibrosis and alcohol-related liver disease. J Hepatol 70:294-304.

Lam GM and Mobarhan S (2004): Central obesity and elevated liver enzymes. Nutr Rev 62:394-399.

Langhammer B, Bergland A and Rydwik E (2018): The importance of physical activity exercise among older people. Biomed Res Int 2018:7856823.

Laramée P, Leonard S, Buchanan-Hughes A, Warnakula S, Daeppen JB and Rehm J (2015): Risk of all-cause mortality in alcohol-dependent individuals: a systematic literature review and meta-analysis. EBioMedicine 2:1394-1404.

Latvala J, Hietala J, Koivisto H, Järvi K, Anttila P and Niemelä O (2005): Immune responses to ethanol metabolites and cytokine profiles differentiate alcoholics with or without liver disease. Am J Gastroenterol 100:1303-1310.

Lau K, Baumeister SE, Lieb W, Meffert PJ, Lerch MM, Mayerle J and Volzke H (2015): The combined effects of alcohol consumption and body mass index on hepatic steatosis in a general population sample of European men and women. Aliment Pharmacol Ther 41:467-476.

Lawlor DA, Sattar N, Smith GD and Ebrahim S (2005): The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. Am J Epidemiol 161:1081-1088.

Lee DH, Ha MH and Christiani DC (2001): Body weight, alcohol consumption and liver enzyme activity--a 4-year follow-up study. Int J Epidemiol 30:766-770.

Lee DH, Blomhoff R and Jacobs DR Jr (2004): Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res 38:535-539.

Lee DH, Silventoinen K, Hu G, Jacobs DR Jr, Jousilahti P, Sundvall J and Tuomilehto J (2006): Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. Eur Heart J 27:2170-2176.

Lee H, Kim SS, You KS, Park W, Yang JH, Kim M and Hayman LL (2014): Asian flushing: genetic and sociocultural factors of alcoholism among East asians. Gastroenterol Nurs 37:327-336.

Lee TH, Kim WR, Benson JT, Therneau TM and Melton LJ 3rd (2008): Serum aminotransferase activity and mortality risk in a United States community. Hepatology 47:880-887.

Li K, Hüsing A and Kaaks R (2014): Lifestyle risk factors and residual life expectancy at age 40: a German cohort study. BMC Med 12:59.

Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, Kaptoge S, Di AE, Stampfer M, Willett WC and Hu FB (2018): Impact of healthy lifestyle factors on life expectancies in the US population. Circulation 138:345-355.

Libby P, Ridker PM, Hansson GK and Leducq Transatlantic Network on Atherothrombosis (2009): Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 54:2129-2138.

Lieber CS (1995): Medical disorders of alcoholism. N Engl J Med 333:1058-1065.

Lieber CS (1997): Cytochrome P-4502E1: its physiological and pathological role. Physiol Rev 77:517-544.

Lieber CS (2004): Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 34:9-19.

Lieber CS (2005): Metabolism of alcohol. Clin Liver Dis 9:1-35.

Lieber CS, Seitz HK, Garro AJ and Worner TM (1979): Alcohol-related diseases and carcinogenesis. Cancer Res 39:2863-2886.

Liikunta: Current Care Guidelines (2016): Working group set up by the Finnish Medical Society Duodecim and the Executive Board of Current Care. The Finnish Medical Society Duodecim, Helsinki. Available online at: https://www.kaypahoito.fi/hoi50075#s7 (Last accessed March 14, 2020).

Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, Amann M, Anderson HR, Andrews KG, Aryee M, Atkinson C, Bacchus LJ, Bahalim AN, Balakrishnan K, Balmes J, Barker-Collo S, Baxter A, Bell ML, Blore JD, Blyth F, Bonner C, Borges G, Bourne R, Boussinesq M, Brauer M, Brooks P, Bruce NG, Brunekreef B, Bryan-Hancock C, Bucello C, Buchbinder R, Bull F, Burnett RT, Byers TE, Calabria B, Carapetis J, Carnahan E, Chafe Z, Charlson F, Chen H, Chen JS, Cheng AT, Child JC, Cohen A, Colson KE, Cowie BC, Darby S, Darling S, Davis A, Degenhardt L, Dentener F, Des Jarlais DC, Devries K, Dherani M, Ding EL, Dorsey ER, Driscoll T, Edmond K, Ali SE, Engell RE, Erwin PJ, Fahimi S, Falder G, Farzadfar F, Ferrari A, Finucane MM, Flaxman S, Fowkes FG, Freedman G, Freeman MK, Gakidou E, Ghosh S, Giovannucci E, Gmel G, Graham K, Grainger R, Grant B, Gunnell D, Gutierrez HR, Hall W, Hoek HW, Hogan A, Hosgood HD3, Hoy D, Hu H, Hubbell BJ, Hutchings SJ, Ibeanusi SE, Jacklyn GL, Jasrasaria R, Jonas JB, Kan H, Kanis JA, Kassebaum N, Kawakami N, Khang YH, Khatibzadeh S, Khoo JP, Kok C, Laden F, Lalloo R, Lan Q, Lathlean T, Leasher JL, Leigh J, Li Y, Lin JK, Lipshultz SE, London S, Lozano R, Lu Y, Mak J, Malekzadeh R, Mallinger L, Marcenes W, March L, Marks R, Martin R, McGale P, McGrath J, Mehta S, Mensah GA, Merriman TR, Micha R, Michaud C, Mishra V, Mohd Hanafiah K, Mokdad AA, Morawska L, Mozaffarian D, Murphy T, Naghavi M, Neal B, Nelson PK, Nolla JM, Norman R, Olives C, Omer SB, Orchard J, Osborne R, Ostro B, Page A, Pandey KD, Parry CD, Passmore E, Patra J, Pearce N, Pelizzari PM, Petzold M, Phillips MR, Pope D, Pope CA 3rd, Powles J, Rao M, Razavi H, Rehfuess EA, Rehm JT, Ritz B, Rivara FP, Roberts T, Robinson C, Rodriguez-Portales JA, Romieu I, Room R, Rosenfeld LC, Roy A, Rushton L, Salomon JA, Sampson U, Sanchez-Riera L, Sanman E, Sapkota A, Seedat S, Shi P, Shield K, Shivakoti R, Singh GM, Sleet DA, Smith E, Smith KR, Stapelberg NJ, Steenland K, Stockl H, Stovner LJ, Straif K, Straney L, Thurston GD, Tran JH, Van Dingenen R, van Donkelaar A, Veerman JL, Vijayakumar L, Weintraub R, Weissman MM, White RA, Whiteford H, Wiersma ST, Wilkinson JD, Williams HC, Williams W, Wilson N, Woolf AD, Yip P, Zielinski JM, Lopez AD, Murray CJL, Ezzati M, AlMazroa MA and Memish ZA (2012): A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380:2224-2260.

Lindsay DG and Astley SB (2002): European research on the functional effects of dietary antioxidants - EUROFEDA. Mol Aspects Med 23:1-38.

Liu B, Balkwill A, Reeves G, Beral V and Million Women Study Collaborators. (2010): Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ 340:c912.

Livingston M and Callinan S (2015): Underreporting in alcohol surveys: whose drinking is underestimated? J Stud Alcohol Drugs 76:158-164.

Loef M and Walach H (2013): Midlife obesity and dementia: meta-analysis and adjusted forecast of dementia prevalence in the United States and China. Obesity (Silver Spring) 21:E51-E55.

Lombardi R, Buzzetti E, Roccarina D and Tsochatzis EA (2015): Non-invasive assessment of liver fibrosis in patients with alcoholic liver disease. World J Gastroenterol 21:11044-11052.

Loomba R, Bettencourt R and Barrett-Connor E (2009): Synergistic association between alcohol intake and body mass index with serum alanine and aspartate

aminotransferase levels in older adults: the Rancho Bernardo Study. Aliment Pharmacol Ther 30:1137-1149.
Loomis AK, Kabadi S, Preiss D, Hyde C, Bonato V, St Louis M, Desai J, Gill JM, Welsh P, Waterworth D and Sattar N (2016): Body mass index and risk of nonalcoholic fatty liver disease: Two electronic health record prospective studies. J Clin Endocrinol Metab 101:945-952.
Loos RJ (2018): The genetics of adiposity. Curr Opin Genet Dev 50:86-95.
Lu Y and Cederbaum AI (2008): CYP2E1 and oxidative liver injury by alcohol. Free Radic Biol Med 44:723-738.
Lucas DL, Brown RA, Wassef M and Giles TD (2005): Alcohol and the cardiovascular system: research challenges and opportunities. J Am Coll Cardiol 45:1916-1924.
Luyckx FH, Desaive C, Thiry A, Dewé W, Scheen AJ, Gielen JE and Lefèbvre PJ (1998): Liver abnormalities in severely obese subjects: effect of drastic weight loss after gastroplasty. Int J Obes Relat Metab Disord 22:222-226.
Mackus M, van de Loo AJAE, Raasveld SJ, Hogewoning A, Sastre Toraño J, Flesch FM, Korte-Bouws GAH, van Neer RHP, Wang X, Nguyen TT, Brookhuis KA, Kraneveld AD, Garssen J and Verster JC (2017): Biomarkers of the alcohol hangover state: ethyl glucuronide (EtG) and ethyl sulfate (EtS). Hum Psychopharmacol 32:e2624.
Mahli A and Hellerbrand C (2016): Alcohol and obesity: a dangerous association for fatty liver disease. Dig Dis 34 Suppl 1:32-39.
Manuel DG, Perez R, Sanmartin C, Taljaard M, Hennessy D, Wilson K, Tanuseputro P, Manson H, Bennett C, Tuna M, Fisher S and Rosella LC (2016): Measuring burden of unhealthy behaviours using a multivariable predictive approach: life expectancy lost in Canada attributable to smoking, alcohol, physical inactivity, and diet. PLoS Med 13:e1002082.
Manzo-Avalos S and Saavedra-Molina A (2010): Cellular and mitochondrial effects of alcohol consumption. Int J Environ Res Public Health 7:4281-4304.
Marcos M, Gómez-Munuera M, Pastor I, González-Sarmiento R and Laso FJ (2009): Tumor necrosis factor polymorphisms and alcoholic liver disease: a HuGE review and meta-analysis. Am J Epidemiol 170:948-956.
Martin-Willett R, Helmuth T, Abraha M, Bryan AD, Hitchcock L, Lee K and Bidwell LC (2020): Validation of a multisubstance online Timeline Followback assessment. Brain Behav 10:e01486.
Mason BJ (2017): Emerging pharmacotherapies for alcohol use disorder. Neuropharmacology 122:244-253.
Mason RJ, Buist AS, Fisher EB, Merchant JA, Samet JM and Welsh CH (1985): Cigarette smoking and health. Am Rev Respir Dis 132:1133-1136.
Mathus-Vliegen EM (2012): Prevalence, pathophysiology, health consequences and treatment options of obesity in the elderly: a guideline. Obes Facts 5:460-483.
Männistö S, Laatikainen T and Vartiainen E (2012): Suomalaisten lihavuus ennen ja nyt. Tutkimuksesta tiiviisti 4. National Institute for Health and Welfare (THL), Helsinki. www.julkari.fi/handle/10024/90885 (Last accessed March 14, 2020).
McCord JM (2000): The evolution of free radicals and oxidative stress. Am J Med 108:652-659.
McGinnis M, Williams-Russo P and Knickman JR (2002): The case for more active policy attention to health promotion. Health Affairs 21:78-93.

McManus DD, Yin X, Gladstone R, Vittinghoff E, Vasan RS, Larson MG, Benjamin EJ and Marcus GM (2016): Alcohol consumption, left atrial diameter, and atrial fibrillation. J Am Heart Assoc 5:e004060.

Meier P and Seitz HK (2008): Age, alcohol metabolism and liver disease. Curr Opin Clin Nutr Metab Care 11:21-26.

Miele L, De Michele T, Marrone G, Antonietta Isgrò M, Basile U, Cefalo C, Biolato M, Maria Vecchio F, Lodovico Rapaccini G, Gasbarrini A, Zuppi C and Grieco A (2017): Enhanced liver fibrosis test as a reliable tool for assessing fibrosis in nonalcoholic fatty liver disease in a clinical setting. Int J Biol Markers 32:e397-e402.

Mikkelsen IM, Kanitz RD, Nilssen O and Huseby NE (1998): Carbohydrate-deficient transferrin: marker of actual alcohol consumption or chronic alcohol misuse? Alcohol Alcohol 33:646-650.

Miller RA and Britigan BE (1997): Role of oxidants in microbial pathophysiology. Clin Microbiol Rev 10:1-18.

Modi AA, Feld JJ, Park Y, Kleiner DE, Everhart JE, Liang TJ and Hoofnagle JH (2010): Increased caffeine consumption is associated with reduced hepatic fibrosis. Hepatology 51:201-209.

Molloy JW, Calcagno CJ, Williams CD, Jones FJ, Torres DM and Harrison SA (2012): Association of coffee and caffeine consumption with fatty liver disease, nonalcoholic steatohepatitis, and degree of hepatic fibrosis. Hepatology 55:429-436.

Moradi H, Streja E and Kalantar-Zadeh K (2017): Serum high density lipoprotein cholesterol level and risk of death: let's avoid the extremes. J Thorac Dis 9:4849-4852.

Moreno C, Mueller S and Szabo G (2019): Non-invasive diagnosis and biomarkers in alcohol-related liver disease. J Hepatol 70:273-283.

Morgan MY, Camilo ME, Luck W, Sherlock S and Hoffbrand AV (1981): Macrocytosis in alcohol-related liver disease: its value for screening. Clin Lab Haematol 3:35-44.

Morisco F, Lembo V, Mazzone G, Camera S and Caporaso N (2014): Coffee and liver health. J Clin Gastroenterol. 48 Suppl 1:S87-90.

Mumtaz S, Schomaker N and Von Roenn N (2019): Pro: noninvasive imaging has replaced biopsy as the gold standard in the evaluation of nonalcoholic fatty liver disease. Clin Liver Dis (Hoboken) 13:111-113.

Musso G, Cassader M, Rosina F and Gambino R (2012): Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. Diabetologia 55:885-904.

Nakanishi N, Suzuki K and Tatara K (2004): Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. Diabetes Care 27:1427-1432.

Nalpas B, Vassault A, Le GA, Lesgourgues B, Ferry N, Lacour B and Berthelot P (1984): Serum activity of mitochondrial aspartate aminotransferase: a sensitive marker of alcoholism with or without alcoholic hepatitis. Hepatology 4:893-896.

National Institute of Alcohol Abuse and Alcoholism (2004): NIAAA Council approves definition of binge drinking. NIAAA Newsletter 3:3. Available online at: https://pubs.niaaa.nih.gov/publications/Newsletter/winter2004/Newsletter_Number3.pdf (Last accessed January 27, 2020).

Naveau S, Giraud V, Borotto E, Aubert A, Capron F and Chaput JC (1997): Excess weight risk factor for alcoholic liver disease. Hepatology 25:108-111.

Naviaux RK (2012): Oxidative shielding or oxidative stress? J Pharmacol Exp Ther 342:608-618.
Nawrot P, Jordan S, Eastwood J, Rotstein J, Hugenholtz A and Feeley M (2003): Effects of caffeine on human health. Food Addit Contam 20:1-30.
Nielsen SJ, Kit BK, Fakhouri T and Ogden CL (2012): Calories consumed from alcoholic beverages by U.S. adults, 2007-2010. NCHS Data Brief :1-8.
Niemelä O (2001): Distribution of ethanol-induced protein adducts in vivo: relationship to tissue injury. Free Radic Biol Med 31:1533-1538.
Niemelä O (2007): Biomarkers in alcoholism. Clin Chim Acta 377:39-49.
Niemelä O (2016): Biomarker-based approaches for assessing alcohol use disorders. Int J Environ Res Public Health 13:166.
Niemelä O and Alatalo P (2010): Biomarkers of alcohol consumption and related liver disease. Scand J Clin Lab Invest 70:305-312.
Niemelä O and Danielsson J (2015): Maksa-arvojen viiterajat tarkistettava. Duodecim 131:1124-1126.
Niemelä O, Niemelä M, Bloigu R, Aalto M and Laatikainen T (2017): Where should the safe limits of alcohol consumption stand in light of liver enzyme abnormalities in alcohol consumers? PLoS One 12:e0188574.
Nilssen O and Førde OH (1994): Seven-year longitudinal population study of change in gamma-glutamyltransferase: the Trømso Study. Am J Epidemiol 139:787-792.
Nilssen O, Førde OH and Brenn T (1990): The Trømso Study. Distribution and population determinants of gamma-glutamyltransferase. Am J Epidemiol 132:318-326.
Nordic Nutrition Recommendations 2012 (2014): Dietary antioxidants. In: Nordic Nutrition Recommendations 2012. Integrating nutrition and physical activity, pp. 323-334. Nordic Council of Ministers, Copenhagen. Available online at: http://norden.diva-portal.org/smash/get/diva2:704251/FULLTEXT01.pdf (Last accessed March 15, 2020).
O'Dwyer C, Mongan D, Millar SR, Rackard M, Galvin B, Long J and Barry J (2019): Drinking patterns and the distribution of alcohol-related harms in Ireland: evidence for the prevention paradox. BMC Public Health 19:1323.
O'Shea RS, Dasarathy S, McCullough AJ and Practice Guideline Committee of the American Association for the Study of Liver Diseases and Practice Parameters Committee of the American College of Gastroentrology (2010): Alcoholic liver disease. Hepatology 51:307-328.
Ofosu A, Ramai D and Reddy M (2018): Non-alcoholic fatty liver disease: controlling an emerging epidemic, challenges, and future directions. Ann Gastroenterol 31:288-295.
Ogden CL, Carroll MD, Kit BK and Flegal KM (2014): Prevalence of childhood and adult obesity in the United States, 2011-2012. JAMA 311:806-814.
Oh S, Shida T, Yamagishi K, Tanaka K, So R, Tsujimoto T and Shoda J (2015): Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: a retrospective study. Hepatology 61:1205-1215.
Ohnishi K and Lieber CS (1977): Reconstitution of the microsomal ethanol-oxidizing system. Qualitative and quantitative changes of cytochrome P-450 after chronic ethanol consumption. J Biol Chem 252:7124-7131.
Okorodudu DO, Jumean MF, Montori VM, Romero-Corral A, Somers VK, Erwin PJ and Lopez-Jimenez F (2010): Diagnostic performance of body mass index to identify obesity as defined by body adiposity: a systematic review and meta-analysis. Int J Obes (Lond) 34:791-799.

Orio L, Antón M, Rodriguez-Rojo IC, Correas Á, García-Bueno B, Corral M, de Fonseca FR, García-Moreno LM, Maestú F and Cadaveira F (2017): Young alcohol binge drinkers have elevated blood endotoxin, peripheral inflammation and low cortisol levels: neuropsychological correlations in women. Addict Biol 23:1130-1144.

Owolabi EO, Ter GD, Adeniyi OV and Ajayi AI (2018): Optimal waist circumference cut-off points for predicting metabolic syndrome among low-income black South African adults. BMC Res Notes 11:22.

Pacifico L, Osborn JF, Bonci E, Pierimarchi P and Chiesa C (2019): Association between vitamin D levels and nonalcoholic fatty liver disease: Potential confounding variables. Mini Rev Med Chem 19:310-332.

Papastergiou V, Tsochatzis E and Burroughs AK (2012): Non-invasive assessment of liver fibrosis. Ann Gastroenterol 25:218-231.

Park EY, Lim MK, Oh JK, Cho H, Bae MJ, Yun EH, Kim DI and Shin HR (2013): Independent and supra-additive effects of alcohol consumption, cigarette smoking, and metabolic syndrome on the elevation of serum liver enzyme levels. PLoS One 8:e63439.

Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J, Lombard M, Alexander G, Ramage J, Dusheiko G, Wheatley M, Gough C, Burt A and Rosenberg W (2010): Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut 59:1245-1251.

Pasala S, Barr T and Messaoudi I (2015): Impact of alcohol abuse on the adaptive immune system. Alcohol Res 37:185-197.

Pascual M, Montesinos J, Marcos M, Torres JL, Costa-Alba P, García-García F, Laso FJ and Guerri C (2017): Gender differences in the inflammatory cytokine and chemokine profiles induced by binge ethanol drinking in adolescence. Addict Biol 22:1829-1841.

Patrick ME (2016): A call for research on high-intensity alcohol use. Alcohol Clin Exp Res 40:256-259.

Patrick ME and Azar B (2018): High-intensity drinking. Alcohol Res 39:49-55.

Patrick ME, Cronce JM, Fairlie AM, Atkins DC and Lee CM (2016): Day-to-day variations in high-intensity drinking, expectancies, and positive and negative alcohol-related consequences. Addict Behav 58:110-116.

Perreault K, Bauman A, Johnson N, Britton A, Rangul V and Stamatakis E (2017): Does physical activity moderate the association between alcohol drinking and all-cause, cancer and cardiovascular diseases mortality? A pooled analysis of eight British population cohorts. Br J Sports Med 51:651-657.

Petroni ML, Brodosi L, Marchignoli F, Musio A and Marchesini G (2019): Moderate alcohol intake in non-alcoholic fatty liver disease: to drink or not to drink? Nutrients 11:3048.

Poynard T, Bedossa P and Opolon P (1997): Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 349:825-832.

Poynard T, Imbert-Bismut F, Munteanu M, Messous D, Myers RP, Thabut D, Ratziu V, Mercadier A, Benhamou Y and Hainque B (2004): Overview of the diagnostic value of biochemical markers of liver fibrosis (FibroTest, HCV FibroSure) and necrosis (ActiTest) in patients with chronic hepatitis C. Comp Hepatol 3:8.

Poynard T, Munteanu M, Deckmyn O, Ngo Y, Drane F, Castille JM, Housset C, Ratziu V and Imbert-Bismut F (2012): Validation of liver fibrosis biomarker (FibroTest) for assessing liver fibrosis progression: proof of concept and first application in a large population. J Hepatol 57:541-548.

Pratt DS and Kaplan MM (2000): Evaluation of abnormal liver-enzyme results in asymptomatic patients. N Engl J Med 342:1266-1271.

Preston SH, Stokes A, Mehta NK and Cao B (2014): Projecting the effect of changes in smoking and obesity on future life expectancy in the United States. Demography 51:27-49.

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.

Ramos-Lopez O, Martinez-Lopez E, Roman S, Fierro NA and Panduro A (2015): Genetic, metabolic and environmental factors involved in the development of liver cirrhosis in Mexico. World J Gastroenterol 21:11552-11566.

Raynard B, Balian A, Fallik D, Capron F, Bedossa P, Chaput JC and Naveau S (2002): Risk factors of fibrosis in alcohol-induced liver disease. Hepatology 35:635-638.

Rehm J, Room R, van den Brink W and Jacobi F (2005): Alcohol use disorders in EU countries and Norway: an overview of the epidemiology. Eur Neuropsychopharmacol 15:377-388.

Rehm J, Samokhvalov AV and Shield KD (2013): Global burden of alcoholic liver diseases. J Hepatol 59:160-168.

Rehm J, Anderson P, Manthey J, Shield KD, Struzzo P, Wojnar M and Gual A (2016): Alcohol use disorders in primary health care: what do we know and where do we go? Alcohol Alcohol 51:422-427.

Rehm J, Gmel GESr, Gmel G, Hasan OSM, Imtiaz S, Popova S, Probst C, Roerecke M, Room R, Samokhvalov AV, Shield KD and Shuper PA (2017): The relationship between different dimensions of alcohol use and the burden of disease-an update. Addiction 112:968-1001.

Renaud S and de Lorgeril M (1992): Wine, alcohol, platelets, and the French paradox for coronary heart disease. Lancet 339:1523-1526.

Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C, Fonseca F, Nicolau J, Koenig W, Anker SD, Kastelein JJP, Cornel JH, Pais P, Pella D, Genest J, Cifkova R, Lorenzatti A, Forster T, Kobalava Z, Vida-Simiti L, Flather M, Shimokawa H, Ogawa H, Dellborg M, Rossi PRF, Troquay RPT, Libby P, Glynn RJ and CANTOS Trial Group (2017): Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 377:1119-1131.

Rinella ME (2015): Nonalcoholic fatty liver disease: a systematic review. JAMA 313:2263-2273.

Roberts CK and Sindhu KK (2009): Oxidative stress and metabolic syndrome. Life Sci 84:705-712.

Rodriguez-Merchan EC (2018): The importance of smoking in orthopedic surgery. Hosp Pract (1995) 46:175-182.

Romaguera D, Tauler P, Bennasar M, Pericas J, Moreno C, Martinez S and Aguilo A (2011): Determinants and patterns of physical activity practice among Spanish university students. J Sports Sci 29:989-997.

Romero-Gómez M, Zelber-Sagi S and Trenell M (2017): Treatment of NAFLD with diet, physical activity and exercise. J Hepatol 67:829-846.

Rooney D, Lye WK, Tan G, Lamoureux EL, Ikram MK, Cheng CY, Kumari N, Zheng YF, Mitchell P, Wang JJ, Wong TY and Sabanayagam C (2015): Body mass index and retinopathy in Asian populations with diabetes mellitus. Acta Diabetol 52:73-80.

Rosa-Oliveira LQ, Presti PF, Antunes IR, Carbonari GC, Imada AC, Maeda MY, Silveira DX and Doering-Silveira J (2011): Reliability and dimensionality of the Short Alcohol Dependence Data (SADD) questionnaire in a clinical sample of hospitalized patients: using the SADD in a general hospital setting. Braz J Psychiatry 33:68-71.

Rosoff DB, Charlet K, Jung J, Lee J, Muench C, Luo A, Longley M, Mauro KL and Lohoff FW (2019): Association of High-Intensity Binge Drinking With Lipid and Liver Function Enzyme Levels. JAMA Netw Open 2:e195844.

Ruhl CE and Everhart JE (2005a): Coffee and tea consumption are associated with a lower incidence of chronic liver disease in the United States. Gastroenterology 129:1928-1936.

Ruhl CE and Everhart JE (2005b): Joint effects of body weight and alcohol on elevated serum alanine aminotransferase in the United States population. Clin Gastroenterol Hepatol 3:1260-1268.

Ruhl CE and Everhart JE (2009): Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology 136:477-485.

Rutten-Jacobs LC, Larsson SC, Malik R, Rannikmae K, Sudlow CL, Dichgans M, Markus HS and Traylor M (2018): Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: cohort study of 306 473 UK Biobank participants. BMJ 363:k4168.

Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H and the Vorarlberg Health Monitoring and Promotion Program Study Group (2005): Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation 112:2130-2137.

Saab S, Mallam D, Cox GA and Tong MJ (2014): Impact of coffee on liver diseases: a systematic review. Liver Int 34:495-504.

Saklayen MG (2018): The global epidemic of the metabolic syndrome. Curr Hypertens Rep 20:12.

Salaspuro M (1987): Use of enzymes for the diagnosis of alcohol-related organ damage. Enzyme 37:87-107.

Salaspuro M (1999): Carbohydrate-deficient transferrin as compared to other markers of alcoholism: a systematic review. Alcohol 19:261-271.

Sánchez-Jiménez BA, Brizuela-Alcántara D, Ramos-Ostos MH, Alva-López LF, Uribe-Esquivel M and Chávez-Tapia NC (2018): Both alcoholic and non-alcoholic steatohepatitis association with cardiovascular risk and liver fibrosis. Alcohol 69:63-67.

Sato N, Lindros KO, Baraona E, Ikejima K, Mezey E, Järveläinen HA and Ramchandani VA (2001): Sex difference in alcohol-related organ injury. Alcohol Clin Exp Res 25:40S-45S.

Saunders JB, Aasland OG, Babor TF, de la Fuente JR and Grant M (1993): Development of the Alcohol Use Disorders Identification Test (AUDIT): WHO collaborative project on early detection of persons with harmful alcohol consumption--II. Addiction 88:791-804.

Schenker S (1997): Medical consequences of alcohol abuse: is gender a factor? Alcohol Clin Exp Res 21:179-181.

Schindhelm RK, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, Heine RJ and Diamant M (2007): Alanine aminotransferase and the 6-year risk of the metabolic syndrome in Caucasian men and women: the Hoorn Study. Diabet Med 24:430-435.

Schwarzinger M, Pollock BG, Hasan OSM, Dufouil C, Rehm J and QalyDays Study Group (2018): Contribution of alcohol use disorders to the burden of dementia in France 2008-13: a nationwide retrospective cohort study. Lancet Public Health 3:e124-e132.

Seitz HK and Stickel F (2007): Molecular mechanisms of alcohol-mediated carcinogenesis. Nat Rev Cancer 7:599-612.

Selzer ML (1971): The Michigan alcoholism screening test: the quest for a new diagnostic instrument. Am J Psychiatry 127:1653-1658.

Setshedi M, Wands JR and Monte SM (2010): Acetaldehyde adducts in alcoholic liver disease. Oxid Med Cell Longev 3:178-185.

Shah M and Paulson D (2016): C-reactive protein level partially mediates the relationship between moderate alcohol use and frailty: the Health and Retirement Study. Age Ageing 45:874-878.

Shah NJ, Royer A and John S (2020): Alcoholic hepatitis. In: StatPearls, StatPearls Publishing, Treasure Island (FL).

Sharma P and Arora A (2020): Clinical presentation of alcoholic liver disease and non-alcoholic fatty liver disease: spectrum and diagnosis. Transl Gastroenterol Hepatol 5:19.

Sheth SG, Flamm SL, Gordon FD and Chopra S (1998): AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 93:44-48.

Shukla L, Sharma P, Ganesha S, Ghadigaonkar D, Thomas E, Kandasamy A, Murthy P and Benegal V (2017): Value of ethyl glucuronide and ethyl sulfate in serum as biomarkers of alcohol consumption. Indian J Psychol Med 39:481-487.

Sierksma A, van der Gaag MS, Kluft C and Hendriks HF (2002): Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur J Clin Nutr 56:1130-1136.

Sipilä P, Rose RJ and Kaprio J (2016): Drinking and mortality: long-term follow-up of drinking-discordant twin pairs. Addiction 111:245-254.

Smith AD, Crippa A, Woodcock J and Brage S (2016): Physical activity and incident type 2 diabetes mellitus: a systematic review and dose-response meta-analysis of prospective cohort studies. Diabetologia 59:2527-2545.

Spanagel R, Durstewitz D, Hansson A, Heinz A, Kiefer F, Köhr G, Matthäus F, Nöthen MM, Noori HR, Obermayer K, Rietschel M, Schloss P, Scholz H, Schumann G, Smolka M, Sommer W, Vengeliene V, Walter H, Wurst W, Zimmermann US, Addiction GWAS Resource Group, Stringer S, Smits Y and Derks EM (2013): A systems medicine research approach for studying alcohol addiction. Addict Biol 18:883-896.

Spear LP and Swartzwelder HS (2014): Adolescent alcohol exposure and persistence of adolescent-typical phenotypes into adulthood: a mini-review. Neurosci Biobehav Rev 45:1-8.

Speisky H, Shackel N, Varghese G, Wade D and Israel Y (1990): Role of hepatic gamma-glutamyltransferase in the degradation of circulating glutathione: studies in the intact guinea pig perfused liver. Hepatology 11:843-849.

Sproston NR and Ashworth JJ (2018): Role of C-reactive protein at sites of inflammation and infection. Front Immunol 9:754.

St George A, Bauman A, Johnston A, Farrell G, Chey T and George J (2009): Independent effects of physical activity in patients with nonalcoholic fatty liver disease. Hepatology 50:68-76.
Statistics Canada (2015): Health at a glance. Available at: www150.statcan.gc.ca/n1 /pub/82-624-x/2012001/article/11676-eng.htm (Last accessed April 6, 2020),
Stewart SH, Mainous AG, III and Gilbert G (2002): Relation between alcohol consumption and C-reactive protein levels in the adult US population. J Am Board Fam Pract 15:437-442.
Stibler H and Hultcrantz R (1987): Carbohydrate-deficient transferrin in serum in patients with liver diseases. Alcohol Clin Exp Res 11:468-473.
Stickel F and Hampe J (2012): Genetic determinants of alcoholic liver disease. Gut 61:150-159.
Stickel F, Datz C, Hampe J and Bataller R (2017): Pathophysiology and Management of Alcoholic Liver Disease: update 2016. Gut Liver 11:173-188.
Stockwell T, Zhao J, Panwar S, Roemer A, Naimi T and Chikritzhs T (2016): Do "moderate" drinkers have reduced mortality risk? A systematic review and meta-analysis of alcohol consumption and all-cause mortality. J Stud Alcohol Drugs 77:185-198.
Styne DM, Arslanian SA, Connor EL, Farooqi IS, Murad MH, Silverstein JH and Yanovski JA (2017): Pediatric obesity-assessment, treatment, and prevention: an endocrine society clinical practice guideline. J Clin Endocrinol Metab 102:709-757.
Sullivan S, Kirk EP, Mittendorfer B, Patterson BW and Klein S (2012): Randomized trial of exercise effect on intrahepatic triglyceride content and lipid kinetics in nonalcoholic fatty liver disease. Hepatology 55:1738-1745.
Sun M and Kisseleva T (2015): Reversibility of liver fibrosis. Clin Res Hepatol Gastroenterol 39 Suppl 1:S60-S63.
Sundberg CJ (2016): Physical activity: what is already being done and how we can avert 1 million deaths annually in future. Br J Sports Med 50:319.
Sundell L, Salomaa V, Vartiainen E, Poikolainen K and Laatikainen T (2008): Increased stroke risk is related to a binge-drinking habit. Stroke 39:3179-3184.
Sureshchandra S, Raus A, Jankeel A, Ligh BJK, Walter NAR, Newman N, Grant KA and Messaoudi I (2019): Dose-dependent effects of chronic alcohol drinking on peripheral immune responses. Sci Rep 9:7847.
Szabo G (1998): Monocytes, alcohol use, and altered immunity. Alcohol Clin Exp Res 22:216S-219S.
Szabo G, Wands JR, Eken A, Osna NA, Weinman SA, Machida K and Joe Wang H (2010): Alcohol and hepatitis C virus – interactions in immune dysfunctions and liver damage. Alcohol Clin Exp Res 34:1675-1686.
Szczygielska A, Widomska S, Jaraszkiewicz M, Knera P and Muc K (2003): Blood lipids profile in obese or overweight patients. Ann Univ Mariae Curie Sklodowska Med 58:343-349.
Takigawa T, Hibino Y, Kimura S, Yamauchi H, Wang B, Wang D and Ogino K (2008): Association between serum gamma-glutamyltransferase and oxidative stress related factors. Hepatogastroenterology 55:50-53.
Tamakoshi A, Tamakoshi K, Lin Y, Yagyu K, Kikuchi S and JACC Study Group (2009): Healthy lifestyle and preventable death: findings from the Japan Collaborative Cohort (JACC) study. Prev Med 48:486-492.
Tamakoshi A, Kawado M, Ozasa K, Tamakoshi K, Lin Y, Yagyu K, Kikuchi S, Hashimoto S and JACC Study Group (2010): Impact of smoking and other lifestyle factors on

life expectancy among japanese: findings from the Japan Collaborative Cohort (JACC) Study. J Epidemiol 20:370-376.
Tanaka K, Tokunaga S, Kono S, Tokudome S, Akamatsu T, Moriyama T and Zakouji H (1998): Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. Int J Epidemiol 27:438-443.
Tapper EB and Parikh ND (2018): Mortality due to cirrhosis and liver cancer in the United States, 1999-2016: observational study. BMJ 362:k2817.
Targher G, Bertolini L, Rodella S, Tessari R, Zenari L, Lippi G and Arcaro G (2007): Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care 30:2119-2121.
Tavakoli HR, Hull M and Okasinski M (2011): Review of current clinical biomarkers for the detection of alcohol dependence. Innov Clin Neurosci 8:26-33.
Taylor CB, Sallis JF and Needle R (1985): The relation of physical activity and exercise to mental health. Public Health Rep 100:195-202.
Teeriniemi AM, Salonurmi T, Jokelainen T, Vähänikkilä H, Alahäivälä T, Karppinen P, Enwald H, Huotari ML, Laitinen J, Oinas-Kukkonen H and Savolainen MJ (2018): A randomized clinical trial of the effectiveness of a Web-based health behaviour change support system and group lifestyle counselling on body weight loss in overweight and obese subjects: 2-year outcomes. J Intern Med 284:534-545.
Terveyden ja hyvinvoinnin laitos (2020): Alkoholi ja puheeksiotto. Helsinki. Available online at: https://thl.fi/fi/web/alkoholi-tupakka-ja-riippuvuudet/ ehkaiseva-paihdetyo/ehkaisevan-paihdetyon-menetelmat/alkoholinkayton-puheeksiotto-ja-mini-interventio/eri-ongelmien-varhainen-tunnistaminen-ja-puheeksiotto/alkoholi (Last accessed July 27, 2020).
Teschke R (2019): Alcoholic liver disease: Current mechanistic aspects with focus on their clinical relevance. Biomedicines 7:68.
Thiele GM, Klassen LW and Tuma DJ (2008): Formation and immunological properties of aldehyde-derived protein adducts following alcohol consumption. Methods Mol Biol 447:235-257.
Thiele M, Madsen BS, Hansen JF, Detlefsen S, Antonsen S and Krag A (2018): Accuracy of the enhanced liver fibrosis test vs fibro test, elastography, and indirect markers in detection of advanced fibrosis in patients with alcoholic liver disease. Gastroenterology 154:1369-1379.
Thompson RP (1986): Measuring the damage--ethanol and the liver. Gut 27:751-755.
Tiikkainen M, Bergholm R, Vehkavaara S, Rissanen A, Häkkinen AM, Tamminen M, Teramo K and Yki-Järvinen H (2003): Effects of identical weight loss on body composition and features of insulin resistance in obese women with high and low liver fat content. Diabetes 52:701-707.
Tohyama Y, Takano T and Yamamura H (2004): B cell responses to oxidative stress. Curr Pharm Des 10:835-839.
Topiwala A, Allan CL, Valkanova V, Zsoldos E, Filippini N, Sexton C, Mahmood A, Fooks P, Singh-Manoux A, Mackay CE, Kivimäki M and Ebmeier KP (2017): Moderate alcohol consumption as risk factor for adverse brain outcomes and cognitive decline: longitudinal cohort study. BMJ 357:j2353.
Torres DM and Harrison SA (2013): Is it time to write a prescription for coffee? Coffee and liver disease. Gastroenterology 144:670-672.

Torruellas C, French SW and Medici V (2014): Diagnosis of alcoholic liver disease. World J Gastroenterol 20:11684-11699.
Traversy G and Chaput JP (2015): Alcohol consumption and obesity: an update. Curr Obes Rep 4:122-130.
Trayhurn P and Wood IS (2004): Adipokines: inflammation and the pleiotropic role of white adipose tissue. Br J Nutr 92:347-355.
Trépo E, Gustot T, Degré D, Lemmers A, Verset L, Demetter P, Ouziel R, Quertinmont E, Vercruysse V, Amininejad L, Deltenre P, Le Moine O, Devière J, Franchimont D and Moreno C (2011): Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease. J Hepatol 55:906-912.
Tsai J, Ford ES, Zhao G, Li C, Greenlund KJ and Croft JB (2012): Co-occurrence of obesity and patterns of alcohol use associated with elevated serum hepatic enzymes in US adults. J Behav Med 35:200-210.
Tsukamoto H, Horne W, Kamimura S, Niemelä O, Parkkila S, Ylä-Herttuala S and Brittenham GM (1995): Experimental liver cirrhosis induced by alcohol and iron. J Clin Invest 96:620-630.
Tucker JA, Vuchinich RE and Sobell MB (1982): Alcohol's effects on human emotions: a review of the stimulation/depression hypothesis. Int J Addict 17:155-180.
Tverdal A and Skurtveit S (2003): Coffee intake and mortality from liver cirrhosis. Ann Epidemiol 13:419-423.
Tynjälä J, Kangastupa P, Laatikainen T, Aalto M and Niemelä O (2012): Effect of age and gender on the relationship between alcohol consumption and serum GGT: time to recalibrate goals for normal ranges. Alcohol Alcohol 47:558-562.
Unger KW and Johnson D Jr (1974): Red blood cell mean corpuscular volume: a potential indicator of alcohol usage in a working population. Am J Med Sci 267:281-289.
Vakili S, Sobell LC, Sobell MB, Simco ER and Agrawal S (2008): Using the Timeline Followback to determine time windows representative of annual alcohol consumption with problem drinkers. Addict Behav 33:1123-1130.
van Faassen E and Niemelä O (2011): Biochemistry of prenatal alcohol exposure. Nova Science Publishers, New York.
van Strien T (2018): Causes of emotional eating and matched treatment of obesity. Curr Diab Rep 18:35.
Vandevijvere S, Chow CC, Hall KD, Umali E and Swinburn BA (2015): Increased food energy supply as a major driver of the obesity epidemic: a global analysis. Bull World Health Organ 93:446-456.
Viel G, Boscolo-Berto R, Cecchetto G, Fais P, Nalesso A and Ferrara SD (2012): Phosphatidylethanol in blood as a marker of chronic alcohol use: a systematic review and meta-analysis. Int J Mol Sci 13:14788-14812.
Volpato S, Pahor M, Ferrucci L, Simonsick EM, Guralnik JM, Kritchevsky SB, Fellin R and Harris TB (2004): Relationship of alcohol intake with inflammatory markers and plasminogen activator inhibitor-1 in well-functioning older adults: the Health, Aging, and Body Composition study. Circulation 109:607-612.
Vonghia L, Michielsen P, Dom G and Francque S (2014): Diagnostic challenges in alcohol use disorder and alcoholic liver disease. World J Gastroenterol 20:8024-8032.
Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS and Lok AS (2003): A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. Hepatology 38:518-526.

Walker RK, Cousins VM, Umoh NA, Jeffress MA, Taghipour D, Al-Rubaiee M and Haddad GE (2013): The good, the bad, and the ugly with alcohol use and abuse on the heart. Alcohol Clin Exp Res 37:1253-1260.

Wannamethee SG and Shaper AG (2010): Cigarette smoking and serum liver enzymes: the role of alcohol and inflammation. Ann Clin Biochem 47:321-326.

Warburton DE and Bredin SS (2016): Reflections on physical activity and health: what should we recommend? Can J Cardiol 32:495-504.

Ware J Jr, Kosinski M and Keller SD (1996): A 12-Item Short-Form Health Survey: construction of scales and preliminary tests of reliability and validity. Med Care 34:220-233.

Warren KR, Hewitt BG and Thomas JD (2011): Fetal alcohol spectrum disorders: research challenges and opportunities. Alcohol Res Health 34:4-14.

Weir DG (1978): The pathophysiology of alcohol and acetaldehyde metabolism in the liver. Eur J Clin Invest 8:263-265.

Welsh JA, Karpen S and Vos MB (2013): Increasing prevalence of nonalcoholic fatty liver disease among United States adolescents, 1988-1994 to 2007-2010. J Pediatr 162:496-500.

Westermeyer J, Yargic I and Thuras P (2004): Michigan assessment-screening test for alcohol and drugs (MAST/AD): evaluation in a clinical sample. Am J Addict 13:151-162.

Whelan G (1992): Biological markers of alcoholism. Aust N Z J Med 22:209-213.

Willcox JK, Ash SL and Catignani GL (2004): Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 44:275-295.

Wilson DF and Matschinsky FM (2020): Ethanol metabolism: the good, the bad, and the ugly. Med Hypotheses 140:109638.

Wilson PW, D'Agostino RB, Sullivan L, Parise H and Kannel WB (2002): Overweight and obesity as determinants of cardiovascular risk: the Framingham experience. Arch Intern Med 162:1867-1872.

Witkiewitz K, Hallgren KA, Kranzler HR, Mann KF, Hasin DS, Falk DE, Litten RZ, O'Malley SS and Anton RF (2017a): Clinical validation of reduced alcohol consumption after treatment for alcohol dependence using the World Health Organization risk drinking levels. Alcohol Clin Exp Res 41:179-186.

Witkiewitz K, Pearson MR, Hallgren KA, Maisto SA, Roos CR, Kirouac M, Wilson AD, Montes KS and Heather N (2017b): Who achieves low risk drinking during alcohol treatment? An analysis of patients in three alcohol clinical trials. Addiction 112:2112-2121.

Wittchen HU (2012): The burden of mood disorders. Science 338:15.

Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, Paige E, Paul DS, Sweeting M, Burgess S, Bell S, Astle W, Stevens D, Koulman A, Selmer RM, Verschuren WMM, Sato S, Njølstad I, Woodward M, Salomaa V, Nordestgaard BG, Yeap BB, Fletcher A, Melander O, Kuller LH, Balkau B, Marmot M, Koenig W, Casiglia E, Cooper C, Arndt V, Franco OH, Wennberg P, Gallacher J, Gómez de la Cámara A, Völzke H, Dahm CC, Dale CE, Bergmann MM, Crespo CJ, van der Schouw YT, Kaaks R, Simons LA, Lagiou P, Schoufour JD, Boer JMA, Key TJ, Rodriguez B, Moreno-Iribas C, Davidson KW, Taylor JO, Sacerdote C, Wallace RB, Quiros JR, Tumino R, Blazer II DG, Linneberg A, Daimon M, Panico S, Howard B, Skeie G, Strandberg T, Weiderpass E, Nietert PJ, Psaty BM, Kromhout D, Salamanca-Fernandez E, Kiechl S, Krumholz HM, Grioni S, Palli D, Huerta JM, Price J, Sundström J, Arriola L, Arima H, Travis RC, Panagiotakos DB, Karakatsani A,

Trichopoulou A, Kühn T, Grobbee DE, Barrett-Connor E, van Schoor N, Boeing H, Overvad K, Kauhanen J, Wareham N, Langenberg C, Forouhi N, Wennberg M, Després J-P, Cushman M, Cooper JA, Rodriguez CJ, Sakurai M, Shaw JE, Knuiman M, Voortman T, Meisinger C, Tjønneland A, Brenner H, Palmieri L, Dallongeville J, Brunner EJ, Assmann G, Trevisan M, Gillum RF, Ford I, Sattar N, Lazo M, Thompson SG, Ferrari P, Leon DA, Davey Smith G, Peto R, Jackson R, Banks E, Di Angelantonio E, Danesh J and for the Emerging Risk Factors Collaboration/EPIC-CVD/UK Biobank Alcohol Study Group (2018): Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet 391:1513-1523.

Woolard R, Liu J, Parsa M, Merriman G, Tarwater P, Alba I, Villalobos S, Ramos R, Bernstein J, Bernstein E, Bond J and Cherpitel CJ (2015): Smoking is associated with increased risk of binge drinking in a young adult hispanic population at the US-Mexico border. Subst Abus 36:318-324.

World Cancer Research Fund: Alcohol and cancer risk. Available online at: https://www.wcrf-uk.org/uk/preventing-cancer/what-can-increase-your-risk-cancer/alcohol-and-cancer-risk (Last accessed March 10, 2020),

World Health Organization (1988): The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol 41:105-114.

World Health Organization (2000a): International guide for monitoring alcohol consumption and related harm. World Health Organization, Geneva, Switzerland. Available online at: https://apps.who.int/iris/handle/10665/66529 (Last accessed March 10, 2020).

World Health Organization (2000b): Obesity: preventing and managing the global epidemic. Report of a WHO consultation. WHO Technical Report Series 894. World Health Organization, Geneva, Switzerland. Available online at: https://www.who.int/nutrition/publications/obesity/WHO_TRS_894/en/ (Last accessed March 8, 2020).

World Health Organization (2018a): Alcohol. World Health Organization, Geneva, Switzerland. Available online at: https://www.who.int/news-room/fact-sheets/detail/alcohol (Last accessed January 24, 2020).

World Health Organization (2018b): Global status report on alcohol and health 2018. World Health Organization, Geneva, Switzerland. Available online at: https://www.who.int/substance_abuse/publications/global_alcohol_report/en/ (Last accessed January 20, 2020).

Wu A, Chanarin I and Levi AJ (1974): Macrocytosis of chronic alcoholism. Lancet 1:829-831.

Wu D, Zhai Q and Shi X (2006): Alcohol-induced oxidative stress and cell responses. J Gastroenterol Hepatol 21 Suppl 3:S26-S29.

Wu D, Wang X, Zhou R, Yang L and Cederbaum AI (2012): Alcohol steatosis and cytotoxicity: the role of cytochrome P4502E1 and autophagy. Free Radic Biol Med 53:1346-1357.

Wurst FM, Kempter C, Seidl S and Alt A (1999): Ethyl glucuronide--a marker of alcohol consumption and a relapse marker with clinical and forensic implications. Alcohol Alcohol 34:71-77.

Xu J, Lai KKY, Verlinsky A, Lugea A, French SW, Cooper MP, Ji C and Tsukamoto H (2011): Synergistic steatohepatitis by moderate obesity and alcohol in mice despite increased adiponectin and p-AMPK. J Hepatol 55:673-682.

Yeomans MR (2010): Alcohol, appetite and energy balance: is alcohol intake a risk factor for obesity? Physiol Behav 100:82-89.

Yki-Järvinen H (2014): Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2:901-910.

Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L and Wymer M (2016): Global epidemiology of nonalcoholic fatty liver disease–Meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 64:73-84.

Yu-Poth S, Zhao G, Etherton T, Naglak M, Jonnalagadda S and Kris-Etherton PM (1999): Effects of the National Cholesterol Education Program's Step I and Step II dietary intervention programs on cardiovascular disease risk factors: a meta-analysis. Am J Clin Nutr 69:632-646.

Yun KE, Shin CY, Yoon YS and Park HS (2009): Elevated alanine aminotransferase levels predict mortality from cardiovascular disease and diabetes in Koreans. Atherosclerosis 205:533-537.

Zaccardi F, Davies MJ, Khunti K and Yates T (2019): Comparative relevance of physical fitness and adiposity on life expectancy: a UK Biobank observational study. Mayo Clin Proc 94:985-994.

Zaitsu M, Takeuchi T, Kobayashi Y and Kawachi I (2020): Light to moderate amount of lifetime alcohol consumption and risk of cancer in Japan. Cancer 126:1031-1040.

Zakhari S (2013): Bermuda Triangle for the liver: alcohol, obesity, and viral hepatitis. J Gastroenterol Hepatol 28 Suppl 1:18-25.

Zhang H and Forman HJ (2009): Redox regulation of gamma-glutamyl transpeptidase. Am J Respir Cell Mol Biol 41:509-515.

Zhao Y and Zhao B (2013): Oxidative stress and the pathogenesis of Alzheimer's disease. Oxid Med Cell Longev 2013:316523.

Zheng H and Chen C (2015): Body mass index and risk of knee osteoarthritis: systematic review and meta-analysis of prospective studies. BMJ Open 5:e007568.

Zheng JS, Sharp SJ, Imamura F, Koulman A, Schulze MB, Ye Z, Griffin J, Guevara M, Huerta JM, Kröger J, Sluijs I, Agudo A, Barricarte A, Boeing H, Colorado-Yohar S, Dow C, Dorronsoro M, Dinesen PT, Fagherazzi G, Franks PW, Feskens EJM, Kühn T, Katzke VA, Key TJ, Khaw KT, de Magistris MS, Mancini FR, Molina-Portillo E, Nilsson PM, Olsen A, Overvad K, Palli D, Quirós JR, Rolandsson O, Ricceri F, Spijkerman AMW, Slimani N, Tagliabue G, Tjønneland A, Tumino R, van der Schouw YT, Langenberg C, Riboli E, Forouhi NG and Wareham NJ (2017): Association between plasma phospholipid saturated fatty acids and metabolic markers of lipid, hepatic, inflammation and glycaemic pathways in eight European countries: a cross-sectional analysis in the EPIC-InterAct study. BMC Med 15:203.

Zhou JY, Jiang ZA, Zhao CY, Zhen Z, Wang W and Nanji AA (2013): Long-term binge and escalating ethanol exposure causes necroinflammation and fibrosis in rat liver. Alcohol Clin Exp Res 37:213-222.

PUBLICATION I

Laboratory test based assessment of WHO alcohol risk drinking levels

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Laboratory test based assessment of WHO alcohol risk drinking levels

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Running head: WHO risk drinking levels and biomarkers

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Abstract

Low-risk thresholds for alcohol use differ across various national guidelines. To assess the novel WHO risk drinking levels in light of alcohol-sensitive common laboratory tests we analysed biomarkers of liver status, inflammation and lipid profiles from a population-based survey of individuals classified to abstainers and different WHO risk drinking levels defined in terms of mean alcohol consumption per day.

The study included 22,327 participants aged 25–74 years from the National FINRISK Study. Data on alcohol use, health status, diet, body weight, and lifestyle (smoking, coffee consumption and physical activity) were recorded from structured interviews. Alcohol data from self-reports covering the past 12 months was used to categorize the participants into subgroups of abstainers and WHO risk drinking categories representing low, moderate, high and very high risk drinkers. Serum liver enzymes (GGT, ALT), C-reactive protein (CRP) and lipid profiles were measured using standard laboratory techniques.

Alcohol risk category was roughly linearly related with the occurrence of elevated values for GGT, ALT and CRP. Alcohol drinking also significantly influenced the incidence of abnormalities in serum lipids. Significantly higher odds for abnormal GGT, ALT and altered lipid profiles remained in alcohol drinkers even after adjustment for age, waist circumference, physical inactivity, smoking, and coffee consumption.

A more systematic use of laboratory tests during treatment of individuals classified to WHO risk drinking categories may improve the assessment of alcohol-related health risks. Follow-ups of biomarker responses may also prove to be useful in health interventions aimed at reducing alcohol consumption.

Key words: ethanol, harm reduction, inflammation, lipid, liver

Introduction

Heavy alcohol drinking is known to associate with a broad range of health problems including both somatic and psychiatric morbidity [1-3]. The thresholds below which alcohol consumption stops being associated with disease risks have, however, remained unclear. While full abstinence has been the most widely accepted outcome in current treatment of alcohol use disorders, recent studies have indicated that any reduction in alcohol consumption could yield significant health benefits [4,5]. Therefore, both European Medicines Agency (EMA) and the U.S. Food and Drug Administration (FDA) currently emphasize reduced drinking or low-risk drinking as alternative endpoints in the treatment of patients with alcohol dependence [5-7].

Current WHO risk drinking protocol defines alcohol consumption as mean ethanol consumption in grams of ethanol per day and classify alcohol consumption into categories of low, medium, high or very high risk drinking [5,8,9]. Recent studies among alcohol-dependent individuals have indicated that such patients could indeed benefit from any reduction in alcohol consumption [5,8]. Studies on clinical or laboratory outcomes of alcohol intake in individuals representing different WHO risk categories have, however, so far been limited.

Recent studies on the relationships between the amount of alcohol consumption and all-cause mortality [10] or liver status [11] have supported the view that alcohol consumption may be associated with health problems even at relatively low drinking levels. A wide variety of common laboratory tests, which are frequently used in health screening programs are also known to be sensitive to heavy alcohol intake [12]. Current studies have indicated that the activities of common liver enzymes are readily increased in early-phase liver disease, which

can result either from alcohol consumption or excess body weight [11-15]. Such findings may also be linked with extra-hepatic health risks, including metabolic syndrome, and cardio- or cerebrovascular events [15-17]. Alcohol consumption is also readily reflected in biomarkers of inflammation [18,19] and serum lipid profiles [20], which may also play a pivotal role in the progression of tissue damage induced by excessive alcohol consumption [21,22].

The purpose of the present study was to compare the occurrence of abnormalities in biomarkers of liver status, inflammation and lipid profiles in a large national FINRISK population health survey of individuals classified to WHO risk drinking categories based on self-reported alcohol consumption from a period of one year preceding blood sampling. Given the limited data available on this subject so far the FINRISK survey of individuals with detailed records on alcohol consumption, diet and other health-related behaviour affords an excellent opportunity to elucidate the associations between alcohol-sensitive biomarkers and the different risk drinking categories.

Materials and methods

Study design, data sources and participants

In this work, we used data collected from a cross-sectional population health survey (The National FINRISK Study) carried out in Finland every five years since 1972. In this study, data from surveys between 1997 and 2007 were used, as previously described [11]. The participants represent a nationally representative age- and gender stratified random sample drawn from the population register according to an international protocol [23]. Clinical examinations included both physical measurements and laboratory tests. Data on alcohol intake, current health status, diet, smoking, current physical activity, medical history and socioeconomic factors were collected using specifically designed and validated questionnaires

for use in a population based health surveys according to the World Health Organization MONICA project protocol [23,24]. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI, kg/m^2) was calculated as a measure of relative body weight. Waist circumference was measured to the nearest 0.5 cm between the lowest rib and the iliac crest while exhaling.

The data on regular alcohol consumption from the past 12 months prior to blood sampling was recorded from self-reports using structured questionnaires, where the responses were assigned to mutually exclusive and collectively exhaustive categories. The questionnaire included information on the types of beverages consumed, the frequency of consumption, and the amounts of various types of ethanol-containing drinks consumed. The ethanol content in different beverages was quantitated in grams of ethanol based on defined portion sizes as follows: regular beer 12 grams (1/3 L), strong beer 15.5 grams (1/3 L), long drink 15.5 grams (1/3 L), spirit 12 grams (4 cL), wine 12 grams (12 cL) and cider 12 grams (1/3 L). The data was available from 22,327 apparently healthy individuals: 10,724 men and 11,603 women (mean age 45 ± 13 years, range 25–74 years) who completed the questionnaires and attended the medical examination. All participants were devoid of any apparent clinical signs of liver disease, ischaemic heart or brain disease or active infection at the time of the study.

The data was subsequently used to categorize the population by gender and drinking habits according to recently established WHO criteria as follows: 1. persons who reported no current alcohol consumption were referred to as non-drinkers (abstainers), 2. low risk drinkers consumed between 1 to 40 grams (men) or 1 to 20 grams (women), 3. moderate risk drinkers consumed 41 to 60 grams (men) or 21 to 40 grams (women), 4. high risk drinkers consumed 61 to 100 grams (men) or 41 to 60 grams (women) and 5. very high risk drinkers consumed

more than 100 grams (men) or more than 60 grams (women) per day. Smoking and coffee consumption were assessed with a set of standardized questions and expressed as the amounts of cigarettes per day and as the intake of standard servings of coffee (cups) per day, respectively. Leisure-time physical activity and the number and total time used for physical exercises were registered using structured questionnaires and the data was used to classify the population into the subgroups of 1. moderate or vigorous activity (over 4 hours of activity per week) 2. light (0.5–4 hours per week), and 3. sedentary activity (less than 0.5 hours per week).

The approval for this study was received from the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. All surveys were conducted in accordance with the Declaration of Helsinki according to the ethical rules of the National Public Health Institute.

Laboratory analyses

Serum ALT and GGT were measured by standard clinical chemical methods following the recommendations of the assay manufacturer on an Abbott Architect clinical chemistry analyzer (Abbott Laboratories, Abbott Park, IL, USA). High-sensitivity CRP was determined using a latex immunoassay (Sentinel Diagnostics, Milan, Italy) with the Abbott Architect c8000 clinical chemistry analyzer. Lipid profiles included determinators of total cholesterol, high-density lipoprotein-associated cholesterol (HDL), low-density lipoprotein associated cholesterol (LDL) and total triglycerides using standard enzymatic methods. All laboratory tests carried out between years 1997 and 2007 were subjects to continuous external quality control programs organized by Labquality, Finland and CDC (Center for Disease Control and Prevention) quality assurance and standardization program for serum lipids. The previously established national cut-offs for the different biomarkers were as follows: ALT (50 U/L men;

35 U/L women), GGT (60 U/L men; 40 U/L women), CRP (3.0 mg/L), cholesterol (5 mmol/L), HDL cholesterol (1.0 mmol/L men, 1.2 mmol/L women), LDL cholesterol (3.0 mmol/L), triglycerides (1.7 mmol/L).

Statistical methods

The main characteristics of the study population in different drinking groups are shown as mean ± SD and the potential linear or quadratic (U-shaped) association in the mean values across the groups was tested by analysis of variance with polynomial contrast. The distribution of abnormal biomarker levels across drinking groups are presented as percentages and the presence of linear trend in proportions was evaluated by Chi-square test for trend. Binary logistic regression was used to estimate the relative risk of abnormal biomarker levels associated with drinking status and the covariates. The estimates are presented as odds ratios (OR) and 95% confidence intervals (CI). As covariates we used age, waist circumference as an index of weight, physical activity and smoking habit as these factors are known to potentially associate with abnormal biomarker levels and showed association in univariate analysis. All factors were entered simultaneously into the multivariable model. Potential multicollinearity among the covariates was examined by calculating the Variance Inflation Factor (VIF) and no evidence was found. The analyses were carried out with IBM SPSS Statistics 22.0 (Armonk, NY: IBM Corp.). A p-value < 0.05 was considered statistically significant.

Results

The main demographic characteristics and lifestyle factors of the participants classified to subgroups according to gender and alcohol consumption from the past 12 months are summarized in Table 1. Of the 22,327 participants, 9.3% of men (n = 10,724) were abstainers,

80.6% were low risk drinkers, 5.4% were moderate risk drinkers, 3.1% were high risk drinkers and 1.6% represented very high risk drinkers. In women (n = 11,603), the corresponding percentages were 14.7%, 79.0%, 4.6%, 1.0% and 0.7%.

Figure 1 summarizes the frequencies of values exceeding the upper normal limits for GGT, ALT, CRP and triglycerides or target ranges for cholesterol, HDL and LDL in the different study subgroups. The individuals with high or very high risk were found to show the highest rates of abnormal values (Figure 1). For GGT and ALT the number of abnormal findings in the different study groups increased in a rather linear manner as a function of alcohol consumption ($p < 0.0005$). In these comparisons, the distribution of abnormal CRP levels was also significantly associated with drinking status in men whereas not in women (Figure 1). A gender-dependent effect of alcohol drinking was also observed in the distributions of serum LDL and triglyceride values outside the target ranges.

Table 2 summarizes the odds ratios (OR) of abnormal biomarker findings according to different drinking categories following adjustment for age, waist circumference, physical activity, smoking and coffee consumption. When compared with abstainers, low risk drinkers ($p<0.0005$ for men; $p < 0.01$ for women), moderate risk drinkers ($p < 0.0005$ for both genders), high risk drinkers ($p< 0.0005$ for both genders) and very high risk drinkers ($p < 0.0005$ for both genders) showed significantly higher ORs of abnormal GGT activities (Table 2). For ALT, the relative risks of abnormal activities were significantly increased in all other alcohol consuming groups except for low risk drinkers of men and low to medium risk drinkers of women (Table 2). In serum lipid profiles, alcohol drinking was associated with lower odds of HDL values outside the target range, whereas increased risks were observed in serum cholesterol and LDL in men, although not in women (Table 2).

Discussion

The present large cross-sectional population-based survey reports on the relationships between abnormalities in alcohol-sensitive biomarkers for liver status, inflammation, lipid profiles and WHO alcohol drinking risk levels. All the biomarkers chosen for the present comparisons are common laboratory tests which are frequently used in health screening programs and may also be expected to be sensitive to alcohol consumption [22]. The present data supports the usefulness of biomarker-based approaches in a more comprehensive assessment of alcohol-attributable health risks.

Reduction in WHO drinking risk levels has been recently suggested as a useful efficacy outcome in clinical trials, which is due to the fact that many alcohol-consuming patients prefer drinking reduction instead of full abstinence as a treatment goal [4]. Improved knowledge on the possible clinical consequences resulting from the presence or absence in such risk categories should thus be important in order to improve treatment compliance and appropriate patient guidance. In accordance with our recent observations on liver enzymes in a population-based sample of individuals classified based on their recent drinking [11], the present study on self-reported regular long-term alcohol consumption indicates that the ORs for abnormal liver enzyme activities increase even in alcohol consumers representing low to moderate risk drinking and further with increasing levels of consumption. Especially GGT levels seemed to be sensitive for discriminating between alcohol risk groups. While reduction of alcohol drinking has been a long-standing target in public health policies, the specific clinical consequences which may be expected to result from lowering the levels of alcohol consumption have so far remained obscure. Thus, further follow-up studies are clearly warranted to explore whether the changes in GGT activities perhaps combined with other

alcohol specific tests, such as CDT, could provide additional clinical value as diagnostic tools for detecting shifts in risk drinking categories at an individual level in a more sensitive and specific manner. GGT assays could also prove to be of value as general health indicators reflecting both hepatic and extra-hepatic health risks [13,17,22,25].

Previous analyses on the patterns of alcohol-attributable health risks have traditionally yielded different types of dose-response curves [10]. While high risk drinking has been unequivocally linked to a wide variety of diseases, the relationship between low to moderate levels of consumption and health problems has remained as an issue of controversy [1,11,26]. While some studies have suggested protective effects of light to moderate drinking [27], more recent studies have provided no evidence of health benefits of drinking alcohol in amounts exceeding 100 grams (~8 drinks) per week [10,11,28-32]. Even light drinking may increase the risk for carcinogenesis in certain tissues [33-35], cognitive decline [32,36], heart problems [37,38] and all-cause mortality [10,30]. However, contrasting views have remained especially on the associations between alcohol use and cardiovascular disease risks with a lower risks of coronary artery disease having been observed among light to moderate drinkers. The pathogenic mechanisms associated with such divergent disease associations have also remained unknown [28]. The cardiovascular effects of alcohol consumption have been suggested to be mediated by the diverse effects of alcohol on blood lipid profiles and the status of inflammation, which both seem to play a major role in the development of atherosclerosis [39-41]. There may also be a mechanistic link between fatty liver and atherosclerosis since GGT enzyme is able to fuel LDL oxidation in coronary plaques [42]. Interestingly, current data suggests that serum CRP, a marker of inflammation, is increased among men with rather moderate levels of alcohol consumption especially when combined with excess body weight or sedentary lifestyle.

The present data lends further support to recent findings based on either liver enzymes [11] or all-cause mortality [10] as outcomes indicating that the thresholds for low-risk alcohol consumption should be at a lower level (below 100 grams per week) than those currently recommended in many national guidelines. However, individuals with other concomitant risk factors appear to need separate attention in such considerations. Based on current data the biomarker responses of liver status [11,43,44] but also those reflecting inflammation and lipid metabolism seem to be significantly driven by other determinants of lifestyle, including obesity, smoking or sedentary lifestyle among alcohol consumers. The individual assessment of health risks and the most appropriate levels of alcohol drinking should therefore include multiple factors of lifestyle and measurements of several biomarkers. It should also be noted that the time windows for detecting the possible harmful consequences of alcohol intake may be different between the various laboratory methods.

Current data also underscores distinct differences between men and women in biomarker responses to alcohol consumption. Women seem to show elevated liver enzyme activities after smaller amounts of alcohol consumption, whereas they seem to be less sensitive to changes in the status of inflammation. Advanced age, the presence of adiposity and sedentary lifestyle may be other important determinants of risk with gender-dependent characteristics [11,45-49]. Recent statistics have indicated that mortality due to liver cirrhosis has been increasing over the past decades mainly driven by deaths due to alcoholic cirrhosis [50]. However, there has also been a rapid and simultaneous increase of non-alcoholic liver disease in most Western countries. This phenomenon has been linked not only with increasing prevalence of adiposity but also with increasing levels of regular yet moderate levels of alcohol consumption [14]. Obviously, there may also be significant interactions with ethanol

intake and high-fat diets [51]. Since in real life situations alcohol use often co-exists with obesity, synergistic health problems due to these two triggers may be expected to occur in an increasing manner and obviously, separate drinking reduction goals may also be justified for existing fatty deposition in tissues [11,15,46,52,53]. In obese individuals, even low to moderate amounts of drinking could increase the risk of additional deposition of triglycerides in tissues, insulin resistance and consequent vascular events. It remains to be established whether the prognostication of such adverse outcomes could also benefit from a more targeted use of selected biomarkers [16,17,24,42,53-57].

The strengths of this study include the large number of study subjects and a comprehensive assessment of clinical factors together with several biomarkers. The evaluation of regular alcohol consumption was carried out based on the data from the past one year allowing an estimate of long term alcohol consumption. Data on other risk factors, such as waist circumference, were included in the statistical models to reduce confounding. The study also included separate assessments for men and women. Nevertheless, our study has some potential limitations. Self-reports on alcohol consumption are prone to bias and could lead to underestimation of the true dose-response associations. The cross-sectional setting of the survey can also be kept as a limitation of this study since lack of follow-up data prevents analyses on the possible shifts between the different risk drinking categories at an individual level. Further studies should also address the possibility whether more alcohol-specific tests, such as CDT, would also improve the possibilities for detecting changes between the different risk groups.

Taken together, our study demonstrates previously unrecognized relationships between alcohol intake and biomarker abnormalities in individuals classified according to the WHO

risk drinking categories. The data may prove to be useful in the clinical assessments of patients based on WHO risk drinking levels and in related public health recommendations.

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Conflicts of interest

None declared

Table 1. Main characteristics of the study population, as classified according to drinking status.

Men	Abstainers	Low	Medium	High	Very high	
Alcohol consumption	0 g/day	1–40 g/day	41–60 g/day	61–100 g/day	> 100 g/day	*p*
N (%)	1001 (9.3)	8648 (80.6)	577 (5.4)	331 (3.1)	167 (1.6)	
Age, years, mean ± SD	55.4 ± 13.6	49.4 ± 13.5	46.9 ± 11.6	47.2 ± 12.2	48.8 ± 11.9	< 0.0005[a]
BMI[c]	27.2 ± 4.2	27.1 ± 4.0	27.4 ± 4.1	27.5 ± 4.4	27.7 ± 4.6	0.057[b]
Waist circumference, cm	96.3 ± 12.1	95.6 ± 11.6	97.3 ± 11.8	97.7 ± 12.5	98.4 ± 12.5	< 0.003[b]
Smoking, cigarettes/day	2.5 ± 7.5	4.6 ± 8.6	8.5 ± 10.8	9.7 ± 11.9	12.9 ± 14.2	< 0.0005[b]
Coffee, cups/day	4.5 ± 3.6	4.6 ± 3.2	4.7 ± 3.3	4.5 ± 3.4	4.4 ± 3.8	0.355[a]
Physical activity, number of exercises per week	2.5 ± 2.4	2.4 ± 2.2	1.9 ± 2.0	2.1 ± 2.1	1.8 ± 1.9	0.014[b]
Women	Abstainers	Low	Medium	High	Very high	
Alcohol consumption	0 g/day	1–20 g/day	21–40 g/day	41–60 g/day	> 60 g/day	*p*
N (%)	1703 (14.7)	9166 (79.0)	535 (4.6)	121 (1.0)	78 (0.7)	
Age, years, mean ± SD	55.0 ± 13.7	46.6 ± 12.9	45.0 ± 12.1	47.3 ± 10.9	50.6 ± 11.1	< 0.0005[a]
BMI	27.8 ± 5.5	26.2 ± 5.0	26.1 ± 5.0	26.2 ± 4.7	27.2 ± 5.2	< 0.0005[a]
Waist circumference, cm	87.4 ± 13.7	83.4 ± 12.8	84.4 ± 13.1	85.3 ± 11.8	88.7 ± 14.1	< 0.0005[a]
Smoking, cigarettes/day	1.0 ± 4.0	2.3 ± 5.5	5.0 ± 7.1	8.4 ± 10.2	10.3 ± 12.4	< 0.0005[b]
Coffee, cups/day	3.7 ± 2.6	3.7 ± 2.5	3.8 ± 2.4	4.1 ± 3.1	3.9 ± 2.9	0.237[b]
Physical activity, number of exercises per week	2.6 ± 2.3	2.5 ± 2.0	2.4 ± 1.9	2.0 ± 2.1	1.8 ± 2.8	0.008[b]

[a] analysis of variance, test for quadratic association
[b] analysis of variance, test for linear association
[c] body mass index

Table 2. Odds ratios (OR) of abnormal biomarker levels in various study subgroups representing different WHO risk drinking levels (low risk, medium risk, high risk and very high risk drinkers) based on alcohol consumption from the past 12 months, as adjusted for participant's age, waist circumference, physical activity, coffee consumption and smoking habit.

	Men		Women	
	Multivariable OR	*p*	Multivariable OR	*p*
GGT				
Abstainers	1.00		1.00	
Low	1.61 (1.28 to 2.03)	< 0.0005	1.29 (1.07 to 1.55)	0.008
Medium	3.77 (2.81 to 5.07)	< 0.0005	2.45 (1.83 to 3.28)	< 0.0005
High	4.42 (3.17 to 6.16)	< 0.0005	3.76 (2.37 to 5.97)	< 0.0005
Very high	6.73 (4.51 to 10.04)	< 0.0005	5.26 (3.13 to 8.82)	< 0.0005
ALT				
Abstainers	1.00		1.00	
Low	1.56 (0.97 to 2.52)	0.069	1.10 (0.78 to 1.55)	0.591
Medium	2.73 (1.55 to 4.83)	0.001	1.63 (0.97 to 2.73)	0.064
High	1.99 (1.03 to 3.84)	0.041	2.52 (1.15 to 5.52)	0.021
Very high	4.29 (1.94 to 9.48)	< 0.0005	3.58 (1.48 to 8.66)	0.005
CRP				
Abstainers	1.00		1.00	
Low	0.98 (0.81 to 1.18)	0.810	0.95 (0.83 to 1.09)	0.467
Medium	1.16 (0.88 to 1.53)	0.289	1.16 (0.90 to 1.49)	0.256
High	1.09 (0.79 to 1.51)	0.608	0.96 (0.59 to 1.54)	0.858
Very high	1.27 (0.85 to 1.91)	0.239	1.04 (0.60 to 1.81)	0.885
Cholesterol				
Abstainers	1.00		1.00	
Low	1.35 (1.17 to 1.57)	< 0.0005	0.97 (0.85 to 1.10)	0.591
Medium	1.89 (1.49 to 2.41)	< 0.0005	1.01 (0.80 to 1.26)	0.966
High	1.50 (1.13 to 2.00)	0.005	1.16 (0.76 to 1.79)	0.487
Very high	1.17 (0.81 to 1.70)	0.399	1.00 (0.59 to 1.70)	0.995
HDL				
Abstainers	1.00		1.00	
Low	0.60 (0.50 to 0.70)	< 0.0005	0.60 (0.52 to 0.69)	< 0.0005
Medium	0.28 (0.21 to 0.39)	< 0.0005	0.32 (0.23 to 0.44)	< 0.0005
High	0.30 (0.21 to 0.44)	< 0.0005	0.29 (0.15 to 0.54)	< 0.0005
Very high	0.21 (0.12 to 0.36)	< 0.0005	0.14 (0.05 to 0.35)	< 0.0005
LDL				
Abstainers	1.00		1.00	
Low	1.25 (1.04 to 1.50)	0.020	0.89 (0.77 to 1.04)	0.154
Medium	1.39 (1.05 to 1.84)	0.022	0.80 (0.62 to 1.03)	0.089
High	1.28 (0.92 to 1.78)	0.152	0.85 (0.52 to 1.37)	0.504
Very high	0.71 (0.47 to 1.08)	0.110	0.79 (0.45 to 1.39)	0.413
Triglycerides				
Abstainers	1.00		1.00	
Low	0.91 (0.78 to 1.06)	0.224	0.76 (0.66 to 0.87)	< 0.0005
Medium	0.97 (0.77 to 1.23)	0.799	0.70 (0.53 to 0.93)	0.013
High	0.87 (0.65 to 1.15)	0.319	0.84 (0.51 to 1.38)	0.479
Very high	0.95 (0.66 to 1.37)	0.779	0.67 (0.38 to 1.20)	0.182

GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; HDL, high-density lipoproteins; LDL, low-density lipoprotein

Figure legend

Figure 1. Distribution of abnormal biomarker findings in groups classified according to WHO risk drinking levels. The cut-offs used for the different laboratory tests were as follows: GGT 60 U/L (men), 40 U/L (women); ALT 50 U/L (men), 35 U/L (women); CRP 3.0 mg/L; Cholesterol 5.0 mmol/L; HDL 1.0 mmol/L (men), 1.2 mmol/L (women); LDL 3.0 mmol/L; triglycerides 1.7 mmol/L. P values for linear trend for each parameter are shown.

References

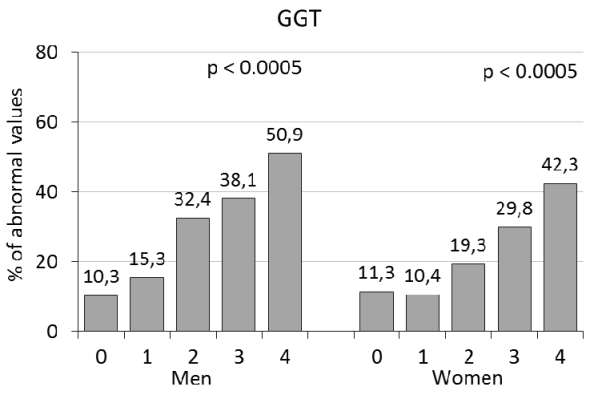
1. Connor JP, Haber PS, Hall WD. Alcohol use disorders. Lancet 2016;387:988-98.
2. Lim SS, Vos T, Flaxman AD, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 2012;380:2224-60.
3. Wittchen HU. The burden of mood disorders. Science 2012;338:15.
4. Hasin DS, Wall M, Witkiewitz K, et al. Change in non-abstinent WHO drinking risk levels and alcohol dependence: a 3 year follow-up study in the US general population. Lancet Psychiatry 2017;4:469-76.
5. Witkiewitz K, Hallgren KA, Kranzler HR, et al. Clinical validation of reduced alcohol consumption after treatment for alcohol dependence using the World Health Organization risk drinking levels. Alcohol Clin Exp Res 2017;41:179-86.
6. European Medicines Agency. Guideline on the development of medicinal products for the treatment of alcohol dependence. London, UK; 2010. Available from: http://www.ema.europa.eu/ema/index.jsp?curl=pages/includes/document/document_detail.jsp?webContentId=WC500074898&mid=WC0b01ac058009a3dc
7. Food and Drug Administration. Alcoholism: Developing Drugs for Treatment. Silver Spring, MD; 2015. Available from: https://www.fda.gov/downloads/drugs/.../ucm433618.pdf
8. Witkiewitz K, Pearson MR, Hallgren KA, et al. Who achieves low risk drinking during alcohol treatment? An analysis of patients in three alcohol clinical trials. Addiction 2017;112:2112-21.
9. World Health Organization. International Guide for Monitoring Alcohol Consumption and Related harm. Geneva, Switzerland; 2000. Available from: whqlibdoc.who.int/hq/2000/WHO_MSD_MSB_00.4.pdf
10. Wood AM, Kaptoge S, Butterworth AS, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet 2018;391:1513-23.

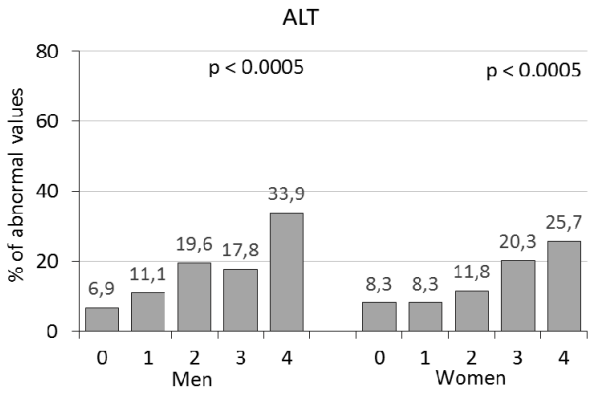
11. Niemelä O, Niemelä M, Bloigu R, et al. Where should the safe limits of alcohol consumption stand in light of liver enzyme abnormalities in alcohol consumers? PLoS One 2017;12:e0188574.
12. Danielsson J, Kangastupa P, Laatikainen T, et al. Impacts of common factors of life style on serum liver enzymes. World J Gastroenterol 2014;20:11743-52.
13. Kim WR, Flamm SL, Di Bisceglie AM, et al. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology 2008;47:1363-70.
14. Lau K, Baumeister SE, Lieb W, et al. The combined effects of alcohol consumption and body mass index on hepatic steatosis in a general population sample of European men and women. Aliment Pharmacol Ther 2015;41:467-76.
15. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology 2009;136:477-85.
16. Lee TH, Kim WR, Benson JT, et al. Serum aminotransferase activity and mortality risk in a United States community. Hepatology 2008;47:880-7.
17. Ruttmann E, Brant LJ, Concin H, et al. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation 2005;112:2130-7.
18. Oliveira A, Rodríguez-Artalejo F, Lopes C. Alcohol intake and systemic markers of inflammation--shape of the association according to sex and body mass index. Alcohol Alcohol 2010;45:119-25.
19. Zheng JS, Sharp SJ, Imamura F, et al. Association between plasma phospholipid saturated fatty acids and metabolic markers of lipid, hepatic, inflammation and glycaemic pathways in eight European countries: a cross-sectional analysis in the EPIC-InterAct study. BMC Med 2017;15:203.
20. Hao G, Wang Z, Zhang L, et al. Relationship between alcohol consumption and serum lipid profiles among middle-aged population in China: a multiple-center cardiovascular epidemiological study. Angiology 2015;66:753-8.
21. Wang HJ, Gao B, Zakhari S, et al. Inflammation in alcoholic liver disease. Annu Rev Nutr 2012;32:343-68.

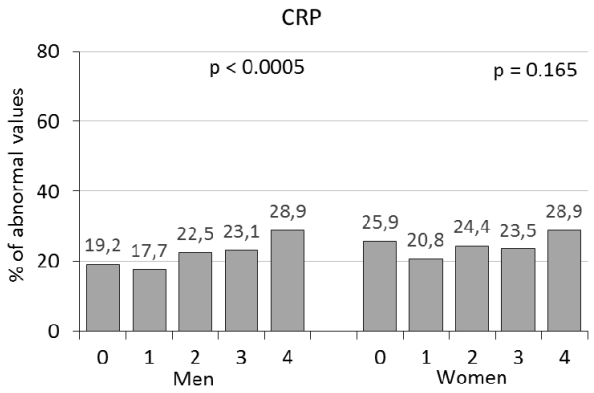
22. Niemelä O. Biomarker-based approaches for assessing alcohol use disorders. Int J Environ Res Public Health 2016;13:166.
23. The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol 1988;41:105-14.
24. Kuulasmaa K, Tolonen H, Cepaitis Z, et al. European Health Risk Monitoring Project. 2006. Available from: http://www.thl.fi/ehrm/ Accessed April 5, 2018.
25. Kazemi-Shirazi L, Endler G, Winkler S, et al. Gamma glutamyltransferase and long-term survival: is it just the liver? Clin Chem 2007;53:940-6.
26. Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. J Hepatol 2013;59:160-8.
27. Di Castelnuovo A, Costanzo S, Bagnardi V, et al. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med 2006;166:2437-45.
28. Holmes MV, Dale CE, Zuccolo L, et al. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 2014;349:g4164.
29. Klatsky AL. Alcohol and cardiovascular diseases: where do we stand today? J Intern Med 2015;278:238-50.
30. Sipilä P, Rose RJ, Kaprio J. Drinking and mortality: long-term follow-up of drinking-discordant twin pairs. Addiction 2016;111:245-54.
31. Stockwell T, Zhao J, Panwar S, et al. Do "Moderate" Drinkers Have Reduced Mortality Risk? A Systematic Review and Meta-Analysis of Alcohol Consumption and All-Cause Mortality. J Stud Alcohol Drugs 2016;77:185-98.
32. Topiwala A, Allan CL, Valkanova V, et al. Moderate alcohol consumption as risk factor for adverse brain outcomes and cognitive decline: longitudinal cohort study. BMJ 2017;357:j2353.
33. Bagnardi V, Rota M, Botteri E, et al. Light alcohol drinking and cancer: a meta-analysis. Ann Oncol 2013;24:301-8.

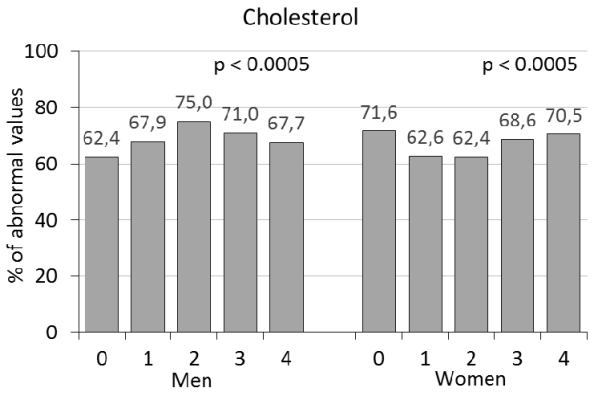
34. Cao Y, Willett WC, Rimm EB, et al. Light to moderate intake of alcohol, drinking patterns, and risk of cancer: results from two prospective US cohort studies. BMJ 2015;351:h4238.
35. Choi YJ, Myung SK, Lee JH. Light alcohol drinking and risk of cancer: a meta-analysis of cohort studies. Cancer Res Treat 2018;50:474-87.
36. Schwarzinger M, Pollock BG, Hasan OSM, et al. Contribution of alcohol use disorders to the burden of dementia in France 2008-13: a nationwide retrospective cohort study. Lancet Public Health 2018;3:e124-32.
37. Catena C, Colussi G, Verheyen ND, et al. Moderate alcohol consumption is associated with left ventricular diastolic dysfunction in nonalcoholic hypertensive patients. Hypertension 2016;68:1208-16.
38. McManus DD, Yin X, Gladstone R, et al. Alcohol consumption, left atrial diameter, and atrial fibrillation. J Am Heart Assoc 2016;5:e004060.
39. Libby P, Ridker PM, Hansson GK, et al. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol 2009;54:2129-38.
40. Moradi H, Streja E, Kalantar-Zadeh K. Serum high density lipoprotein cholesterol level and risk of death: let's avoid the extremes. J Thorac Dis 2017;9:4849-52.
41. Ridker PM, Everett BM, Thuren T, et al. Anti-inflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med 2017;377:1119-31.
42. Kozakova M, Palombo C, Eng MP, et al. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. Hepatology 2012;55:1406-15.
43. Breitling LP, Raum E, Müller H, et al. Synergism between smoking and alcohol consumption with respect to serum gamma-glutamyltransferase. Hepatology 2009;49:802-8.
44. Conigrave KM, Degenhardt LJ, Whitfield JB, et al. CDT, GGT, and AST as markers of alcohol use: the WHO/ISBRA collaborative project. Alcohol Clin Exp Res 2002;26:332-9.
45. Caro AA, Cederbaum AI. Oxidative stress, toxicology, and pharmacology of CYP2E1. Annu Rev Pharmacol Toxicol 2004;44:27-42.
46. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology 1998;114:842-5.
47. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239-47.

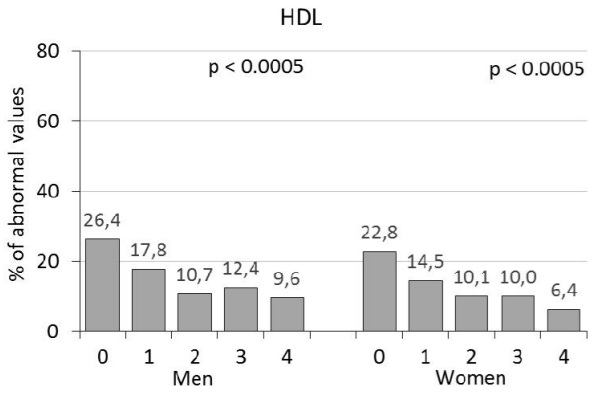
48. Lee DH, Blomhoff R, Jacobs DR Jr. Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radic Res 2004;38:535-9.
49. Loomba R, Bettencourt R, Barrett-Connor E. Synergistic association between alcohol intake and body mass index with serum alanine and aspartate aminotransferase levels in older adults: the Rancho Bernardo Study. Aliment Pharmacol Ther 2009;30:1137-49.
50. Tapper EB, Parikh NH. Mortality due to cirrhosis and liver cancer in the United States, 1999-2016:observational study. BMJ 2018;362:k2817.
51. Tsukamoto H, Horne W, Kamimura S, et al. Experimental liver cirrhosis induced by alcohol and iron. J Clin Invest 1995;96:620-30.
52. Liu B, Balkwill A, Reeves G, et al. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ 2010;340:c912.
53. Tsai J, Ford ES, Zhao G, et al. Co-occurrence of obesity and patterns of alcohol use associated with elevated serum hepatic enzymes in US adults. J Behav Med 2012;35:200-10.
54. Fraser A, Harris R, Sattar N, et al. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care. 2009;32:741-50.
55. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology 2010;52:1156-61.
56. Lee DH, Silventoinen K, Hu G, et al. Serum gamma-glutamyltransferase predicts non-fatal myocardial infarction and fatal coronary heart disease among 28,838 middle-aged men and women. Eur Heart J 2006;27:2170-6.
57. Targher G, Bertolini L, Rodella S, et al. Nonalcoholic fatty liver disease is independently associated with an increased incidence of cardiovascular events in type 2 diabetic patients. Diabetes Care 2007;30:2119-21.

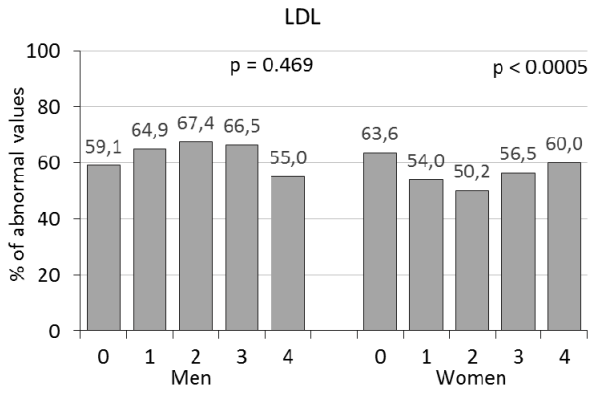


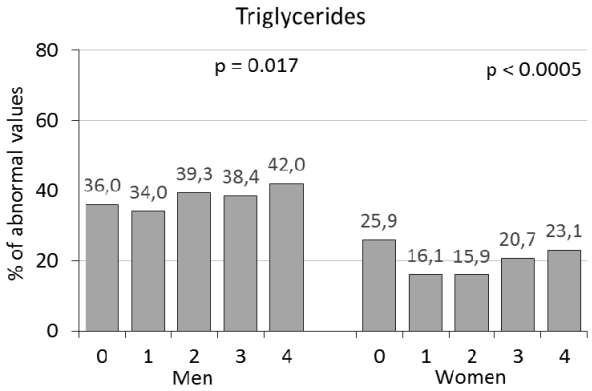












Alcohol consumption categories:

0 = Abstainers, 0 g/day

1 = Low risk drinkers, men 1–40 g/day, women 1–20 g/day

2 = Medium risk drinkers, men 41–60 g/day, women 21–40 g/day

3 = High risk drinkers, men 61–100 g/day, women 41–60 g/day

4 = Very high risk drinkers, men > 100 g/day, women > 60 g/day

PUBLICATION II

Liver enzymes in alcohol consumers with or without binge drinking

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Liver enzymes in alcohol consumers with or without binge drinking

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ABSTRACT

Background: While alcohol use is linked with a wide variety of health problems, the question of whether differences in drinking patterns could yield different outcomes has remained unclear.
Patients and methods: We measured liver enzymes (ALT, GGT) from alcohol consumers with or without binge drinking from a population-based sample in Finland, where binge-type drinking is common. Data on alcohol use, diet, body weight, lifestyle (smoking, coffee consumption, physical activity), and health status were collected from 19225 subjects (9492 men, 9733 women), aged 25–74 years. The participants were subsequently classified to subgroups, both according to the frequencies of binge drinking and the amounts of regular alcohol intake (low-, medium-, and high-risk drinking).
Results: The quantity of regular alcohol use was roughly linearly related with GGT and ALT activities. ANOVA analyses of the trends according to the frequency of binge drinking showed a significant GGT increase in both men ($p < 0.0005$) and women ($p < 0.0005$), and a significant increase of ALT in men ($p < 0.0005$). In those with low-risk overall consumption, markedly higher GGT ($p < 0.0005$) and ALT ($p < 0.0005$) occurred in those with binge drinking more than once a month, compared with those with no such occasions. Binge drinking occurring $\leq$1/month also resulted in higher GGT ($p < 0.0005$) and ALT ($p < 0.05$) activities.
Conclusions: These results emphasize possible adverse consequences of binge drinking on hepatic function even in those with low-risk overall consumption. The pattern of drinking should be more systematically implicated in clinical recommendations for drinking reduction.

Introduction

Alcohol use is a leading cause of addiction and disease throughout the world (Connor, Haber, & Hall, 2016; GBD 2016 Alcohol and Drug Use Collaborators, 2018 a,b; Lim et al., 2012; Spanagel et al., 2013; Wittchen, 2012). While the relationships between total cumulative alcohol consumption and adverse health effects have been well established, only a few studies have separately investigated the specific characteristics of the ethanol effects brought about by different patterns of intake. Therefore, the contributions of repeated episodes of binge-type drinking when several drinks are consumed within short periods of time have remained especially poorly defined (Rehm, Samokhvalov, & Shield, 2013).

Recent studies have supported the view that regular alcohol consumption in amounts exceeding 100 g of ethanol per week leads to an increase in all-cause mortality and signs of hepatotoxicity (Niemelä, Niemelä, Bloigu, Aalto, & Laatikainen, 2017; Wood et al., 2018). It may, however, be hypothesized that repeated heavy drinking occasions could further potentiate the risks for negative health effects, since binge drinking has been suggested to readily lead to stimulation of inflammatory cascades and oxidative stress (Hillbom, Saloheimo, & Juvela, 2011; Li et al., 2017; Orio et al., 2017; Rehm & Shield, 2013). Current guidelines define binge drinking as a pattern of drinking that typically consists of occasional heavy

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drinking that exceeds 60 g of alcohol for men or 40 g of alcohol for women on one occasion (National Institute of Alcohol Abuse and Alcoholism, 2004; World Health Organization, 2000). On the other hand, chronic drinking can be classified to categories of low-, medium-, or high-risk drinking, based on mean ethanol consumption defined in g of ethanol per day with gender-specific cutoffs, as recently recommended by the World Health Organization (Witkiewitz et al., 2017). In the latter protocol, low-risk drinking represents drinking in amounts below 40 g (men) or 20 g (women) per day.

In the present work, we explored the joint and individual effects of heavy drinking occasions and regular alcohol consumption on biomarkers of liver status in a large population health survey, the National FINRISK Study. The patterns of alcohol drinking are known to show a notable variation between communities. Since Finland represents a country with a high prevalence of binge drinking (Levola & Aalto, 2015), the FINRISK survey of individuals, which includes detailed records on alcohol consumption, diet, and other health-related behavior, affords an excellent opportunity to examine the associations between the different patterns of drinking and health outcomes.

Materials and methods

Study design, data sources, and participants

Data were collected from a cross-sectional population health survey (The National FINRISK Study), which has been carried out in Finland every five years since 1972. In this study, data from surveys between 1997 and 2007 were used, as previously described (Niemelä et al., 2017). A nationally representative age- and gender-stratified random sample was drawn from the population register following an international protocol (The World Health Organization MONICA project, monitoring trends and determinants in cardiovascular disease: a major international collaboration; WHO MONICA project principal investigators, 1988) (World Health Organization, 1988). Clinical examinations included physical measurements, laboratory tests, and detailed questionnaires on the amounts and patterns of alcohol intake, current health status, diet, smoking, physical activity, medical history, and socioeconomic factors (Kuulasmaa et al., 2006). Data were available from 19225 apparently healthy individuals: 9492 men and 9733 women (mean age 45 ± 13 years, range 25–74 years) who completed the questionnaires, attended the medical examination, and were devoid of any apparent clinical signs of liver disease, ischemic heart or brain disease, or active infection at the time of the study.

Data on self-reported alcohol consumption were recorded from the past 12 months prior to blood sampling using structured questionnaires on the types of beverages consumed, the frequency of consumption, and the amounts of ethanol-containing drinks. The amount of ethanol in different beverages was quantified in g of ethanol based on defined portion sizes as follows: regular beer 12 g (1/3 L), strong beer 15.5 g (1/3 L), long drink 15.5 g (1/3 L), spirit 12 g (4 cL), wine 12 g (12 cL), and cider 12 g (1/3 L). Binge drinking was defined as a pattern of drinking, which typically had consisted of occasional heavy drinking exceeding 60 g of alcohol for men or 40 g of alcohol for women on one occasion (National Institute of Alcohol Abuse and Alcoholism, 2004; World Health Organization, 2000). Such drinking within a relatively short period of time typically results in blood alcohol levels above 0.08 g/100 ml. Based on the frequency of such episodes, the material was divided to subgroups of those with no episodes of binge drinking and to those with different numbers of binge episodes, as indicated. Data on total alcohol consumption from the period of 12 months prior to sampling were used to further categorize the material according to the recently established WHO risk drinking protocol (Witkiewitz et al., 2017) as follows: 1) those who consumed between 1 and 40 g (men) or 1–20 g (women) per day represented low-risk drinkers, 2) medium-risk drinkers consumed 41–60 g (men) or 21–40 g (women) per day, 3) high-risk drinkers consumed 61–100 g (men) or 41–60 g (women) per day. Individuals exceeding the levels of high-risk drinking (very high-risk drinkers) ($n = 245$, 167 men, 78 women) were excluded due to the fact that they all represented individuals with high levels of both total alcohol consumption and high numbers of binge drinking occasions.

Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI, kg/m^2) was calculated as a measure of relative body weight. Waist circumference was measured to the nearest 0.5 cm between the lowest rib and the iliac crest while exhaling. Smoking and coffee consumption were assessed with a set of standardized questions and expressed as the amounts of cigarettes per day and as the intake of standard servings of coffee (cups) per day, respectively. For statistical analyses, smoking habits were classified into subgroups as follows: 1) no smoking, 2) smoking 1–19 cigarettes per day, and 3) smoking ≥20 cigarettes per day. For coffee consumption, the subgroups were the following: 1) no consumption, 2) coffee consumption 1–4 cups per day, and 3) coffee consumption ≥4 cups per day. Leisure-time physical activity, the number of exercises and total time used for physical exercises were registered using structured questionnaires, and the data were used to classify the population into subgroups of 1) moderate or vigorous activity (over 4 h of activity per week), 2) light (0.5–4 h per week), and 3) sedentary activity (less than 0.5 h per week).

The approval for this study was received from the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. All surveys were conducted in accordance with the Declaration of Helsinki according to the ethical rules of the National Public Health Institute.

Laboratory analyses

Serum ALT and GGT were measured by standard clinical chemical methods following the recommendations of the assay manufacturer on an Abbott Architect clinical chemistry analyzer (Abbott Laboratories; Abbott Park, Illinois, United States). High-sensitivity C-reactive protein (CRP), an index of inflammation, was determined using a latex immunoassay (Sentinel Diagnostics; Milan, Italy) with the Abbott Architect c8000 clinical chemistry analyzer. The upper normal limits for the assays were as follows: ALT – 50 U/L men, 35 U/L women; GGT – 60 U/L men, 40 U/L women; CRP – 3.0 mg/L.

Statistical methods

Values are expressed as mean ± SD or mean ± 95% confidence interval (CI). The main characteristics were compared using analysis of variance (ANOVA). A logarithmic transformation of GGT and ALT was used to obtain non-skewed distributions. ANOVA was performed to assess the trend in GGT and ALT activities across the ordered groups of heavy drinking occasions. Comparisons of GGT and ALT activities between the groups representing the different drinking patterns were carried out using analysis of covariance (ANCOVA). As covariates, we used age and BMI as continuous variables, and smoking, physical activity, and coffee consumption as categorical variables (categories as described above). The association between GGT and ALT levels above the upper normal limit and the different drinking patterns was evaluated by means of logistic regression, while simultaneously adjusting for aforementioned covariates. The Breslow–Day test was used to assess whether the

effect of binge drinking was homogenous across the different BMI categories (<25, 25–29.99, and ≥30). For the analyses, IBM SPSS Statistics 22.0 (Armonk, New York: IBM Corp.) software was used. A p value < 0.05 was considered statistically significant.

Results

The main demographic and lifestyle characteristics of the participants classified to subgroups according to gender, the amounts of regular alcohol consumption, and the frequencies of heavy drinking occasions are summarized in Table 1. The data on regular alcohol consumption indicated that among the 19 225 participants, 90.6% of men ($n = 8597$) were low-risk drinkers consuming an average of below 40 g of ethanol per day, 6.0% ($n = 570$) were moderate-risk drinkers, and 3.4% ($n = 325$) represented high-risk drinkers. In women ($n = 9733$), the corresponding percentages in the different alcohol-drinking risk groups were 93.8%, 5.1%, and 1.1%. Those with higher numbers of heavy drinking occasions were younger than those with low numbers of such occasions ($p < 0.001$ for both genders). Smoking was also more common in those with higher levels of alcohol consumption ($p < 0.001$ for both genders) and higher numbers of heavy drinking occasions ($p < 0.001$ for both genders), whereas for body weight, coffee consumption, and physical activity, no clear patterns were seen in the corresponding comparisons.

Fig. 1 demonstrates the medians and interquartile ranges of GGT and ALT activities in the total study population, classified according to the frequency of heavy drinking occasions. The ANOVA analyses of the trends across the subgroups showed significant increases for GGT in both men ($p < 0.0005$) and women ($p < 0.0005$) in association with increasing frequencies of binge drinking. For ALT, a significant trend was observed in men ($p < 0.0005$), whereas no such trend was observed for women. In individual comparisons of each group with binge episodes to those reporting no such episodes, a significant increase in GGT and ALT in men was first observed in the group reporting heavy drinking occasions 2–3 times per year ($p < 0.05$ for both comparisons). A notable increase in liver enzymes was found in those with heavy drinking occasions at least once a month ($p < 0.0005$ for both GGT and ALT). For women, a significant increase in GGT values was noted in subgroups reporting heavy drinking occasions once a week or more often ($p < 0.0005$).

Fig. 2 shows the adjusted geometric means of GGT and ALT in the different study subgroups classified according to both the total alcohol consumption and the frequency of heavy drinking occasions. The activities of the liver enzymes increased in a rather linear manner as a function of total regular alcohol consumption, with the individuals with the highest amounts of total ethanol intake showing the highest activities. The patterns of drinking were found to further influence the activities, such that among individuals with low-risk total alcohol consumption, the individuals reporting heavy drinking occasions more than once a month showed elevated GGT ($p < 0.0005$) and ALT ($p < 0.0005$) activities significantly more often than those reporting no such occasions. Episodes of heavy drinking once a month or less were also associated with significantly higher GGT ($p < 0.0005$) and ALT ($p < 0.05$) values than those in individuals without any such episodes. In subgroups representing medium- or high-risk drinkers, such differences were not evident.

Table 2 summarizes the ORs of GGT and ALT activities exceeding the upper normal limits according to the different drinking categories following adjustment for age, BMI, smoking, physical activity, and coffee consumption. When compared with those reporting no episodes of binge drinking, GGT ($p < 0.0005$ for both genders) and ALT ($p < 0.02$ for men) showed significantly higher odds for exceeding the upper thresholds in low-risk drinkers with a history of binge drinking. The effect of binge drinking on GGT and ALT levels above the upper normal limit was also found to be homogeneous across BMI categories for both genders (p values varying from 0.18 to 0.94). In additional logistic regression analyses done by adding alcohol consumption reported from the previous week (reflecting recent drinking) to our previous model as adjusting variable (categorized as with or without recent drinking), we reached similar conclusions on higher odds for elevated liver enzymes in those with binge drinking than in those without binge drinking (data not shown).

In men with low overall levels of alcohol consumption, the levels of C-reactive protein (CRP), a marker of inflammation, were also slightly higher in those with binge drinking (1.36; 1.29–1.44 mg/L) than in those without such episodes (1.25; 1.17–1.34 mg/L) ($p < 0.05$), whereas in women, no significant differences were observed. In the present population, total alcohol consumption recorded from the past 12 months correlated positively with GGT ($r = 0.224$, $p < 0.01$), ALT ($r = 0.132$, $p < 0.01$), and CRP ($r = 0.037$, $p < 0.01$). The number of binge drinking episodes

Table 1
Main characteristics of the study population, as classified according to the amounts and patterns of drinking

Men							
Amount of drinking	≤40 g/day (low risk)			41–60 g/day (medium risk)		61–100 g/day (high risk)	
Binge drinking episodes	None	≤1/month	>1/month	≤1/month	>1/month	≤1/month	>1/month
n (%)	1248 (13.1)	5249 (55.3)	2100 (22.1)	174 (1.8)	396 (4.2)	53 (0.6)	272 (2.9)
Age, years, mean ± SD	58.6 ± 11.8	48.9 ± 13.1	45.0 ± 12.7	51.7 ± 11.3	44.6 ± 11.1	54.7 ± 10.8	45.4 ± 11.7
BMI	27.3 ± 4.0	27.1 ± 3.9	27.1 ± 4.2	27.9 ± 4.1	27.2 ± 4.1	27.5 ± 3.7	27.5 ± 4.5
Waist circumference, cm	96.7 ± 11.5	95.4 ± 11.3	95.4 ± 12.2	99.0 ± 10.9	96.4 ± 12.0	98.8 ± 11.4	97.5 ± 12.7
Smoking, cigarettes/day	2.1 ± 6.8	4.1 ± 8.1	7.2 ± 10.0	7.4 ± 11.2	8.9 ± 10.4	8.5 ± 12.1	10.0 ± 11.9
Coffee, cups/day	4.1 ± 3.3	4.7 ± 3.2	4.7 ± 3.1	4.2 ± 3.2	4.9 ± 3.3	4.6 ± 3.4	4.5 ± 3.4
Physical activity, number of exercises per week	2.7 ± 2.3	2.4 ± 2.1	2.3 ± 2.2	2.0 ± 1.8	1.9 ± 2.1	1.8 ± 1.9	2.0 ± 2.1
Women							
Amount of drinking	≤20 g/day (low risk)			21–40 g/day (medium risk)		41–60 g/day (high risk)	
Binge drinking episodes	None	≤1/month	>1/month	≤1/month	>1/month	≤1/month	>1/month
n (%)	3152 (32.4)	5439 (55.9)	535 (5.5)	274 (2.8)	224 (2.3)	42 (0.4)	67 (0.7)
Age, years, mean ± SD	53.8 ± 12.0	43.2 ± 11.7	40.1 ± 11.4	46.7 ± 10.9	40.7 ± 11.7	49.1 ± 9.9	44.7 ± 10.8
BMI	27.3 ± 5.2	25.7 ± 4.8	25.6 ± 5.0	26.2 ± 5.1	25.9 ± 4.9	27.3 ± 4.9	25.6 ± 4.5
Waist circumference, cm	85.8 ± 13.3	82.2 ± 12.3	82.1 ± 13.0	84.6 ± 13.1	83.9 ± 13.0	88.8 ± 12.1	83.6 ± 10.9
Smoking, cigarettes/day	1.1 ± 3.8	2.7 ± 5.9	5.5 ± 7.2	4.6 ± 7.4	5.6 ± 6.6	7.2 ± 9.3	9.8 ± 10.9
Coffee, cups/day	3.6 ± 2.3	3.7 ± 2.5	4.1 ± 2.8	3.8 ± 2.4	3.9 ± 2.5	3.9 ± 2.6	4.2 ± 3.5
Physical activity, number of exercises per week	2.5 ± 2.2	2.5 ± 2.0	2.3 ± 1.8	2.5 ± 2.0	2.3 ± 1.8	1.9 ± 1.9	1.8 ± 2.0

BMI, body mass index.

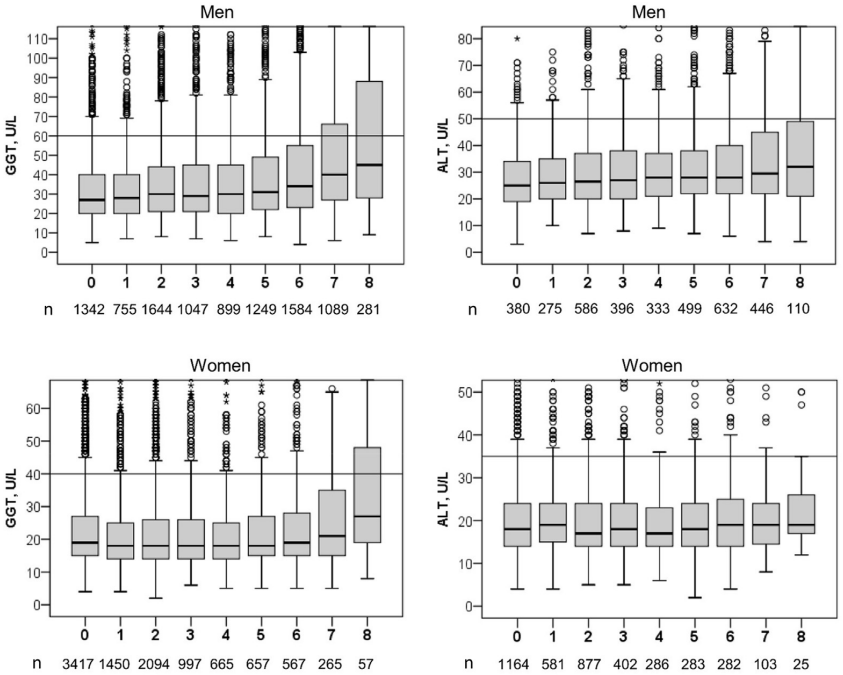

Fig. 1. Median and interquartile ranges of liver enzyme activities in the study population classified according to the number of heavy drinking occasions as follows: 0 = no episodes, 1 = once a year; 2 = 2–3 times per year; 3 = 4–5 times per year; 4 = once per 2 months; 5 = once a month; 6 = two times per month; 7 = once per week; 8 = two times per week or more. The ANOVA analyses of the trends across the subgroups with different levels of binge drinking showed significant GGT increases in both men ($p < 0.0005$) and women ($p < 0.0005$) and increased ALT activities in men ($p < 0.0005$). In individual comparisons of each binge drinking subgroup with those reporting no binge drinking, a significant increase in GGT and ALT in men was first observed in group 2 (reporting heavy drinking occasions 2–3 times per year) ($p < 0.05$ for both comparisons). In women, a significant increase in GGT values was noted in subgroups 7–8 reporting heavy drinking occasions once a week or more often ($p < 0.0005$ for both comparisons). The upper normal limits (ALT – 50 U/L men, 35 U/L women; GGT – 60 U/L men, 40 U/L women) are indicated by solid lines in each figure. n = number of observations in each subgroup.

was also found to correlate with GGT ($r = 0.158$, $p < 0.01$) and ALT ($r = 0.115$, $p < 0.01$) activities.

Discussion

While excessive alcohol consumption is known to cause both addiction and substantial health loss, comparisons between the health effects brought about by repeated episodes of heavy drinking or regular total alcohol consumption have been limited. Therefore, the present data from a large cross-sectional population-based health survey is unique and demonstrates that even in individuals with low-risk overall alcohol consumption, occasions of heavy drinking may lead to an extra burden to hepatic tissue and increased activities of liver-derived enzymes.

Recent findings from large international collaborations have indicated that all-cause mortality increases significantly when regular alcohol consumption exceeds the levels of 100 g (~8 drinks) per week (GBD 2016 Alcohol and Drug Use Collaborators, 2018b; Wood et al., 2018). Therefore, many national guidelines currently recommend lowering the thresholds for risky alcohol consumption. Based on the present data, individuals who habitually engage in heavy drinking occasions may need separate attention in alcohol control policies, and the frequency of alcohol binge episodes should also be recorded in a more systematic manner in the follow-up of alcohol-consuming patients. Those engaged in heavy drinking occasions appear to show increased activity of both GGT and ALT, despite relatively low total alcohol consumption levels. It remains to be established whether the increases in liver enzymes could also be related to higher odds for incident liver disease (Alatalo et al., 2008; Lawlor et al., 2014) or possible extrahepatic disease risks (Hillbom et al., 2011; Kazemi-Shirazi et al., 2007; Sundell, Salomaa, Vartiainen, Poikolainen, & Laatikainen, 2008) in individual patients.

Based on current findings, it is tempting to speculate that differences in the prevalence of heavy drinking occasions in different societies could also explain some previous findings on the alcohol-attributable health outcomes that had notable differences in dose–response curves (Connor et al., 2016; Wood et al., 2018). For instance, several studies from populations following Mediterranean diets have proposed cardio-protective properties for light to moderate drinking (Di Castelnuovo et al., 2006), whereas a number of other studies have found no evidence of such benefits (Holmes et al., 2014; Klatsky, 2015; Niemelä et al., 2017; Sipilä, Rose, & Kaprio, 2016; Stockwell et al., 2016; Topiwala et al., 2017; Wood et al., 2018). Therefore, it is of interest to note that the possible beneficial health effects related to light to moderate drinking have been observed primarily from societies with a low prevalence of binge drinking (Di Castelnuovo et al., 2006; Renaud & de Lorgeril, 1992).

Alcohol exerts its toxic effects through multiple biochemical mechanisms (Lieber, 1995). So far, however, relatively little has been learned about the specific pathogenic features of binge-type drinking. Recent studies have demonstrated that even young binge drinkers show elevated levels of blood endotoxin, activation of inflammatory cascades, enhanced oxidative stress, and lipid

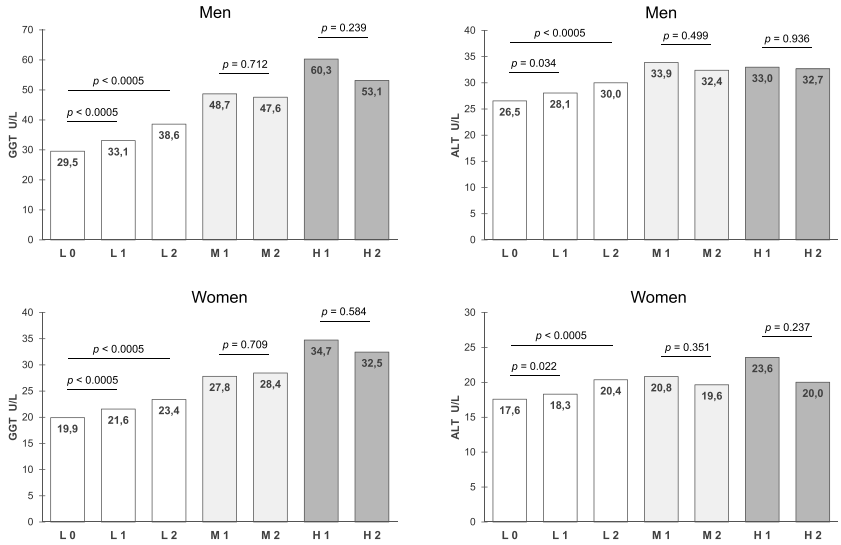

Fig. 2. Geometric mean values of GGT and ALT (adjusted for age, BMI, smoking, physical activity, and coffee consumption) in groups classified according to both alcohol drinking levels and episodes of binge drinking. L = low-risk drinking, 1–40 g (men) or 1–20 g (women) per day; M = medium-risk drinking, 41–60 g (men) or 21–40 g (women) per day; H = high-risk drinking, 61–100 g (men) or 41–60 g (women) per day. Episodes of binge drinking were classified to: 0, those with no episodes of heavy drinking; 1, those with heavy drinking occasions once a month or less and 2, those with heavy drinking more than once a month.

peroxidation (Guerri & Pascual, 2010; Orio et al., 2017), as well as increased markers of neuroinflammation (Ezquer et al., 2018). Generation of oxidative stress has been linked with activation of GGT enzyme, and several lines of evidence have also suggested a role for GGT as a biomarker of oxidative stress (Kazemi-Shirazi et al., 2007; Lee, Blomhoff, & Jacobs, 2004; Speisky, Shackel, Varghese, Wade, & Israel, 1990). Its activation seems to be related to the development of superoxide ion, unintended oxidation of lipoproteins, and generation of pro-inflammatory status in the body (Danielsson, Kangastupa, Laatikainen, Aalto, & Niemelä, 2014; Emdin, Pompella, & Paolicchi, 2005; Kozakova et al., 2012). Alcoholics with recent drinking have been shown to present with higher levels of circulating neutrophils, which also correlate with serum liver enzyme activities (Li et al., 2017). Chronic plus binge type drinking also markedly induces liver inflammation and injury through upregulation of pro-inflammatory cytokines and induction of E-selectin (Bertola, Park, & Gao, 2013; Cai et al., 2017).

Based on current data, the changes in the activities of GGT and ALT enzymes could also be used as biomarkers for detecting the individuals needing the closest monitoring in the assessment of alcohol-related health risks. Follow-up of liver enzyme activities may also prove to be of value in monitoring both hepatic and extrahepatic health risks, including cardio- or cerebrovascular events and metabolic syndrome (Kazemi-Shirazi et al., 2007; Kim, Flamm, Di Bisceglie, Bodenheimer, & Public Policy Committee of the American Association for the Study of Liver Disease, 2008; Niemelä, 2016; Ruhl & Everhart, 2009; Ruttmann et al., 2005). A more systematic use of liver enzyme measurements in addition to alcohol self-reports in the follow-up of alcohol patients could thus help to yield a more comprehensive approach for improving

Table 2
Odds ratios (OR) for liver enzymes exceeding the upper normal limits in individuals with low-, medium-, or high-risk drinking and different levels of binge drinking (as adjusted for age, BMI, smoking, physical activity, and coffee consumption)

	Binge drinking episodes	Low risk		Medium risk		High risk	
		OR (95% CI)	*p* value[a]	OR (95% CI)	*p* value[a]	OR (95% CI)	*p* value[a]
Men							
GGT	None	1.00	<0.0005				
	≤1/month	1.49 (1.21–1.83)		1.00	0.791	1.00	0.347
	>1/month	2.63 (2.09–3.30)		1.06 (0.69–1.62)		0.71 (0.35–1.45)	
ALT	None	1.00	0.015				
	≤1/month	1.49 (0.92–2.44)		1.00	0.524	1.00	0.802
	>1/month	1.99 (1.18–3.34)		1.28 (0.60–2.72)		1.21 (0.26–5.57)	
Women							
GGT	None	1.00	<0.0005				
	≤1/month	1.48 (1.25–1.74)		1.00	0.597	1.00	0.874
	>1/month	2.41 (1.78–3.26)		1.15 (0.68–1.94)		0.93 (0.37–2.31)	
ALT	None	1.00	0.272				
	≤1/month	1.16 (0.86–1.56)		1.00	0.085	1.00	0.174
	>1/month	1.56 (0.91–2.67)		0.47 (0.19–1.14)		0.26 (0.03–2.05)	

[a] Likelihood Ratio test.

treatment adherence and for offering specifically targeted support aimed at drinking reduction. In clinical settings, more emphasis should also be placed on changes occurring in the low range of liver enzyme activities.

While the overall biomarker responses to episodes of binge drinking appeared relatively similar between genders, men seem to show relatively greater sensitivities for elevations in liver enzymes in response to heavy drinking occasions. Although the primary mechanisms underlying such observations remain unknown at this time, it is possible that alcohol use stimulates oxidative stress in a gender-dependent manner (Finkel & Holbrook, 2000; Zhang & Forman, 2009). GGT plays a pivotal role in the metabolism of glutathione (GSH), and elevated activities could be related to an attempt to maintain intracellular GSH levels during oxidative stress, which could also be considered as a protective mechanism against alcohol toxicity (Emdin et al., 2005; Speisky et al., 1990; Zhang & Forman, 2009). Women, however, seem to show elevated liver enzyme activities following smaller actual quantities of total alcohol consumption. Women are also known to be more vulnerable to alcohol addiction, alcohol-induced liver disease, and central nervous system effects (Alfonso-Loeches, Pascual, & Guerri, 2013; Hillbom et al., 2011; Liu, Balkwill, Reeves, Beral, & Million Women Study Collaborators, 2010; Schwarzinger et al., 2018). Previous studies have also suggested that the immune and inflammatory consequences of binge drinking may be more pronounced among women (Orio et al., 2017; Pascual et al., 2017). In the present work, the responses in CRP, an acute inflammatory protein, to binge drinking was found, however to occur in a slightly more sensitive manner among men.

Not surprisingly, those engaged more frequently in heavy drinking occasions were younger than those with a lower number of such episodes. In accordance with previous observations, heavy drinking occasions and smoking also appeared to be highly concomitant behaviors, especially in young adults (Harrison, Desai, & McKee, 2008; Woolard et al., 2015). There may also be significant synergistic effects between alcohol use and smoking in creating hepatotoxic effects (Breitling, Raum, Müller, Rothenbacher, & Brenner, 2009; Park et al., 2013). It may therefore be assumed that interventions aimed at reducing smoking could also affect binge drinking and vice versa. While physical activity, the presence or absence of obesity (Alatalo et al., 2008), and coffee consumption (Goh, Chow, Wang, Yuan, & Koh, 2014; Xiao, Sinha, Graubard, & Freedman, 2014) have also been suggested as factors influencing liver enzyme activities in alcohol consumers, in the present material such variables were not found to affect the conclusion reached on the effects of binge drinking on liver enzyme activities.

The strengths of this study include the large number of study subjects and separate assessments for men and women. The questionnaire used in this study covered the evaluation of both regular alcohol consumption and the frequencies of heavy drinking occasions from the previous year, allowing the assessment of single and joint effects of regular or binge-type drinking on liver outcomes. Various possible covariates, such as age, smoking, waist circumference, BMI, physical activity, or coffee consumption were also included in the multivariable analyses. Nevertheless, our study has some potential limitations. Self-reports are prone to the shortcomings of this memory-dependent channel, and it is possible that the alcohol recall techniques overestimate the proportion of those not drinking alcohol at all. This could also lead to underestimation of the true dose–response associations (Livingston & Callinan, 2015). The cross-sectional setting of the survey and lack of follow-up data can also be considered as limitations of this study.

Taken together, our study demonstrates distinct differences in liver enzyme responses in alcohol consumers with or without binge drinking. These data should be considered in health guidelines related to the amounts and patterns of alcohol drinking and in efforts aimed at reduction of population-level alcohol consumption.

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Conflict of interest

The authors declare that they have no conflicts of interest.

Author contributions

ON, TL, and MA designed the study, UN, AB, and RB performed data analyses. UN and ON drafted the manuscript, and all authors revised and approved the final version.

References

Alatalo, P. I., Koivisto, H. M., Hietala, J. P., Puukka, K. S., Bloigu, R., & Niemelä, O. J. (2008). Effect of moderate alcohol consumption on liver enzymes increases with increasing body mass index. *American Journal of Clinical Nutrition, 88*, 1097–1103. https://doi.org/10.1093/ajcn/88.4.1097.

Alfonso-Loeches, S., Pascual, M., & Guerri, C. (2013). Gender differences in alcohol-induced neurotoxicity and brain damage. *Toxicology, 311*, 27–34. https://doi.org/10.1016/j.tox.2013.03.001.

Bertola, A., Park, O., & Gao, B. (2013). Chronic plus binge ethanol feeding synergistically induces neutrophil infiltration and liver injury: A critical role for E-selectin. *Hepatology, 58*, 1814–1823. https://doi.org/10.1002/hep.26419.

Breitling, L. P., Raum, E., Müller, H., Rothenbacher, D., & Brenner, H. (2009). Synergism between smoking and alcohol consumption with respect to serum gamma-glutamyltransferase. *Hepatology, 49*, 802–808. https://doi.org/10.1002/hep.22727.

Cai, Y., Xu, M.-J., Koritzinsky, E. H., Zhou, Z., Wang, W., Cao, H., et al. (2017). Mitochondrial DNA-enriched microparticles promote acute-on-chronic alcoholic neutrophilia and hepatotoxicity. *Journal of Clinical Investigation Insight, 2*, e92634. https://doi.org/10.1172/jci.insight.92634. pii.

Connor, J. P., Haber, P. S., & Hall, W. D. (2016). Alcohol use disorders. *Lancet, 387*, 988–998. https://doi.org/10.1016/S0140-6736(15)00122-1.

Danielsson, J., Kangastupa, P., Laatikainen, T., Aalto, M., & Niemelä, O. (2014). Impacts of common factors of life style on serum liver enzymes. *World Journal of Gastroenterology, 20*, 11743–11752. https://doi.org/10.3748/wjg.v20.i33.11743.

Di Castelnuovo, A., Costanzo, S., Bagnardi, V., Donati, M. B., Iacoviello, L., & de Gaetano, G. (2006). Alcohol dosing and total mortality in men and women: An updated meta-analysis of 34 prospective studies. *Archives of Internal Medicine, 166*, 2437–2445. https://doi.org/10.1001/archinte.166.22.2437.

Emdin, M., Pompella, A., & Paolicchi, A. (2005). Gamma-glutamyltransferase, atherosclerosis, and cardiovascular disease: Triggering oxidative stress within the plaque. *Circulation, 112*, 2078–2080. https://doi.org/10.1161/CIRCULATIONAHA.105.571919.

Ezquer, F., Morales, P., Quintanilla, M. E., Santapau, D., Lespay-Rebolledo, C., Ezquer, M., et al. (2018). Intravenous administration of anti-inflammatory mesenchymal stem cell spheroids reduces chronic alcohol intake and abolishes binge-drinking. *Scientific Reports, 8*, 4325. https://doi.org/10.1038/s41598-018-22750-7.

Finkel, T., & Holbrook, N. J. (2000). Oxidants, oxidative stress and the biology of ageing. *Nature, 408*, 239–247. https://doi.org/10.1038/35041687.

GBD 2016 Alcohol and Drug Use Collaborators. (2018a). The global burden of disease attributable to alcohol and drug use in 195 countries and territories, 1990-2016: A systematic analysis for the global burden of disease study 2016. *Lancet Psychiatry, 5*, 987–1012. https://doi.org/10.1016/S2215-0366(18)30337-7.

GBD 2016 Alcohol and Drug Use Collaborators. (2018b). Alcohol use and burden for 195 countries and territories, 1990-2016: A systematic analysis for the global burden of disease study 2016. *Lancet, 392*, 1015–1035. https://doi.org/10.1016/S0140-6736(18)31310-2.

Goh, G. B., Chow, W. C., Wang, R., Yuan, J. M., & Koh, W. P. (2014). Coffee, alcohol and other beverages in relation to cirrhosis mortality: The Singapore Chinese health study. *Hepatology, 60*, 661–669. https://doi.org/10.1002/hep.27054.

Guerri, C., & Pascual, M. (2010). Mechanisms involved in the neurotoxic, cognitive, and neurobehavioral effects of alcohol consumption during adolescence. *Alcohol, 44*, 15–26. https://doi.org/10.1016/j.alcohol.2009.10.003.

Harrison, E. L., Desai, R. A., & McKee, S. A. (2008). Nondaily smoking and alcohol use, hazardous drinking, and alcohol diagnoses among young adults: Findings from the NESARC. *Alcoholism: Clinical and Experimental Research, 32*, 2081–2087. https://doi.org/10.1111/j.1530-0277.2008.00796.x.

Hillbom, M., Saloheimo, P., & Juvela, S. (2011). Alcohol consumption, blood pressure, and the risk of stroke. *Current Hypertension Reports, 13*, 208–213. https://doi.org/10.1007/s11906-011-0194-y.

Holmes, M. V., Dale, C. E., Zuccolo, L., Silverwood, R. J., Guo, Y., Ye, Z., et al. (2014). Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. *BMJ, 349*, g4164. https://doi.org/10.1136/bmj.g4164.

Kazemi-Shirazi, L., Endler, G., Winkler, S., Schickbauer, T., Wagner, O., & Marsik, C. (2007). Gamma glutamyltransferase and long-term survival: Is it just the liver? *Clinical Chemistry, 53*, 940–946. https://doi.org/10.1373/clinchem.2006.081620.

Kim, W. R., Flamm, S. L., Di Bisceglie, A. M., Bodenheimer, H. C., & Public Policy Committee of the American Association for the Study of Liver Disease. (2008). Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. *Hepatology, 47*, 1363–1370. https://doi.org/10.1002/hep.22109.

Klatsky, A. L. (2015). Alcohol and cardiovascular diseases: Where do we stand today? *Journal of Internal Medicine, 278*, 238–250. https://doi.org/10.1111/joim.12390.

Kozakova, M., Palombo, C., Eng, M. P., Dekker, J., Flyvbjerg, A., Mitrakou, A., et al. (2012). Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. *Hepatology, 55*, 1406–1415. https://doi.org/10.1002/hep.25555.

Kuulasmaa, K., Tolonen, H., Cepaitis, Z., Laatikainen, T., Nissinen, A., Vartiainen, E., et al. (2006). *European health risk monitoring project*. Helsinki: Finnish National Public Health Institute (KTL). Retrieved from www.thl.fi/ehrm.

Lawlor, D. A., Benn, M., Zuccolo, L., De Silva, N. M., Tybjaerg-Hansen, A., Smith, G. D., et al. (2014). ADH1B and ADH1C genotype, alcohol consumption and biomarkers of liver function: Findings from a mendelian randomization study in 58,313 european origin Danes. *PLoS One, 9*, e114294. https://doi.org/10.1371/journal.pone.0114294.

Lee, D. H., Blomhoff, R., & Jacobs, D. R., Jr. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? *Free Radical Research, 38*, 535–539.

Levola, J., & Aalto, M. (2015). Screening for at-risk drinking in a population reporting symptoms of depression: A validation of the AUDIT, AUDIT-C, and AUDIT-3. *Alcoholism: Clinical and Experimental Research, 39*, 1186–1192. https://doi.org/10.1111/acer.12763.

Lieber, C. S. (1995). Medical disorders of alcoholism. *New England Journal of Medicine, 333*, 1058–1065. https://doi.org/10.1056/NEJM199510193331607.

Li, M., He, Y., Zhou, Z., Ramirez, T., Gao, Y., Gao, Y., et al. (2017). MicroRNA-223 ameliorates alcoholic liver injury by inhibiting the IL-6-p47phox-oxidative stress pathway in neutrophils. *Gut, 66*, 705–715. https://doi.org/10.1136/gutjnl-2016-311861.

Lim, S. S., Vos, T., Flaxman, A. D., Danaei, G., Shibuya, K., Adair-Rohani, H., et al. (2012). A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: A systematic analysis for the global burden of disease study 2010. *Lancet, 380*, 2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8.

Liu, B., Balkwill, A., Reeves, G., Beral, V., & Million Women Study Collaborators. (2010). Body mass index and risk of liver cirrhosis in middle aged UK women: Prospective study. *BMJ, 340*, c912. https://doi.org/10.1136/bmj.c912.

Livingston, M., & Callinan, S. (2015). Underreporting in alcohol surveys: Whose drinking is underestimated? *Journal of Studies on Alcohol and Drugs, 76*, 158–164.

National Institute of Alcohol Abuse and Alcoholism. (2004). NIAAA Council approves definition of binge drinking. *NIAAA Newsletter, 3*, 3.

Niemelä, O. (2016). Biomarker-based approaches for assessing alcohol use disorders. *International Journal of Environmental Research and Public Health, 13*, 166. https://doi.org/10.3390/ijerph13020166.

Niemelä, O., Niemelä, M., Bloigu, R., Aalto, M., & Laatikainen, T. (2017). Where should the safe limits of alcohol consumption stand in light of liver enzyme abnormalities in alcohol consumers? *PLoS One, 12*, e0188574. https://doi.org/10.1371/journal.pone.0188574.

Orio, L., Antón, M., Rodriguez-Rojo, I. C., Correas, Á., García-Bueno, B., Corral, M., et al. (2017). Young alcohol binge drinkers have elevated blood endotoxin, peripheral inflammation and low cortisol levels: Neuropsychological correlations in women. *Addiction Biology, 23*, 1130–1144. https://doi.org/10.1111/adb.12543.

Park, E. Y., Lim, M. K., Oh, J. K., Cho, H., Bae, M. J., Yun, E. H., et al. (2013). Independent and supra-additive effects of alcohol consumption, cigarette smoking, and metabolic syndrome on the elevation of serum liver enzyme levels. *PLoS One, 8*, e63439. https://doi.org/10.1371/journal.pone.0063439.

Pascual, M., Montesinos, J., Marcos, M., Torres, J. L., Costa-Alba, P., García-García, F., et al. (2017). Gender differences in the inflammatory cytokine and chemokine profiles induced by binge ethanol drinking in adolescence. *Addiction Biology, 22*, 1829–1841. https://doi.org/10.1111/adb.12461.

Rehm, J., Samokhvalov, A. V., & Shield, K. D. (2013). Global burden of alcoholic liver diseases. *Journal of Hepatology, 59*, 160–168. https://doi.org/10.1016/j.jhep.2013.03.007.

Rehm, J., & Shield, K. D. (2013). The impact of confounding and alcohol consumption patterns on the calculated risks of alcohol-related diseases. *Addiction, 108*, 1544–1545. https://doi.org/10.1111/add.12074.

Renaud, S., & de Lorgeril, M. (1992). Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet, 339*, 1523–1526.

Ruhl, C. E., & Everhart, J. E. (2009). Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. *Gastroenterology, 136*, 477–485. https://doi.org/10.1053/j.gastro.2008.10.052.

Ruttmann, E., Brant, L. J., Concin, H., Diem, G., Rapp, K., Ulmer, H., et al. (2005). Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: An epidemiological investigation in a cohort of 163,944 Austrian adults. *Circulation, 112*, 2130–2137. https://doi.org/10.1161/CIRCULATIONAHA.105.552547.

Schwarzinger, M., Pollock, B. G., Hasan, O. S. M., Dufouil, C., Rehm, J., & QalyDays Study Group. (2018). Contribution of alcohol use disorders to the burden of dementia in France 2008-13: A nationwide retrospective cohort study. *Lancet Public Health, 3*, e124–e132. https://doi.org/10.1016/S2468-2667(18)30022-7.

Sipilä, P., Rose, R. J., & Kaprio, J. (2016). Drinking and mortality: Long-term follow-up of drinking-discordant twin pairs. *Addiction, 111*, 245–254. https://doi.org/10.1111/add.13152.

Spanagel, R., Durstewitz, D., Hansson, A., Heinz, A., Kiefer, F., Köhr, G., et al. (2013). A systems medicine research approach for studying alcohol addiction. *Addiction Biology, 18*, 883–896. https://doi.org/10.1111/adb.12109.

Speisky, H., Shackel, N., Varghese, G., Wade, D., & Israel, Y. (1990). Role of hepatic gamma-glutamyltransferase in the degradation of circulating glutathione: Studies in the intact Guinea pig perfused liver. *Hepatology, 11*, 843–849.

Stockwell, T., Zhao, J., Panwar, S., Roemer, A., Naimi, T., & Chikritzhs, T. (2016). Do "moderate" drinkers have reduced mortality risk? A systematic review and meta-analysis of alcohol consumption and all-cause mortality. *Journal of Studies on Alcohol and Drugs, 77*, 185–198.

Sundell, L., Salomaa, V., Vartiainen, E., Poikolainen, K., & Laatikainen, T. (2008). Increased stroke risk is related to a binge-drinking habit. *Stroke, 39*, 3179–3184. https://doi.org/10.1161/STROKEAHA.108.520817.

Topiwala, A., Allan, C. L., Valkanova, V., Zsoldos, E., Filippini, N., Sexton, C., et al. (2017). Moderate alcohol consumption as risk factor for adverse brain outcomes and cognitive decline: Longitudinal cohort study. *BMJ, 357*, j2353. https://doi.org/10.1136/bmj.j2353.

Witkiewitz, K., Hallgren, K. A., Kranzler, H. R., Mann, K. F., Hasin, D. S., Falk, D. E., et al. (2017). Clinical validation of reduced alcohol consumption after treatment for alcohol dependence using the World Health Organization risk drinking levels. *Alcoholism: Clinical and Experimental Research, 41*, 179–186. https://doi.org/10.1111/acer.13272.

Wittchen, H. U. (2012). The burden of mood disorders. *Science, 338*, 15. https://doi.org/10.1126/science.1230817.

Wood, A. M., Kaptoge, S., Butterworth, A. S., Willeit, P., Warnakula, S., Bolton, T., et al. (2018). Risk thresholds for alcohol consumption: Combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. *Lancet, 391*, 1513–1523. https://doi.org/10.1016/S0140-6736(18)30134-X.

Woolard, R., Liu, J., Parsa, M., Merriman, G., Tarwater, P., Alba, I., et al. (2015). Smoking is associated with increased risk of binge drinking in a young adult hispanic population at the US-Mexico border. *Substance Abuse, 36*, 318–324. https://doi.org/10.1080/08897077.2014.987945.

World Health Organization. (1988). The world health organization MONICA project (monitoring trends and determinants in cardiovascular disease): A major international collaboration. WHO MONICA project principal investigators. *Journal of Clinical Epidemiology, 41*, 105–114.

World Health Organization. (2000). *International guide for monitoring alcohol consumption and related harm*. Geneva, Switzerland: World Health Organization. Retrieved from https://apps.who.int/iris/bitstream/handle/10665/66529/WHO_MSD_MSB_00.4.pdf.

Xiao, Q., Sinha, R., Graubard, B. I., & Freedman, N. D. (2014). Inverse associations of total and decaffeinated coffee with liver enzyme levels in National Health and Nutrition Examination Survey 1999-2010. *Hepatology, 60*, 2091–2098. https://doi.org/10.1002/hep.27367.

Zhang, H., & Forman, H. J. (2009). Redox regulation of gamma-glutamyl transpeptidase. *American Journal of Respiratory Cell and Molecular Biology, 41*, 509–515. https://doi.org/10.1165/rcmb.2009-0169TR.

PUBLICATION III

Impacts of unfavourable lifestyle factors on biomarkers of liver function, inflammation and lipid status

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RESEARCH ARTICLE

Impacts of unfavourable lifestyle factors on biomarkers of liver function, inflammation and lipid status

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Data Availability Statement: THL Biobank administrates and grants access to the FINRISK data to research projects that are of high scientific quality and impact, are ethically conducted, and that correspond with the research areas of THL Biobank. All data are available for application at https://thl.fi/en/web/thl-biobank/for-researchers/sample-collections/the-national-finrisk-study-1992-2012. The name of dataset is the National FINRISK Study 1992-2012. Interested researchers can replicate our study findings in their entirety by directly obtaining the data and following the

Abstract

Background

Adopting a healthy lifestyle is associated with prolonged life expectancy. The main modifiable lifestyle-related risk factors are hazardous alcohol drinking, smoking, excess body weight and lack of physical activity. Our aim was to estimate the impact of unfavourable lifestyle factors on abnormalities in laboratory tests reflecting liver status, inflammation and lipid metabolism in a population-based cross-sectional study.

Methods

The study included 22,273 participants (10,561 men, 11,712 women) aged 25–74 years from the National FINRISK Study. Data on alcohol use, smoking, body weight, and physical activity were recorded from structured interviews. The risk scores for the various life style factors were established on a 0–8 scale and used to stratify the population in classes to allow estimates of their joint effects. Serum liver enzymes (GGT, ALT), C-reactive protein (CRP) and lipid profiles were measured using standard laboratory techniques.

Results

Consistent dose-response relationships were observed between the number of unfavourable risk factors and serum levels of GGT, ALT, CRP, cholesterol, HDL, LDL and triglycerides ($p < 0.0005$ for linear trend in all comparisons). When compared with those with zero risk factors, the multivariable-adjusted odds ratios (ORs) for abnormalities in all biomarkers were significantly higher in those with a sum of risk score two or more. The most striking increases in ORs in the group with the highest numbers of risk factors were observed among men in serum GGT: 26.6 (12.4–57.0), ALT: 40.3 (5.3–307.8), CRP: 16.2 (7.8–33.7) and serum triglycerides: 14.4 (8.6–24.0).

protocol in the Methods section. The authors did not have any special access privileges that others would not have. More information: finriski(at)thl.fi.

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Conclusions

The data support the view that the presence of unfavourable life style risk factors is associated with distinct abnormalities in laboratory tests for liver function, inflammation and lipid status. Such biomarkers may prove to be of value in the assessment of interventions aimed at reducing unfavourable risk factors and in helping individuals in long-term maintenance of lifestyle modifications.

Introduction

Heavy alcohol drinking, smoking, excess body weight and lack of physical exercise are common modifiable risk factors of lifestyle, which may all contribute to the incidence of chronic diseases and premature death [1–5]. There may also be synergistic and additive interactions between such factors in individuals with clustering of unfavourable lifestyle factors [3, 4, 6]. Therefore, interventions aimed at reducing the number of risk factors has been recognized as an important target in both personalized medicine and public health policies [7]. Recent studies have estimated that adopting a healthy lifestyle even at the age of 50 could add more than a decade to life suggesting significant therapeutic potential for lifestyle interventions [3, 8].

A large body of evidence indicates that the occurrence of increased gamma-glutamyltransferase (GGT), and alanine aminotransferase (ALT) enzyme activities in apparently healthy individuals may often be attributed to unhealthy lifestyle factors, such as alcohol consumption or excess body weight [9–13]. The increases in these liver enzymes may also associate with extra-hepatic disease risks, including metabolic syndrome, and cardio- or cerebrovascular events [13–15]. While the biochemical pathways underlying such observations have remained unclear, previous findings have suggested that inflammatory processes [16–18], oxidative stress [19, 20] and generation of abnormal lipid profiles [21] are key pathogenic factors in the sequence of events leading to hepatotoxicity [22] or other adverse health effects, such as incident stroke [5], in individuals presenting with various clusters of risk factors.

So far, only few studies have been available to examine the individual and joint impacts of the various unfavourable life style factors on biochemical indices of health. Considering this issue, we aimed to investigate the combined effects of various lifestyle-related factors on biomarkers of liver status (ALT, GGT), inflammation (C-reactive protein) and lipid metabolism (cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides) in a large national FINRISK population-based study, which includes detailed records on alcohol consumption, smoking, physical activity and health status. It is assumed that further understanding of the biomarker behaviour in response to various types of unhealthy behaviours may improve our possibilities for interventions aimed at adopting more favourable lifestyles.

Materials and methods

Study design, data sources and participants

The study collects extensive data from a cross-sectional population health survey (The National FINRISK Study) carried out in Finland in 1997, 2002 and 2007. In each survey year an age- and gender stratified random sample was drawn from the population register according to an international protocol [23]. Clinical examinations included physical measurements, laboratory tests and detailed questionnaires gathering information on current health status, alcohol intake, diet, smoking, physical activity, medical history and socioeconomic factors [23,

24]. Body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively. Body mass index (BMI, kg/m^2) was calculated as a measure of relative body weight. The data was available from 22,273 apparently healthy individuals: 10,561 men and 11,712 women (mean age 49 ± 13 years, range 25–74 years) who completed the questionnaires and attended the medical examination. The study excluded individuals with any apparent clinical signs of liver disease, ischaemic heart or brain disease or active infection at the time of blood sampling.

The questionnaire used here for registering information on health and lifestyle has been previously developed and validated for use in international population-based health surveys [23–25]. The responses to each question on alcohol consumption, smoking, physical activity and coffee consumption are assigned to mutually exclusive and collectively exhaustive categories [25]. Data on alcohol consumption was registered from the past 12 months prior to blood sampling and included information on the types of beverages consumed as well as the amounts and frequencies of consumption. The ethanol content in different beverages was quantitated in grams of ethanol based on defined portion sizes as follows: regular beer 12 grams (1/3 L), strong beer 15.5 grams (1/3 L), long drink 15.5 grams (1/3 L), spirit 12 grams (4 cL), wine 12 grams (12 cL) and cider 12 grams (1/3 L). Information on smoking habits was collected with a set of standardized questions and the data was expressed as the amounts of cigarettes per day. Habitual physical activity including both the number and total time used for physical exercises were also registered from each participant. Coffee consumption was assessed with a set of standardized questions and expressed as the intake of standard servings of coffee (cups) per day.

The data obtained from the questionnaires was subsequently used to define scores for low risk (= 0), medium risk (= 1) and high risk (= 2) categories for each individual risk factor following recent work on health-related risk assessment in relation to alcohol consumption, smoking, BMI status and physical activity [3, 8, 26–28]. In this work, the variables were, however, categorized into three ordinal levels to yield increased statistical power as compared to previously used dichotomous classification [3]. For alcohol consumption the scores were defined as follows: 0 = no consumption; 1 = alcohol consumption between 1–14 (men) or 1–7 (women) standard drinks per week; 2 = alcohol consumption exceeding 14 drinks (men) or 7 drinks (women) per week. For smoking 0 = no smoking, 1 = 1–19 cigarettes per day, 2 = $\geq$ 20 cigarettes per day; for BMI 0 = BMI $<$ 25; 1 = BMI $\geq$ 25 and $<$ 30; 2 = BMI $\geq$ 30. For physical activity 0 represents those with physical activity over 4 hours per week; 1 = those with physical activity between 0.5 and 4 hours per week and 2 = those with physical activity less than 30 min/week. The sum of these scores provided a total number of risk factors, with higher scores (maximum = 8) indicating an unhealthier lifestyle.

The approval for the data collection was received from the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District in 2002 and 2007 and from the Ethics Committee of the National Public Health Institute in 1997. All surveys were conducted in accordance with the Declaration of Helsinki according to the ethical rules of the National Public Health Institute.

Laboratory analyses

Serum liver enzymes (ALT and GGT) were measured by standard clinical chemical methods on an Abbott Architect clinical chemistry analyzer following the recommendations of the assay manufacturer (Abbott Laboratories, Abbott Park, IL, USA). High-sensitivity CRP, a biomarker of inflammation, was determined using a latex immunoassay (Sentinel Diagnostics, Milan, Italy) with the Abbott Architect c8000 clinical chemistry analyzer. Lipid profiles included determinations of total cholesterol, high-density lipoprotein-associated cholesterol (HDL), low-density lipoprotein (LDL) and total triglycerides using standard enzymatic

methods. All laboratory tests were subjects to continuous external quality control programs organized by Labquality, Finland and CDC (Center for Disease Control and Prevention) quality assurance and standardization program for serum lipids. The cut-offs for the normal limits of the different markers were as follows: ALT (50 U/L men; 35 U/L women), GGT (60 U/L men; 40 U/L women), CRP (3.0 mg/L), cholesterol (5 mmol/L), HDL cholesterol (1.0 mmol/L men, 1.2 mmol/L women), LDL cholesterol (3.0 mmol/L), triglycerides (1.7 mmol/L).

Statistical methods

The main characteristics were examined using analysis of variance (ANOVA) with polynomial contrasts to reveal possible trends across increasing risk score categories. The distribution of abnormal biomarker levels across the risk categories were analysed by chi-square test for trend. Binary logistic regression was used to estimate the odds ratios (ORs) of abnormal biomarker levels associated with the risk score categories, adjusting for age and coffee consumption, as these factors are known to potentially associate with abnormal biomarker levels and showed association in univariate analysis. All factors were entered simultaneously into the multivariable model. Potential multicollinearity among the covariates was examined by calculating the Variance Inflation Factor (VIF) and no evidence was found. Correlations between the risk scores and various biomarkers were calculated using Spearman's rank correlation coefficients. The analyses were carried out with IBM SPSS Statistics 24.0 (Armonk, NY: IBM Corp.). A p-value < 0.05 was considered statistically significant.

Results

The main demographic characteristics of the participants classified to subgroups according to the distribution of unfavourable lifestyle risk factor scores and gender are summarized in Table 1. Higher levels of alcohol consumption, increased body weight, smoking and physical inactivity were found to characterize the individuals with high risk scores. Age of the participants showed a quadratic association between the risk scores such that the highest mean ages were noted in the middle portion of the risk score categories ($p < 0.0005$ for both genders). Coffee consumption was found to increase with increasing number of risk factor scores in both men and women ($p < 0.0005$ for linear trend in both genders).

Fig 1 demonstrates the median and interquartile ranges for the various biomarkers in groups with different risk factor status. Consistent dose-response relationships were observed between the number of unfavourable risk factors and biomarker levels in all biomarkers. The frequencies of values exceeding the upper normal limits for GGT, ALT, CRP and triglycerides or deviations from the target ranges for serum lipids in the different subgroups are summarized in Table 2. The occurrence of abnormal findings in each laboratory parameter was found to increase in a rather linear and significant manner as a function of the risk score status ($p < 0.0005$ for all comparisons).

Table 3 summarizes the multivariable relative risks of abnormal biomarker findings according to different risk categories. The biomarkers of liver status, inflammation and lipid profiles were all found to react to life-style associated risk factors in a sensitive manner and to show significant associations with the number of risk scores when compared with participants with zero risk factors. The most striking increases in ORs in the group with the highest numbers of risk factors were observed for men in serum GGT: 26.6 (12.4–57.0), ALT: 40.3 (5.3–307.8), CRP: 16.2 (7.8–33.7) and serum triglycerides: 14.4 (8.6–24.0). When using BMI as a covariate in the binary logistic regression analyses, similar findings on ORs for abnormal biomarker status were observed, except for the lack of significance for HDL cholesterol in men and for HDL-, LDL- and total cholesterol in women (data not shown).

Table 1. Main characteristics of the participants, as classified according to lifestyle risk factor scores.

Men								
Risk score	0	1	2	3	4	5	6	7–8
n (%)	217 (2.1)	1131 (10.7)	2321 (22.0)	2737 (25.9)	2181 (20.7)	1213 (11.5)	563 (5.3)	198 (1.9)
Age, years, mean ± SD	44.1 ± 14.3	47.5 ± 14.3	50.0 ± 14.3	51.2 ± 13.5	50.1 ± 13.2	49.3 ± 12.2	47.9 ± 11.7	47.4 ± 10.4
Alcohol consumption, g/day	0.0 ± 0.0	4.4 ± 6.3	6.9 ± 8.8	10.2 ± 13.0	15.3 ± 17.6	22.9 ± 25.1	33.0 ± 30.0	41.9 ± 29.8
BMI	23.1 ± 1.4	24.1 ± 2.1	25.6 ± 2.8	27.2 ± 3.3	28.6 ± 4.4	29.3 ± 4.7	29.7 ± 5.1	31.7 ± 4.1
Waist circumference, cm	82.8 ± 5.7	86.9 ± 7.0	91.3 ± 8.7	96.2 ± 9.9	100.1 ± 12.2	102.1 ± 12.6	103.1 ± 13.2	108.4 ± 11.1
Smoking, cigarettes/day	0.0 ± 0.0	0.3 ± 1.8	1.0 ± 3.4	2.7 ± 6.2	5.8 ± 8.9	11.4 ± 11.0	18.2 ± 12.0	24.3 ± 9.4
Coffee, cups/day	3.6 ± 2.9	3.9 ± 2.7	4.0 ± 2.7	4.5 ± 3.0	5.0 ± 3.5	5.3 ± 3.6	5.7 ± 3.7	5.9 ± 4.4
Physical activity, number of exercises per week	4.3 ± 2.6	3.5 ± 2.1	2.9 ± 2.0	2.4 ± 2.0	2.0 ± 2.2	1.4 ± 1.7	1.3 ± 2.1	0.9 ± 1.8
Women								
Risk score	0	1	2	3	4	5	6	7–8
n (%)	447 (3.8)	1939 (16.6)	3183 (27.2)	3004 (25.6)	1945 (16.6)	816 (7.0)	297 (2.5)	81 (0.7)
Age, years, mean ± SD	41.5 ± 12.5	44.8 ± 13.4	47.8 ± 13.5	49.5 ± 13.2	49.9 ± 13.1	48.8 ± 12.2	47.5 ± 11.0	48.4 ± 11.4
Alcohol consumption, g/day	0.0 ± 0.0	1.9 ± 3.2	3.3 ± 5.0	4.6 ± 7.5	6.4 ± 8.0	12.3 ± 11.9	16.8 ± 15.2	22.8 ± 18.7
BMI	22.4 ± 1.6	23.0 ± 2.4	24.7 ± 3.3	27.4 ± 4.9	29.9 ± 5.7	30.7 ± 6.0	31.0 ± 5.7	33.3 ± 4.7
Waist circumference, cm	73.8 ± 5.8	75.7 ± 7.3	79.7 ± 9.1	86.3 ± 12.2	92.4 ± 14.0	94.6 ± 14.5	95.9 ± 14.0	102.1 ± 12.0
Smoking, cigarettes/day	0.0 ± 0.0	0.2 ± 1.2	0.9 ± 3.1	1.9 ± 4.6	3.6 ± 6.2	6.8 ± 8.3	14.6 ± 11.0	18.8 ± 6.7
Coffee, cups/day	3.1 ± 2.5	3.2 ± 2.4	3.5 ± 2.4	3.8 ± 2.4	4.0 ± 2.6	4.2 ± 2.9	4.9 ± 3.1	4.5 ± 2.9
Physical activity, number of exercises per week	3.7 ± 1.8	3.2 ± 2.1	2.7 ± 2.1	2.4 ± 2.0	2.0 ± 2.0	1.7 ± 1.9	1.3 ± 2.0	0.8 ± 0.8

BMI, body mass index; n, number of observations

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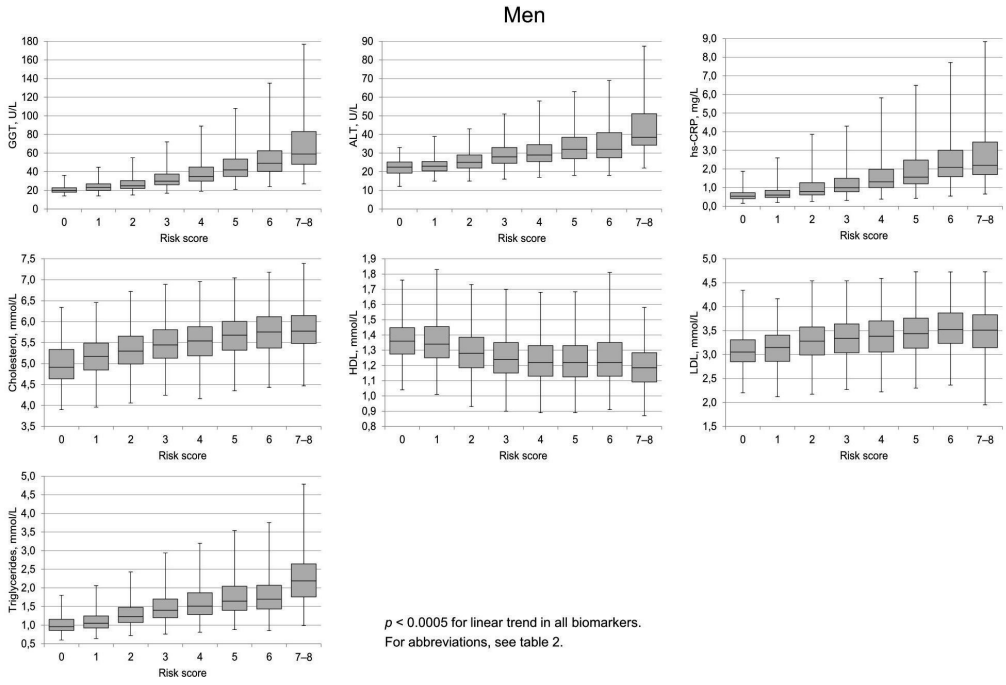
The strongest correlations between the numbers of various unfavourable risk factors and laboratory tests were observed for serum GGT ($r_s = 0.381$ for men; $r_s = 0.311$ for women); ALT ($r_s = 0.252$ for men; $r_s = 0.166$ for women), CRP ($r_s = 0.308$ for men; $r_s = 0.293$ for women) and serum triglycerides ($r_s = 0.274$ for men, $r_s = 0.258$ for women ($p < 0.0001$ for all comparisons).

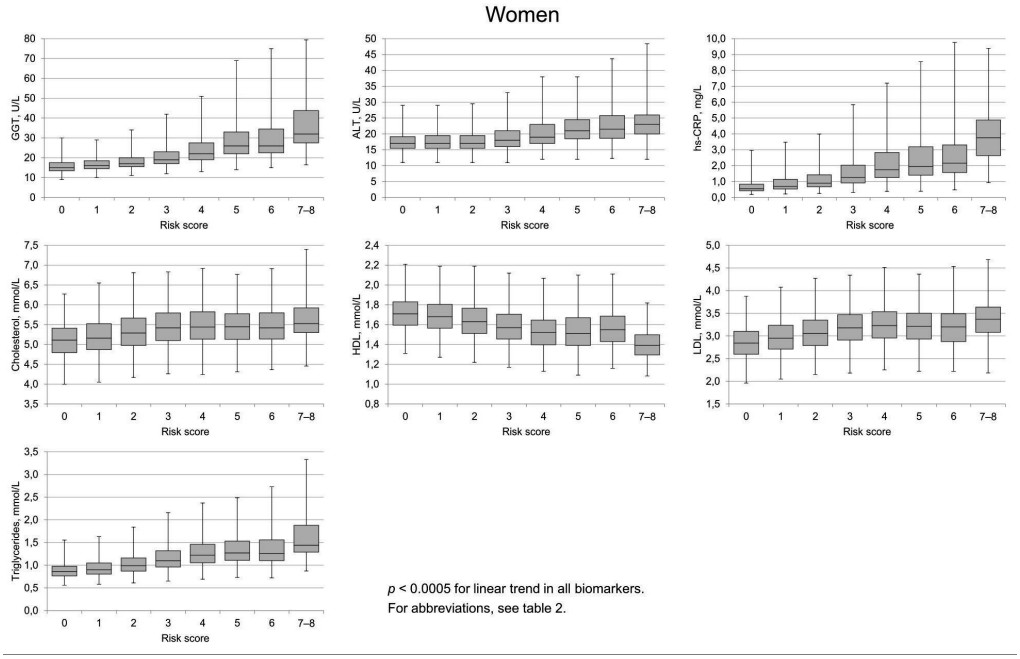
Discussion

The present cross-sectional observational study among a large population-based sample of individuals indicate that unfavourable lifestyle factors increase the risk for abnormalities in biomarkers for liver status, inflammation and lipid profiles in a rather linear and significant manner, which supports the view that profound health benefits could be achieved following the habits of a healthy lifestyle. According to recent observations adherence to favourable lifestyle factors significantly prolongs residual life expectancy [3] and reduces the burden of various chronic diseases [5, 26, 27]. Our data further indicates that laboratory parameters could be used as tools in patient advice and guidance during interventions aimed at achieving a more favourable lifestyle. The biomarkers chosen for the present comparisons appear to be sensitive indicators of adverse biomedical effects related to lifestyle and could therefore also be used in the follow-up of individual patients for long-term maintenance of lifestyle modifications.

Recent findings in lifestyle medicine have indicated that the main determinants for adopting a healthy life style include alcohol drinking in moderation, weight control, not smoking, and taking regular exercise [3, 6, 26, 27]. These studies have also emphasized the benefits of avoiding combinations of unfavourable risk factors, which is also in accordance with the present findings using biomarker levels as outcome measures. Previous studies on alcohol consumption as an individual lifestyle risk factor have recently concluded that regular alcohol



$p < 0.0005$ for linear trend in all biomarkers.
For abbreviations, see table 2.



$p < 0.0005$ for linear trend in all biomarkers.
For abbreviations, see table 2.

Fig 1. Biomarkers of liver function, inflammation and lipid status in individuals with varying lifestyle risk factor status. The data for liver enzymes (GGT, ALT), hs-CRP (biomarker for inflammation) and lipid profiles (cholesterol, HDL, LDL, triglycerides) are shown for both men and women as medians and interquartile ranges. The box represents the middle 50% of the values and the whiskers go down to the 10^{th} percentile and up to the 90^{th}. The scores for the individual risk factors were defined as follows: Alcohol consumption, 0 = no consumption; 1 = alcohol consumption between 1–14 (men) or 1–7 (women) standard drinks per week; 2 = alcohol consumption exceeding 14 drinks (men) or 7 drinks (women) per week Smoking, 0 = no smoking, 1 = 1–19 cigarettes per day, 2 = ≥ 20 cigarettes per day BMI, 0 = BMI < 25; 1 = BMI ≥ 25 and < 30; 2 = ≥ 30 Physical activity, 0 = physical activity over 4 hours per week; 1 = physical activity between 0.5 and 4 hours per week; 2 = physical activity less than 30 min per week. The sum of the above scores provided a total number of risk factors, with higher scores (maximum = 8) indicating an unhealthier lifestyle.

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drinking in amounts exceeding 8 standard drinks per week would lower residual life expectancy at the age of 40 years by 0.5 years, the levels of 30 drinks per week leading to a loss of 4–5 years [26–28]. In individuals with excess body weight even smaller levels of alcohol consumption increase the relative risk of hepatotoxicity, as reflected in elevated liver enzyme activities, fatty changes in the liver and increased rates of mortality due to liver cirrhosis [11, 12, 29]. Previous studies have also reported significant synergistic effect of smoking and alcohol use in increasing liver enzyme activities [30, 31].

Based on current findings lifestyle intervention could be effective when treating patients with liver problems [32–34]. However, the likelihood for a wide variety of other clinical conditions, such as heart diseases, diabetes or cancer are also significantly driven by lifestyle [3, 8, 26, 27]. Typical pathophysiological characteristics associated with lifestyle and disease risks seem to include chronic inflammation, oxidative stress and altered fatty acid metabolism [9, 18, 34]. Thus, it may be expected that systematic measurements of conventional biomarkers reflecting liver status, inflammation and lipid profiles could also offer a significant contribution to the comprehensive assessment of such patients and help in elucidating the mechanisms behind the adverse effects of various behavioural phenotypes. Previously, changes in liver enzyme activities have been shown to be associated with both hepatic and extrahepatic disease risks, including cardio- and cerebrovascular risks, deposition of triglycerides in tissues and the

Table 2. The proportion (%) of abnormal biomarker findings in individuals classified according to the number of life-style associated risk factor scores.

Men									
Risk score	0	1	2	3	4	5	6	7–8	p^a
GGT ≥ 60 U/L	3.7	5.8	8.2	14.7	20.8	29.2	38.2	49.5	< 0.0005
ALT ≥ 50 U/L	1.4	2.8	6.0	11.3	14.7	18.8	25.3	31.8	< 0.0005
CRP-hs ≥ 3 mg/L	4.2	8.9	13.3	16.0	22.0	28.0	33.8	42.1	< 0.0005
Cholesterol ≥ 5 mmol/L	47.0	57.0	64.1	69.0	69.4	73.6	74.7	82.3	< 0.0005
HDL ≤ 1 mmol/L	7.8	9.7	15.9	19.0	21.2	21.0	21.6	25.3	< 0.0005
LDL ≥ 5 mmol/L	52.9	56.3	61.4	64.8	66.3	68.0	73.5	66.9	< 0.0005
Triglycerides ≥ 1.7 mmol/L	12.0	16.8	25.7	35.6	41.4	47.8	50.3	65.7	< 0.0005
Women									
Risk score	0	1	2	3	4	5	6	7–8	p^a
GGT ≥ 40 U/L	5.8	4.7	7.1	11.5	16.4	25.5	30.6	33.3	< 0.0005
ALT ≥ 35 U/L	3.7	5.8	6.0	9.0	12.7	14.2	16.4	11.1	< 0.0005
CRP-hs ≥ 3 mg/L	9.7	12.0	15.1	23.0	33.3	35.8	39.4	58.0	< 0.0005
Cholesterol ≥ 5 mmol/L	54.9	56.9	62.5	67.1	68.9	67.5	67.0	76.5	< 0.0005
HDL ≤ 1.2 mmol/L	6.3	8.9	12.0	16.4	22.1	23.4	20.1	30.0	< 0.0005
LDL ≥ 5 mmol/L	44.5	47.3	53.5	58.8	60.7	60.3	59.9	71.2	< 0.0005
Triglycerides ≥ 1.7 mmol/L	6.7	8.9	12.7	19.7	25.2	29.0	31.0	43.2	< 0.0005

[a], *p* for linear trend

GGT, gamma-glutamyltransferase; ALT, alanine aminotransferase; CRP, C-reactive protein; HDL, high-density lipoproteins; LDL, low-density lipoprotein

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Table 3. Odds ratios (ORs) for abnormal biomarker status according to individual lifestyle risk factor scores, as adjusted for age and coffee consumption.

		Men	Women
	Risk score	OR (95% CI)	OR (95% CI)
GGT	0		
	1	1.5 (0.7 to 3.1)	0.7 (0.4 to 1.1)
	2	2.1 (1.0 to 4.4)[a]	1.0 (0.6 to 1.5)
	3	4.2 (2.1 to 8.7)[c]	1.6 (1.1 to 2.4)[a]
	4	6.7 (3.3 to 13.7)[c]	2.4 (1.6 to 3.7)[c]
	5	10.5 (5.1 to 21.5)[c]	4.6 (3.0 to 7.1)[c]
	6	16.6 (8.0 to 34.4)[c]	6.6 (4.1 to 10.6)[c]
	7–8	26.6 (12.4 to 57.0)[c]	7.0 (3.8 to 13.1)[c]
ALT	0		
	1	2.1 (0.3 to 16.4)	1.6 (0.7 to 3.8)
	2	5.0 (0.7 to 37.2)	1.6 (0.7 to 3.8)
	3	11.3 (1.5 to 82.4)[a]	2.6 (1.1 to 6.0)[a]
	4	15.6 (2.1 to 114.4)[b]	3.8 (1.6 to 8.8)[b]
	5	20.8 (2.8 to 153.0)[b]	4.4 (1.8 to 10.4)[b]
	6	30.0 (4.0 to 222.4)[b]	5.4 (2.1 to 14.1)[c]
	7–8	40.3 (5.3 to 307.8)[c]	3.5 (0.8 to 15.0)
CRP	0		
	1	2.0 (1.0 to 4.0)	1.2 (0.9 to 1.8)
	2	3.0 (1.5 to 5.8)[b]	1.6 (1.2 to 2.3)[b]
	3	3.6 (1.8 to 7.1)[c]	2.7 (2.0 to 3.8)[c]
	4	5.6 (2.8 to 11.0)[c]	4.7 (3.3 to 6.5)[c]
	5	7.9 (4.0 to 15.7)[c]	5.4 (3.8 to 7.6)[c]
	6	11.1 (5.5 to 22.2)[c]	6.6 (4.4 to 9.8)[c]
	7–8	16.2 (7.8 to 33.7)[c]	13.7 (7.9 to 23.7)[c]
Chol	0		
	1	1.4 (1.0 to 1.9)[a]	0.9 (0.7 to 1.1)
	2	1.8 (1.4 to 2.4)[c]	1.0 (0.8 to 1.2)
	3	2.1 (1.6 to 2.8)[c]	1.1 (0.9 to 1.4)
	4	2.2 (1.7 to 3.0)[c]	1.2 (1.0 to 1.5)
	5	2.8 (2.1 to 3.8)[c]	1.2 (0.9 to 1.5)
	6	3.0 (2.1 to 4.2)[c]	1.2 (0.9 to 1.7)
	7–8	4.9 (3.1 to 7.8)[c]	1.9 (1.1 to 3.4)[a]
HDL	0		
	1	1.3 (0.7 to 2.1)	1.5 (1.0 to 2.3)
	2	2.2 (1.3 to 3.6)[b]	2.1 (1.4 to 3.1)[c]
	3	2.7 (1.7 to 4.5)[c]	2.9 (2.0 to 4.4)[c]
	4	3.2 (1.9 to 5.3)[c]	4.2 (2.8 to 6.4)[c]
	5	3.2 (1.9 to 5.3)[c]	4.7 (3.0 to 7.1)[c]
	6	3.3 (1.9 to 5.7)[c]	3.9 (2.4 to 6.4)[c]
	7–8	4.1 (2.3 to 7.5)[c]	6.6 (3.5 to 12.2)[c]
LDL	0		
	1	1.1 (0.8 to 1.6)	1.0 (0.7 to 1.3)
	2	1.4 (1.0 to 1.9)	1.1 (0.9 to 1.4)
	3	1.5 (1.1 to 2.2)[a]	1.3 (1.0 to 1.7)[a]
	4	1.6 (1.2 to 2.3)[b]	1.4 (1.1 to 1.9)[b]

(*Continued*)

Table 3. (Continued)

		Men	Women
	Risk score	OR (95% CI)	OR (95% CI)
	5	1.8 (1.3 to 2.6)[b]	1.4 (1.1 to 1.9)[a]
	6	2.3 (1.5 to 3.4)[c]	1.4 (1.0 to 2.1)
	7–8	1.7 (1.1 to 2.9)[a]	2.5 (1.3 to 4.8)[b]
Trigl	0		
	1	1.5 (0.9 to 2.3)	1.2 (0.8 to 1.8)
	2	2.5 (1.6 to 3.8)[c]	1.6 (1.1 to 2.4)[a]
	3	3.9 (2.6 to 6.0)[c]	2.7 (1.8 to 4.0)[c]
	4	5.1 (3.3 to 7.9)[c]	3.8 (2.5 to 5.5)[c]
	5	6.7 (4.4 to 10.4)[c]	4.8 (3.2 to 7.3)[c]
	6	7.6 (4.8 to 11.9)[c]	5.8 (3.7 to 9.2)[c]
	7–8	14.4 (8.6 to 24.0)[c]	9.7 (5.4 to 17.4)[c]

[a], $p < 0.05$

[b], $p < 0.01$

[c], $p < 0.001$. For abbreviations, see Table 2.

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development of insulin resistance [10, 15, 35, 36]. Based on the present analysis which excluded individuals with clinically apparent diseases at the time of the study the biomarker responses appear to represent early changes in the sequence of events leading from risk exposure to possible disease outcomes. It should further be noted that in this material similar conclusions on a significant linear relationships between the sum of lifestyle risk factors and current biomarker levels were also reached by further exclusions of individuals with any previous history of cardiac or cerebrovascular diseases, chronic inflammatory diseases, diabetes or abnormal oral glucose test (data not shown).

Previous studies have suggested possible mechanistic links between hepatic and extrahepatic disease outcomes, as supported by findings indicating that GGT enzyme is able to fuel LDL oxidation in coronary plaques [37]. In accordance with this view, alcohol and its reactive metabolites are known to exert toxic effects virtually in all tissues and even relatively low levels of chronic drinking may increase the risk for carcinogenesis [38–40], cognitive decline [41, 42], cardiac dysfunction [43–45] and all-cause mortality [28, 46], which may also associate with abnormalities in blood lipid profiles and indices of inflammation [47–49]. Based on the present data abnormalities in serum CRP, a widely used clinical biomarker of inflammation, and lipid profiles appear to follow the burden of unfavourable risk factors and abnormalities in markers of liver function in a sensitive manner. Although CRP alone may be considered as a relatively unspecific biomarker of inflammation, previous studies have shown that CRP levels predict cardiovascular events even in individuals without any atherosclerotic manifestations or conventional risk factors [50, 51]. Evidence also suggests that CRP is an important regulator of inflammatory processes [51].

Physical inactivity and sedentary behaviour are typical characteristics of an unhealthy lifestyle and increasingly common causes of health problems across the world [3, 6, 32, 52–55]. The present biomarker-based data also underscores the benefits of physical activity as an independent and significant part of a favourable lifestyle. The individuals engaged in moderate or vigorous physical activity show significantly lower risks for biomarker abnormalities than the corresponding groups of those with low or sedentary activity even in the presence of other risk factors. The data also supports the view that physical exercise could also be used as a

therapeutic approach to counteract life-style associated adverse metabolic and obesogenic effects and possibly confer long-term benefits to lifestyle-associated disease burden in general [54, 56–58]. Previously, moderate to vigorous physical activity was found to improve the degree of hepatic steatosis in fatty liver disease through reducing inflammation and oxidative stress and altering lipid metabolism even in the absence of any detectable weight reduction [34]. Interestingly, recent UK biobank based study has also concluded that physically active individuals have longer life expectancies across the different levels and indices of adiposity than those with low levels of activity [58].

Based on current data the biomarker responses to factors of lifestyle seem to be significantly driven by their joint effects. However, it should be emphasized that there may also be other types of unhealthy behaviours, such as particular dietary patterns, which may contribute to adverse health effects [3, 8, 26, 27]. Unfortunately, in this work we did not have sufficient information available on the exact compositions of the diet. Here the unfavourable lifestyle factors were, however, found to be associated with an increasing trend of coffee consumption in the high risk subgroups, which is in accordance with previous observations indicating that heavy smoking may be related with increased coffee intake [59]. Interestingly, coffee consumption has been previously shown to be associated with a reduced risk for both all-cause and cause-specific mortality [60]. Lower levels of liver-derived enzymes have also been found to occur in alcohol consumers with high levels of coffee consumption when compared to those with no coffee consumption suggesting possible hepatoprotective effects of coffee intake [12, 60].

Previous work has also emphasized the role of high-fat diets in aggravating inflammation, oxidative stress and metabolic aberrations [18–20]. High carbohydrate and processed/red meat consumption together with insufficient vegetable, fruit or vitamin intake are other important dietary components which may associate with adverse metabolic and hepatic effects [18, 26, 27, 32, 61]. Thus, the individual assessment of health risks should include considerations of the quality of the diet which may include several synergistic triggers for adverse health effects, as also previously reported from both experimental animal models [20] and human studies [12, 13, 18, 62–67]. In real life situations simultaneous adherence to several low-risk lifestyle-related factors may, however, be difficult. Thus, there is an obvious need for improved national health policies emphasizing tools for health care outcome measurements. The present findings suggest a possible expanded role for clinical laboratory information in the follow-up of patients presenting with unfavourable lifestyle risk factors.

Following previous work on lifestyle factors and health risks [3], we used BMI here as a part of the risk factor scoring system instead of using it as a covariate. This may be justified to prevent over-adjustment due to controlling for a variable which may be on a causal pathway between exposure and outcome. In this work the lack of information on the quality of the diet may further support the choice of using BMI as part of the lifestyle-related index. This approach was also supported by additional analyses using BMI as a covariate where similar conclusions were also reached on a linear relationships between the sum of lifestyle risk factors and biomarker levels, except for a lack of significance for HDL-cholesterol in men and for HDL-, LDL- and total cholesterol in women.

The strengths of this study include the large number of study subjects and a comprehensive assessment of various lifestyle risk factors together with several biomarkers. The study also included separate assessments for both genders. Nevertheless, our study has some potential limitations. Due to the observational and cross-sectional nature of the study and lack of follow-up data it is difficult to derive any causal relationships. The lifestyle factors were self-reported and thus underreporting and biased recall may occur particularly in the parameters pertaining to less socially desirable behaviours. The association between the current risk

factors, the quality of the diet and biomarker responses clearly warrant future studies in large follow-up materials. Future studies are also needed to examine the effect of lifestyle factors on indices of inflammation using a wider selection of biomarkers.

Nevertheless, our study demonstrates previously unrecognized relationships between life style risk factors and biomarker abnormalities, which may prove to be useful in public health recommendations. The data also suggests a potential for using biomarker-based algorithms in a comprehensive assessment of interventions aimed at reducing the risks, which based on recent findings seem to have a major impact on life expectancies and disease outcomes.

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References

1. Behrens G, Fischer B, Kohler S, Park Y, Hollenbeck AR, Leitzmann MF. Healthy lifestyle behaviors and decreased risk of mortality in a large prospective study of U.S. women and men. Eur J Epidemiol. 2013; 28: 361–372. https://doi.org/10.1007/s10654-013-9796-9 PMID: 23532745
2. Connor JP, Haber PS, Hall WD. Alcohol use disorders. Lancet. 2016; 387: 988–998. https://doi.org/10.1016/S0140-6736(15)00122-1 PMID: 26343838
3. Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018; 138: 345–355. https://doi.org/10.1161/CIRCULATIONAHA.117.032047 PMID: 29712712
4. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012; 380: 2224–2260. https://doi.org/10.1016/S0140-6736(12)61766-8 PMID: 23245609
5. Rutten-Jacobs LC, Larsson SC, Malik R, Rannikmae K, Sudlow CL, Dichgans M et al. Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: cohort study of 306 473 UK Biobank participants. BMJ. 2018; 363: k4168. https://doi.org/10.1136/bmj.k4168 PMID: 30355576

6. Romaguera D, Tauler P, Bennasar M, Pericas J, Moreno C, Martinez S et al. Determinants and patterns of physical activity practice among Spanish university students. J Sports Sci. 2011; 29: 989–997. https://doi.org/10.1080/02640414.2011.578149 PMID: 21590579
7. McGinnis M, Williams-Russo P, Knickman JR. The case for more active policy attention to health promotion. Health Affairs. 2002; 21: 78–93. https://doi.org/10.1377/hlthaff.21.2.78 PMID: 11900188
8. Tamakoshi A, Tamakoshi K, Lin Y, Yagyu K, Kikuchi S, JACC Study Group. Healthy lifestyle and preventable death: findings from the Japan Collaborative Cohort (JACC) Study. Prev Med. 2009; 48: 486–492. https://doi.org/10.1016/j.ypmed.2009.02.017 PMID: 19254743
9. Danielsson J, Kangastupa P, Laatikainen T, Aalto M, Niemelä O. Impacts of common factors of life style on serum liver enzymes. World J Gastroenterol. 2014; 20: 11743–11752. https://doi.org/10.3748/wjg.v20.i33.11743 PMID: 25206278
10. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 2008; 47: 1363–1370. https://doi.org/10.1002/hep.22109 PMID: 18366115
11. Lau K, Baumeister SE, Lieb W, Meffert PJ, Lerch MM, Mayerle J et al. The combined effects of alcohol consumption and body mass index on hepatic steatosis in a general population sample of European men and women. Aliment Pharmacol Ther. 2015; 41: 467–476. https://doi.org/10.1111/apt.13067 PMID: 25588768
12. Niemelä O, Niemelä M, Bloigu R, Aalto M, Laatikainen T. Where should the safe limits of alcohol consumption stand in light of liver enzyme abnormalities in alcohol consumers? PLoS One. 2017; 12: e0188574. https://doi.org/10.1371/journal.pone.0188574 PMID: 29206836
13. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology. 2009; 136: 477–485. https://doi.org/10.1053/j.gastro.2008.10.052 PMID: 19100265
14. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ III. Serum aminotransferase activity and mortality risk in a United States community. Hepatology. 2008; 47: 880–887. https://doi.org/10.1002/hep.22090 PMID: 18302294
15. Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H et al. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation. 2005; 112: 2130–2137. https://doi.org/10.1161/CIRCULATIONAHA.105.552547 PMID: 16186419
16. Latvala J, Hietala J, Koivisto H, Järvi K, Anttila P, Niemelä O. Immune responses to ethanol metabolites and cytokine profiles differentiate alcoholics with or without liver disease. Am J Gastroenterol. 2005; 100: 1303–1310. https://doi.org/10.1111/j.1572-0241.2005.41509.x PMID: 15929761
17. Oliveira A, Rodríguez-Artalejo F, Lopes C. Alcohol intake and systemic markers of inflammation—shape of the association according to sex and body mass index. Alcohol Alcohol. 2010; 45: 119–125. https://doi.org/10.1093/alcalc/agp092 PMID: 20083478
18. Zheng JS, Sharp SJ, Imamura F, Koulman A, Schulze MB, Ye Z et al. Association between plasma phospholipid saturated fatty acids and metabolic markers of lipid, hepatic, inflammation and glycaemic pathways in eight European countries: a cross-sectional analysis in the EPIC-InterAct study. BMC Med. 2017; 15: 203. https://doi.org/10.1186/s12916-017-0968-4 PMID: 29145892
19. Caro AA, Cederbaum AI. Oxidative stress, toxicology, and pharmacology of CYP2E1. Annu Rev Pharmacol Toxicol. 2004; 44: 27–42. https://doi.org/10.1146/annurev.pharmtox.44.101802.121704 PMID: 14744237
20. Tsukamoto H, Horne W, Kamimura S, Niemelä O, Parkkila S, Ylä-Herttuala S et al. Experimental liver cirrhosis induced by alcohol and iron. J Clin Invest. 1995; 96: 620–630. https://doi.org/10.1172/JCI118077 PMID: 7615836
21. Hao G, Wang Z, Zhang L, Chen Z, Wang X, Guo M et al. Relationship between alcohol consumption and serum lipid profiles among middle-aged population in China: a multiple-center cardiovascular epidemiological study. Angiology. 2015; 66: 753–758. https://doi.org/10.1177/0003319714549557 PMID: 25192699
22. Wang HJ, Gao B, Zakhari S, Nagy LE. Inflammation in alcoholic liver disease. Annu Rev Nutr. 2012; 32: 343–368. https://doi.org/10.1146/annurev-nutr-072610-145138 PMID: 22524187
23. The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988; 41: 105–114. https://doi.org/10.1016/0895-4356(88)90084-4 PMID: 3335877
24. Kuulasmaa K, Tolonen H, Cepaitis Z, Laatikainen T, Nissinen A, Vartiainen E et al. European health risk monitoring project. Finnish National Public Health Institute (KTL), Helsinki. 2006. Available from: www.thl.fi/ehrm (11.9.2018).

25. Luepker RV, Evans A, McKeigue P, Srinath Reddy K. Cardiovascular survey methods. World Health Organization WHO), Geneva. 2004. Available from: https://apps.who.int/iris/handle/10665/42569 (18.10.2018).
26. Li K, Hüsing A, Kaaks R. Lifestyle risk factors and residual life expectancy at age 40: a German cohort study. BMC Med. 2014; 12: 59. https://doi.org/10.1186/1741-7015-12-59 PMID: 24708705
27. Manuel DG, Perez R, Sanmartin C, Taljaard M, Hennessy D, Wilson K et al. Measuring burden of unhealthy behaviours using a multivariable predictive approach: life expectancy lost in Canada attributable to smoking, alcohol, physical inactivity, and diet. PLoS Med. 2016; 13: e1002082. https://doi.org/10.1371/journal.pmed.1002082 PMID: 27529741
28. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 2018; 391: 1513–1523. https://doi.org/10.1016/S0140-6736(18)30134-X PMID: 29676281
29. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999–2016: observational study. BMJ. 2018; 362: k2817. https://doi.org/10.1136/bmj.k2817 PMID: 30021785
30. Breitling LP, Raum E, Müller H, Rothenbacher D, Brenner H. Synergism between smoking and alcohol consumption with respect to serum gamma-glutamyltransferase. Hepatology. 2009; 49: 802–808. https://doi.org/10.1002/hep.22727 PMID: 19152425
31. Park EY, Lim MK, Oh JK, Cho H, Bae MJ, Yun EH et al. Independent and supra-additive effects of alcohol consumption, cigarette smoking, and metabolic syndrome on the elevation of serum liver enzyme levels. PLoS One. 2013; 8: e63439. https://doi.org/10.1371/journal.pone.0063439 PMID: 23667618
32. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol. 2017; 67: 829–846. https://doi.org/10.1016/j.jhep.2017.05.016 PMID: 28545937
33. Teeriniemi AM, Salonurmi T, Jokelainen T, Vähänikkilä H, Alahäivälä T, Karppinen P et al. A randomized clinical trial of the effectiveness of a Web-based health behaviour change support system and group lifestyle counselling on body weight loss in overweight and obese subjects: 2-year outcomes. J Intern Med. 2018; 284: 534–545. https://doi.org/10.1111/joim.12802 PMID: 29974563
34. Oh S, Shida T, Yamagishi K, Tanaka K, So R, Tsujimoto T et al. Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: a retrospective study. Hepatology. 2015; 61: 1205–1215. https://doi.org/10.1002/hep.27544 PMID: 25271091
35. Kazemi-Shirazi L, Endler G, Winkler S, Schickbauer T, Wagner O, Marsik C. Gamma glutamyltransferase and long-term survival: is it just the liver? Clin Chem. 2007; 53: 940–946. https://doi.org/10.1373/clinchem.2006.081620 PMID: 17384006
36. Niemelä O. Biomarker-based approaches for assessing alcohol use disorders. Int J Environ Res Public Health. 2016; 13: 166. https://doi.org/10.3390/ijerph13020166 PMID: 26828506
37. Kozakova M, Palombo C, Eng MP, Dekker J, Flyvbjerg A, Mitrakou A et al. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. Hepatology. 2012; 55: 1406–1415. https://doi.org/10.1002/hep.25555 PMID: 22334565
38. Bagnardi V, Rota M, Botteri E, Tramacere I, Islami F, Fedirko V et al. Light alcohol drinking and cancer: a meta-analysis. Ann Oncol. 2013; 24: 301–308. https://doi.org/10.1093/annonc/mds337 PMID: 22910838
39. Cao Y, Willett WC, Rimm EB, Stampfer MJ, Giovannucci EL. Light to moderate intake of alcohol, drinking patterns, and risk of cancer: results from two prospective US cohort studies. BMJ. 2015; 351: h4238. https://doi.org/10.1136/bmj.h4238 PMID: 26286216
40. Choi YJ, Myung SK, Lee JH. Light alcohol drinking and risk of cancer: a meta-analysis of cohort studies. Cancer Res Treat. 2018; 50: 474–487. https://doi.org/10.4143/crt.2017.094 PMID: 28546524
41. Schwarzinger M, Pollock BG, Hasan OSM, Dufouil C, Rehm J, QalyDays Study Group. Contribution of alcohol use disorders to the burden of dementia in France 2008–13: a nationwide retrospective cohort study. Lancet Public Health. 2018; 3: e124–e132. https://doi.org/10.1016/S2468-2667(18)30022-7 PMID: 29475810
42. Topiwala A, Allan CL, Valkanova V, Zsoldos E, Filippini N, Sexton C et al. Moderate alcohol consumption as risk factor for adverse brain outcomes and cognitive decline: longitudinal cohort study. BMJ. 2017; 357: j2353. https://doi.org/10.1136/bmj.j2353 PMID: 28588063
43. Catena C, Colussi G, Verheyen ND, Novello M, Fagotto V, Soardo G et al. Moderate alcohol consumption is associated with left ventricular diastolic dysfunction in nonalcoholic hypertensive patients. Hypertension. 2016; 68: 1208–1216. https://doi.org/10.1161/HYPERTENSIONAHA.116.08145 PMID: 27672023
44. Klatsky AL. Alcohol and cardiovascular diseases: where do we stand today? J Intern Med. 2015; 278: 238–250. https://doi.org/10.1111/joim.12390 PMID: 26158548

45. McManus DD, Yin X, Gladstone R, Vittinghoff E, Vasan RS, Larson MG et al. Alcohol consumption, left atrial diameter, and atrial fibrillation. J Am Heart Assoc. 2016; 5: e004060. https://doi.org/10.1161/JAHA.116.004060 PMID: 27628571

46. Sipilä P, Rose RJ, Kaprio J. Drinking and mortality: long-term follow-up of drinking-discordant twin pairs. Addiction. 2016; 111: 245–254. https://doi.org/10.1111/add.13152 PMID: 26359785

47. Libby P, Ridker PM, Hansson GK, Leducq Transatlantic Network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009; 54: 2129–2138. https://doi.org/10.1016/j.jacc.2009.09.009 PMID: 19942084

48. Moradi H, Streja E, Kalantar-Zadeh K. Serum high density lipoprotein cholesterol level and risk of death: let's avoid the extremes. J Thorac Dis. 2017; 9: 4849–4852. https://doi.org/10.21037/jtd.2017.10.155 PMID: 29312674

49. Ridker PM, Everett BM, Thuren T, MacFadyen JG, Chang WH, Ballantyne C et al. Antiinflammatory therapy with canakinumab for atherosclerotic disease. N Engl J Med. 2017; 377: 1119–1131. https://doi.org/10.1056/NEJMoa1707914 PMID: 28845751

50. Koenig W. C-reactive protein and cardiovascular risk: will the controversy end after CANTOS? Clin Chem. 2017; 63: 1897–1898. https://doi.org/10.1373/clinchem.2017.273243 PMID: 28931518

51. Sproston NR, Ashworth JJ. Role of C-reactive protein at sites of inflammation and infection. Front Immunol. 2018; 9: 754. https://doi.org/10.3389/fimmu.2018.00754 PMID: 29706967

52. Sundberg CJ. Physical activity: what is already being done and how we can avert 1 million deaths annually in future. Br J Sports Med. 2016; 50: 319. https://doi.org/10.1136/bjsports-2016-096026 PMID: 26936163

53. Warburton DE, Bredin SS. Reflections on physical activity and health: what should we recommend? Can J Cardiol. 2016; 32: 495–504. https://doi.org/10.1016/j.cjca.2016.01.024 PMID: 26995692

54. Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K et al. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the Global Burden of Disease Study 2013. BMJ. 2016; 354: i3857. https://doi.org/10.1136/bmj.i3857 PMID: 27510511

55. Smith AD, Crippa A, Woodcock J, Brage S. Physical activity and incident type 2 diabetes mellitus: a systematic review and dose-response meta-analysis of prospective cohort studies. Diabetologia. 2016; 59: 2527–2545. https://doi.org/10.1007/s00125-016-4079-0 PMID: 27747395

56. Lawlor DA, Sattar N, Smith GD, Ebrahim S. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. Am J Epidemiol. 2005; 161: 1081–1088. https://doi.org/10.1093/aje/kwi125 PMID: 15901629

57. Perreault K, Bauman A, Johnson N, Britton A, Rangul V, Stamatakis E. Does physical activity moderate the association between alcohol drinking and all-cause, cancer and cardiovascular diseases mortality? A pooled analysis of eight British population cohorts. Br J Sports Med. 2017; 51: 651–657. https://doi.org/10.1136/bjsports-2016-096194 PMID: 27581162

58. Zaccardi F, Davies MJ, Khunti K, Yates T4. Comparative relevance of physical fitness and adiposity on life expectancy: a UK biobank observational study. Mayo Clin Proc. 2019;Epub ahead of print. https://doi.org/10.1016/j.mayocp.2018.10.029 PMID: 31079962

59. Bjørngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafò MR et al. Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. Int J Epidemiol. 2017; 46: 1958–1967. https://doi.org/10.1093/ije/dyx147 PMID: 29025033

60. Gunter MJ, Murphy N, Cross AJ, Dossus L, Dartois L, Fagherazzi G et al. Coffee drinking and mortality in 10 European countries: a multinational cohort study. Ann Intern Med. 2017; 167: 236–247. https://doi.org/10.7326/M16-2945 PMID: 28693038

61. Halsted CH, Villanueva JA, Devlin AM, Niemelä O, Parkkila S, Garrow TA et al. Folate deficiency disturbs hepatic methionine metabolism and promotes liver injury in the ethanol-fed micropig. Proc Natl Acad Sci U S A. 2002; 99: 10072–10077. https://doi.org/10.1073/pnas.112336399 PMID: 12122204

62. Day CP, James OF. Steatohepatitis: a tale of two "hits"? Gastroenterology. 1998; 114: 842–845. https://doi.org/10.1016/S0016-5085(98)70599-2 PMID: 9547102

63. Fraser A, Harris R, Sattar N, Ebrahim S, Davey Smith G, Lawlor DA. Alanine aminotransferase, gamma-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. Diabetes Care. 2009; 32: 741–750. https://doi.org/10.2337/dc08-1870 PMID: 19131466

64. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology. 2010; 52: 1156–1161. https://doi.org/10.1002/hep.23789 PMID: 20658466

65. Joshi PK, Pirastu N, Kentistou KA, Fischer K, Hofer E, Schraut KE et al. Genome-wide meta-analysis associates HLA-DQA1/DRB1 and LPA and lifestyle factors with human longevity. Nat Commun. 2017; 8: 910. https://doi.org/10.1038/s41467-017-00934-5 PMID: 29030599

66. Liu B, Balkwill A, Reeves G, Beral V, Million Women Study Collaborators. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ. 2010; 340: c912. https://doi.org/10.1136/bmj.c912 PMID: 20223875

67. Tsai J, Ford ES, Zhao G, Li C, Greenlund KJ, Croft JB. Co-occurrence of obesity and patterns of alcohol use associated with elevated serum hepatic enzymes in US adults. J Behav Med. 2012; 35: 200–210. https://doi.org/10.1007/s10865-011-9353-5 PMID: 21626151

PUBLICATION IV

Combined effects of lifestyle risk factors on fatty liver index

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RESEARCH ARTICLE Open Access

Combined effects of lifestyle risk factors on fatty liver index

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Abstract

Background: Factors of lifestyle may have a major impact on liver-related morbidity and mortality.
We examined independent and joint effects of lifestyle risk factors on fatty liver index (FLI), a biomarker of hepatic steatosis, in a population-based cross-sectional national health survey.

Methods: The study included 12,368 participants (5784 men, 6584 women) aged 25–74 years. Quantitative estimates of alcohol use, smoking, adiposity and physical activity were used to establish a total score of risk factors, with higher scores indicating an unhealthier lifestyle. FLI was calculated based on an algorithm including body mass index, waist circumference, serum gamma-glutamyltransferase and triglycerides.

Results: The occurrence of FLI ≥ 60% indicating fatty liver increased from 2.4% in men with zero risk factors to 81.9% in those with a total risk score of 7–8 ($p < 0.0005$ for linear trend) and in women from 0 to 73.5% ($p < 0.0005$). The most striking individual impacts on the likelihood for FLI above 60% were observed for physical inactivity ($p < 0.0005$ for both genders) and alcohol consumption ($p < 0.0005$ for men). Interestingly, coffee consumption was also found to increase with increasing risk factor scores ($p < 0.0005$ for linear trend in both genders).

Conclusions: The data indicates that unfavorable combinations of lifestyle risk factors lead to a high likelihood of hepatic steatosis. Use of FLI as a diagnostic tool may benefit the assessment of interventions aimed at maintaining a healthy lifestyle and prevention of liver-related morbidity.

Keywords: Alcohol, NAFLD, Obesity, Physical activity, Steatosis

Background

Excessive alcohol use, smoking, and lack of physical activity are typical risk factors of lifestyle, which may contribute to adiposity, fatty deposition in the liver and increased all-cause mortality [1–4]. Furthermore, several risk factors are often present concomitantly in the same individual [5, 6]. Recent studies have concluded that simultaneous adherence to multiple healthy lifestyle factors could significantly prolong life expectancy suggesting substantial therapeutic implications for interventions focusing on basic lifestyle factors [1, 7, 8].

In current societies, hepatic steatosis is a highly common manifestation of health problems driven by behavioral factors. Building of too much fat in the liver may lead to a wide variety of clinical symptoms ranging from asymptomatic increases in biomarkers of liver function to liver cirrhosis [2, 9–11]. Recent studies have indicated that elevated alanine aminotransferase (ALT) and gamma-glutamyltransferase (GGT) activities are common in obese individuals with mild to moderate alcohol consumption suggesting cumulative hepatotoxic effects for adiposity and alcohol use [6, 9, 10, 12–14]. Smoking

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together with alcohol use may also have synergistic effects in increasing the odds of abnormal GGT levels [15, 16]. The increases in liver enzymes under such conditions also appear to associate with systemic inflammation, abnormal lipid status and increased risk for both hepatic and extra-hepatic complications, including cardio- and cerebrovascular diseases [14, 17–19].

Recent advances in research on liver diseases have led to the introduction of various algorithms designed for assessing individual disease risks in a non-invasive manner. Fatty liver index (FLI) is an algorithm designed for the prediction of fatty liver, which in previous external validation studies involving comparisons with ultrasonography data, has been shown to be more accurate for the identification of fatty liver than any of the conventional biomarkers of liver function [11, 20]. So far, no data have, however, been available on the impacts of unhealthy behaviors on FLI. In this work, we aimed to investigate the individual and joint effects of various lifestyle risk factors on FLI in a large Finnish population-based cohort (the National FINRISK study) encompassing detailed records on alcohol use, smoking habits, physical activity and other health-related behavior. Improved knowledge on the associations between FLI, as a proxy for fatty liver, and various risk factors of lifestyle may be assumed to provide new tools for clinical management and counseling regarding factors of lifestyle in patients with suspected hepatic steatosis.

Methods

Study design

Data from a cross-sectional population health survey (The National FINRISK Study) carried out in six geographical areas in Finland in years 1997, 2002 and 2007 were used [13, 21, 22]. The material includes a nationally representative age- and gender stratified sample, which was drawn from the population register according to an international protocol [21]. Clinical examinations comprised physical measurements, laboratory analyses and detailed questionnaires encompassing alcohol intake, smoking, coffee consumption, physical activity, medical history, current health status and socioeconomic factors [21, 22]. Body mass index (BMI, kg/m^2) was calculated as an index of relative body weight based on body weight and height, which were measured to the nearest 0.1 kg and 0.1 cm, respectively. Waist circumference (to the nearest 0.5 cm) was obtained from the measurements between the lowest rib and iliac crest while the study subject was at minimal respiration.

Data on alcohol use from the past 12 months was collected through questionnaires gathering information on the types of beverages, the frequency of consumption, and the amounts of each type of ethanol-containing standard drink (corresponding to 12 g of ethanol) [18]. Information on smoking was gathered with standardized questionnaires and the data was given as the number of cigarettes per day. Leisure-time physical activity including the number and total time used for physical exercises were registered using specifically designed structured questionnaires, as previously described [21, 22]. Coffee consumption as derived from the sets of standardized questions were expressed as the amounts of standard coffee servings (cups) per day.

The responses to each question on alcohol consumption, smoking, physical activity and coffee consumption were assigned to mutually exclusive and collectively exhaustive categories [21, 22]. The data was subsequently used to categorize the subjects into three ordinal levels to define scores for low risk (= 0), medium risk (= 1) and high risk (= 2) for each lifestyle factor, as previously described [1, 13]. For scoring alcohol consumption the currently recommended national limits of low-risk alcohol consumption were followed: 0 = no consumption; 1 = alcohol consumption between 1 and 14 (men) or 1–7 (women) standard drinks per week (low risk consumption); 2 = alcohol consumption exceeding 14 drinks (men) or 7 drinks (women) per week (high risk consumption). For smoking 0 = no smoking, 1 = 1–19 cigarettes per day, 2 = ≥ 20 cigarettes per day; for BMI 0 = < 25; 1 = ≥ 25 and < 30 (overweight); 2 = ≥ 30 (obesity). For physical activity, score = 0 refers to those with physical activity over 4 h per week; 1 = physical activity between 0.5 and 4 h per week and 2 = physical activity less than 30 min/week. The sum of the above scores provided the total number of risk factors, with higher scores indicating an unhealthier lifestyle.

The data was available from 12,368 participants (5784 men, 6584 women, mean age 49 ± 13 years, range 25–74 years) who completed the questionnaires and attended the medical examination. The study excluded individuals with any apparent clinical signs of liver disease, diabetes or abnormal oral glucose test, ischemic heart or brain disease, chronic inflammatory diseases, malignancy or active infection at the time of blood sampling. The investigation was performed with the understanding and written informed consent of each individual and was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. All surveys were conducted in accordance with the Declaration of Helsinki according to the ethical rules of the National Public Health Institute.

Laboratory analyses

Serum ALT and GGT were analyzed by standard clinical chemical methods on an Abbott Architect analyzer following the instructions of the manufacturer (Abbott Laboratories, Abbott Park, IL, USA).

Assays of high-sensitivity C-reactive protein (CRP) were carried out using a latex immunoassay (Sentinel Diagnostics, Milan, Italy) on Abbott Architect c8000 analyzer. Determinations of total cholesterol, high-density lipoprotein-associated cholesterol (HDL), low-density lipoprotein associated cholesterol (LDL) and total triglycerides were based on standard enzymatic methods. All laboratory tests were subjects to continuous external quality control programs organized by Labquality, Finland and CDC (Center for Disease Control and Prevention) quality assurance and standardization program for serum lipids. The cut-offs for the normal limits of the parameters were as follows: ALT (50 U/L men; 35 U/L women), GGT (60 U/L men; 40 U/L women), CRP (3.0 mg/L), cholesterol (5 mmol/L), HDL cholesterol (1.0 mmol/L men, 1.2 mmol/L women), LDL cholesterol (3.0 mmol/L), triglycerides (1.7 mmol/L).

Fatty liver index

Fatty liver index is a predictor algorithm for fatty liver disease, which was computed based on BMI, waist circumference, triglycerides and GGT, as previously described by Bedogni and coworkers [20]. In this algorithm, FLI scores below 30 exclude fatty liver, scores below 30 and 60 remain inconclusive whereas scores of 60 and above indicate that fatty liver is present [20].

Statistical methods

The study variables are reported as mean ± standard deviation (SD) or geometric means with 95% confidence intervals, as indicated. For parameters with skewed distributions a logarithmic transformation was performed. Comparisons between the variables were carried out using analysis of variance (ANOVA) with polynomial contrasts to reveal possible trends across the ordinally increasing risk score categories. The distribution of findings exceeding the cut-offs for FLI and other biomarkers in various risk categories were analyzed by chi-square test for trend. Multinomial logistic regression was used to estimate the odds for abnormal FLI according to the individual number of lifestyle risk factor scores, adjusting for BMI, age and coffee consumption. To evaluate the individual impact of the lifestyle risk factors as predictors of abnormal FLI (≥ 60) multivariate binary logistic regression with likelihood ratio test was performed and estimates are presented as odds ratios (OR). The differences in proportions between men and women were tested using Pearson chi-square test and Fisher's exact test as appropriate. Correlations between the study variables were calculated using Spearman's rank correlation coefficients. The analyses were carried out with IBM SPSS Statistics 24.0 (Armonk, NY: IBM Corp.). A p-value < 0.05 was considered statistically significant.

Results

Table 1 summarizes the main clinical characteristics of the subjects classified according to the score of lifestyle risk factors and gender. Higher quantities of alcohol intake, excess body weight, higher levels of cigarette smoking and physical inactivity were found to characterize the individuals with increased risk scores. In men, there was a quadratic trend between age and ordinal lifestyle risk score categories, the highest mean ages being noted in the middle section of the risk categories ($p < 0.01$) whereas in women a linear trend was observed ($p < 0.0005$). There was also a significant association between coffee consumption and increasing risk factor scores ($p < 0.0005$ for linear trend in both genders). Among the individual components of the risk factor score, a significant association was found to exist between coffee consumption and smoking status. Coffee consumption ≥4 cups/day was found in 52.3% of non-smokers, 70.9% of those smoking 1–19 cigarettes per day and in 84.4% of those smoking ≥20 cigarettes/day ($p < 0.0005$).

The data on the clinical and laboratory parameters in subgroups with different lifestyle risk factor status are summarized in Table 2. The proportions of individuals with FLI ≥ 60 (indicating that fatty liver is present) and the percentages of individuals exceeding the reference limits in the individual components of the FLI (BMI, waist circumference, serum triglycerides and GGT) as well as in biomarkers of liver function (ALT), inflammation (CRP) and lipid status (cholesterol, HDL-cholesterol, LDL-cholesterol) are also shown. Distinct dose-response relationships were observed between the number of unfavorable risk factors, FLI levels and biomarker data in all comparisons. In those with zero risk factors FLI below 30 (ruling out fatty liver) was observed in 87.5% of men and 98.5% of women (Fig. 1). While in both genders the increase in the amount of risk factors was found to lead to a sharp increase in the prevalence of FLI 60 or above suggesting fatty liver, the changes among men were found to occur in a more sensitive manner ($p < 0.0005$ for differences in proportions) (Fig. 1).

Figure 2 demonstrates the rates of abnormal FLI results in the study population classified according to risk factor scores based on alcohol consumption, smoking and physical inactivity as independent individual components of risk factor classification (score range 0–6). In comparisons to those with zero risk factors, a significant increase in the occurrence of abnormal FLI was found in those with one or more risk factors ($p < 0.0005$ for all comparisons). In these analyses, the FLI responses were also found to be more pronounced among men. The data on multinomial logistic regression analysis for increased FLI, as adjusted for BMI, age and coffee

Table 1 Main characteristics of the study population, as categorized to subgroups according to the number of lifestyle risk factor scores

Men								
Risk score	0	1	2	3	4	5	6	7–8
N (%)	168 (2.9)	740 (12.8)	1392 (24.1)	1413 (24.4)	1068 (18.5)	615 (10.6)	294 (5.1)	94 (1.6)
Age, years, mean ± SD	41.8 ± 13.8	44.1 ± 13.4	45.5 ± 13.6	46.2 ± 12.9	44.1 ± 12.2	45.4 ± 11.7	44.4 ± 11.0	43.7 ± 9.7
Alcohol use, g/day	0.0 ± 0.0	4.9 ± 6.5	7.8 ± 9.0	11.5 ± 13.8	17.0 ± 19.1	23.6 ± 26.4	34.2 ± 30.5	44.7 ± 30.5
Smoking, cigarettes/day	0.0 ± 0.0	0.3 ± 1.7	1.2 ± 3.7	3.3 ± 6.7	7.2 ± 9.5	13.1 ± 11.0	18.9 ± 11.7	23.8 ± 8.5
Body mass index	23.1 ± 1.3	23.9 ± 2.0	25.3 ± 2.7	26.6 ± 3.1	27.5 ± 4.0	28.2 ± 4.3	28.6 ± 4.9	30.8 ± 3.7
Waist circumference, cm	82.5 ± 5.7	86.0 ± 6.7	89.8 ± 8.4	94.1 ± 9.1	96.3 ± 11.3	98.7 ± 11.9	100.2 ± 12.9	105.8 ± 11.1
Physical activity, exercises/week	4.1 ± 1.8	3.4 ± 1.9	2.8 ± 1.9	2.3 ± 2.0	1.7 ± 1.7	1.4 ± 1.7	1.3 ± 2.3	0.6 ± 0.9
Coffee, cups/day	3.7 ± 2.8	3.9 ± 2.9	4.1 ± 2.8	4.7 ± 3.1	5.3 ± 3.3	5.9 ± 3.8	6.0 ± 4.1	6.7 ± 4.7
Women								
Risk score	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7–8**
N (%)	338 (5.1)	1286 (19.5)	1877 (28.5)	1596 (24.2)	923 (14.0)	391 (5.9)	139 (2.1)	34 (0.5)
Age, years, mean ± SD	39.6 ± 11.9	42.3 ± 12.7	44.0 ± 12.6	45.1 ± 12.4	44.9 ± 12.5	44.3 ± 11.1	44.5 ± 10.4	47.0 ± 11.6
Alcohol consumption, g/day	0.0 ± 0.0	2.2 ± 3.4	3.9 ± 5.5	5.3 ± 7.1	7.7 ± 8.8	13.8 ± 12.3	15.6 ± 13.2	19.4 ± 12.9
Smoking, cigarettes/day	0.0 ± 0.0	0.2 ± 1.3	1.1 ± 3.4	2.3 ± 4.9	4.8 ± 6.8	7.8 ± 8.5	14.5 ± 10.3	17.4 ± 6.8
Body mass index	22.3 ± 1.6	22.8 ± 2.4	24.1 ± 3.2	26.2 ± 4.4	28.3 ± 5.1	29.1 ± 5.7	30.3 ± 5.5	32.8 ± 3.5
Waist circumference, cm	73.6 ± 5.9	75.0 ± 7.1	78.2 ± 8.7	83.0 ± 11.2	88.5 ± 13.0	90.1 ± 13.7	93.3 ± 13.1	99.9 ± 11.1
Physical activity, exercises/week	3.8 ± 1.8	3.2 ± 2.1	2.5 ± 2.0	2.3 ± 2.0	2.0 ± 2.0	1.6 ± 1.9	0.9 ± 1.3	0.8 ± 0.9
Coffee, cups/day	3.0 ± 2.3	3.2 ± 2.4	3.6 ± 2.4	3.9 ± 2.4	4.3 ± 2.7	4.5 ± 3.0	5.4 ± 3.5	4.4 ± 3.0

consumption, are summarized in Table 3. The risk score status was associated with significant increases in ORs for FLI 60 and above in the groups with one or more risk factors. The most striking influences on the likelihood of abnormal FLI were observed for lack of physical activity ($p < 0.0005$ for both genders) and alcohol consumption exceeding current low risk drinking limits in men (14 drinks per week) ($p < 0.0005$) (Table 4).

In the analyses of correlations between FLI and the various study parameters, significant correlations were found to emerge between FLI and serum ALT ($R_s = 0.512$ for men; $R_s = 0.322$ for women) and CRP ($R_s = 0.429$ for men; $R_s = 0.479$ for women) ($p < 0.001$ for all comparisons).

Discussion

The present findings indicate that combinations of unfavorable determinants in lifestyle markedly increase the risk for fatty liver, as assessed using a recently developed predictor algorithm, FLI. The rather linear association between abnormal FLI and combined lifestyle risk factor status supports the view that significant benefits on liver health could be gained from simultaneous adherence to multiple low-risk lifestyle-related factors and from systematic behavior change support systems for individuals presenting with high-risk lifestyles [1–4, 7, 8]. Based on recent population surveys successful lifestyle interventions could lead to a striking reduction in mortality from both hepatic and extrahepatic causes [1, 2, 4, 17, 19]. Current data indicates that FLI, a non-invasive biomarker of steatosis, could perhaps be used as a clinical tool for patient guidance and motivation during interventions aimed at maintaining long-term lifestyle changes that promote the loss of liver fat.

Fatty liver is currently a highly common condition in high income countries being estimated to affect at least 25–30% of adults in general population and over 70% of those with gross obesity or diabetes [23–25]. Therefore, greater awareness of this phenomenon is important to prevent a looming public health crisis. Building of excess fat in liver cells has been regarded as the hepatic manifestation of the metabolic syndrome, which associates with cerebro- and cardiovascular disease risks, tissue triglyceride deposition, hyperinsulinemia and insulin resistance [10, 19, 23, 26–28]. Therefore, new non-invasive tools for detecting hepatic steatosis in an early phase are needed to prevent progression of liver disease and associated metabolic comorbidities. Although the FLI algorithm has recently been shown to improve the identification of fatty liver when compared with other non-invasive methods [11, 20, 29–31], as yet, only few studies have been available on the clinical applications of FLI or the effects of lifestyle factors on FLI.

Table 2 Fatty liver index (FLI), its individual components and various biomarkers of liver function, inflammation and lipid status according to lifestyle risk factor scores. The percentages show the proportions of individuals exceeding the cut-offs for each parameter

Men															
Risk score	0		1		2		3		4		5		6		7–8	
FLI	18.0 ± 12.3	2.4%	25.4 ± 17.0	5.5%	37.2 ± 22.7	18.0%	49.4 ± 25.4	37.6%	55.7 ± 27.7	46.7%	62.6 ± 27.8	59.0%	67.0 ± 26.2	63.3%	79.9 ± 21.2	81.9%
BMI, kg/m^2	23.1 ± 1.3	0.0%	23.9 ± 2.0	21.4%	25.3 ± 2.7	54.1%	26.6 ± 3.1	73.5%	27.5 ± 4.0	76.4%	28.2 ± 4.3	78.4%	28.6 ± 4.9	83.0%	30.8 ± 3.7	100.0%
Waist, cm	82.5 ± 5.7	4.2%	86.0 ± 6.7	13.0%	89.8 ± 8.4	33.5%	94.1 ± 9.1	52.7%	96.3 ± 11.3	57.0%	98.7 ± 11.9	63.7%	100.2 ± 12.9	72.4%	105.8 ± 11.1	85.1%
GGT, U/L	20.7 (19–22)	1.8%	23.3 (22–24)	5.1%	25.6 (25–26)	5.4%	31.0 (30–32)	12.1%	35.3 (34–37)	18.7%	40.6 (39–43)	24.2%	48.6 (45–53)	36.7%	56.7 (49–65)	41.5%
Trigl, mmol/L	1.02 (1.0–1.1)	12.5%	1.06 (1.0–1.1)	14.3%	1.24 (1.2–1.3)	24.4%	1.36 (1.3–1.4)	31.2%	1.46 (1.4–1.5)	35.8%	1.60 (1.5–1.7)	41.8%	1.68 (1.6–1.8)	47.8%	2.05 (1.8–2.3)	59.6%
ALT, U/L	22.1 (20–25)	0.0%	23.4 (22–24)	2.9%	25.8 (25–27)	6.7%	28.6 (28–30)	11.5%	30.6 (29–32)	16.3%	31.4 (29–33)	15.9%	34.1 (31–38)	25.0%	43.7 (38–51)	32.0%
CRP, mg/L	0.53 (0.5–0.6)	4.9%	0.62 (0.6–0.7)	7.2%	0.81 (0.8–0.9)	11.6%	0.93 (0.9–1.0)	12.1%	1.19 (1.1–1.3)	16.9%	1.38 (1.3–1.5)	21.9%	1.74 (1.5–2.0)	31.0%	2.09 (1.7–2.6)	35.5%
Chol, mmol/L	4.94 (4.8–5.1)	45.8%	5.09 (5.0–5.2)	55.8%	5.28 (5.2–5.3)	64.5%	5.50 (5.4–5.6)	72.3%	5.49 (5.4–5.6)	70.7%	5.60 (5.5–5.7)	73.3%	5.73 (5.6–5.9)	74.8%	5.96 (5.7–6.2)	84.0%
HDL, mmol/L	1.33 (1.3–1.4)	9.5%	1.36 (1.3–1.4)	8.7%	1.29 (1.3–1.3)	15.7%	1.27 (1.3–1.3)	15.6%	1.26 (1.2–1.3)	17.5%	1.26 (1.2–1.3)	18.6%	1.25 (1.2–1.3)	20.5%	1.23 (1.2–1.3)	16.0%
LDL, mmol/L	3.03 (2.9–3.2)	49.5%	3.04 (3.0–3.1)	57.0%	3.27 (3.2–3.3)	65.8%	3.39 (3.3–3.4)	71.4%	3.39 (3.3–3.5)	70.3%	3.36 (3.3–3.5)	68.7%	3.60 (3.5–3.7)	77.9%	3.52 (3.3–3.8)	70.3%
Women																
Risk score	**0**		**1**		**2**		**3**		**4**		**5**		**6**		**7–8**	
FLI	8.4 ± 6.8	0.0%	11.1 ± 10.7	0.2%	17.0 ± 16.5	3.5%	27.9 ± 24.3	13.2%	40.6 ± 28.4	27.7%	45.3 ± 30.7	34.3%	54.5 ± 29.3	43.9%	71.1 ± 23.1	73.5%
BMI, kg/m2	22.3 ± 1.6	0.0%	22.8 ± 2.4	11.7%	24.1 ± 3.2	34.8%	26.2 ± 4.4	60.0%	28.3 ± 5.1	75.9%	29.1 ± 5.7	76.0%	30.3 ± 5.5	87.8%	32.8 ± 3.5	100.0%
Waist, cm	73.6 ± 5.9	15.4%	75.0 ± 7.1	24.3%	78.2 ± 8.7	39.1%	83.0 ± 11.2	58.7%	88.5 ± 13.0	73.8%	90.1 ± 13.7	74.9%	93.3 ± 13.1	84.9%	99.9 ± 11.1	97.1%
GGT, U/L	15.8 (15–17)	5.9%	16.3 (16–17)	4.3%	17.5 (17–18)	4.7%	19.1 (19–20)	8.9%	21.9 (21–23)	10.6%	25.2 (24–27)	17.6%	28.4 (25–32)	25.9%	33.3 (27–41)	26.5%
Trigl, mmol/L	0.87 (0.8–0.9)	5.6%	0.90 (0.9–0.9)	6.4%	0.95 (0.9–1.0)	8.0%	1.04 (1.0–1.1)	13.3%	1.12 (1.1–1.2)	17.0%	1.15 (1.1–1.2)	19.5%	1.24 (1.1–1.3)	25.9%	1.50 (1.3–1.8)	32.4%
ALT, U/L	17.0 (16–18)	3.3%	17.4 (17–18)	6.6%	17.5 (17–18)	4.4%	18.1 (18–19)	6.7%	19.3 (19–20)	9.2%	20.2 (19–22)	8.3%	22.1 (19–25)	15.3%	24.1 (17–34)	9.1%
CRP, mg/L	0.64 (0.6–0.7)	9.1%	0.76 (0.7–0.8)	10.8%	0.83 (0.8–0.9)	12.4%	1.07 (1.0–1.1)	17.7%	1.42 (1.3–1.5)	27.0%	1.43 (1.3–1.6)	26.6%	2.05 (1.7–2.5)	37.2%	2.99 (2.2–4.1)	52.9%
Chol, mmol/L	5.01 (4.9–5.1)	52.7%	5.09 (5.0–5.1)	53.3%	5.21 (5.2–5.3)	59.4%	5.32 (5.3–5.4)	62.6%	5.30 (5.2–5.4)	63.8%	5.29 (5.2–5.4)	61.4%	5.39 (5.2–5.5)	66.2%	5.67 (5.3–6.1)	76.5%
HDL, mmol/L	1.66 (1.6–1.7)	5.9%	1.62 (1.6–1.6)	7.7%	1.60 (1.6–1.6)	10.4%	1.55 (1.5–1.6)	13.6%	1.50 (1.5–1.5)	19.8%	1.50 (1.5–1.5)	21.9%	1.50 (1.4–1.6)	20.3%	1.34 (1.2–1.4)	26.5%
LDL, mmol/L	2.76 (2.7–2.9)	43.0%	2.87 (2.8–2.9)	43.7%	2.96 (2.9–3.0)	50.8%	3.07 (3.0–3.1)	57.0%	3.11 (3.0–3.2)	57.7%	3.08 (3.0–3.2)	55.6%	3.15 (3.0–3.3)	60.4%	3.29 (2.9–3.7)	72.7%

The values are expressed as mean ± SD (FLI, BMI, waist circumference) or as geometric means and 95% confidence intervals (GGT, triglycerides, ALT, CRP, cholesterol, HDL, LDL)
$p < 0.0005$ for linear trend for both mean values and proportions, except $p = 0.001$ for ALT data on proportions among women

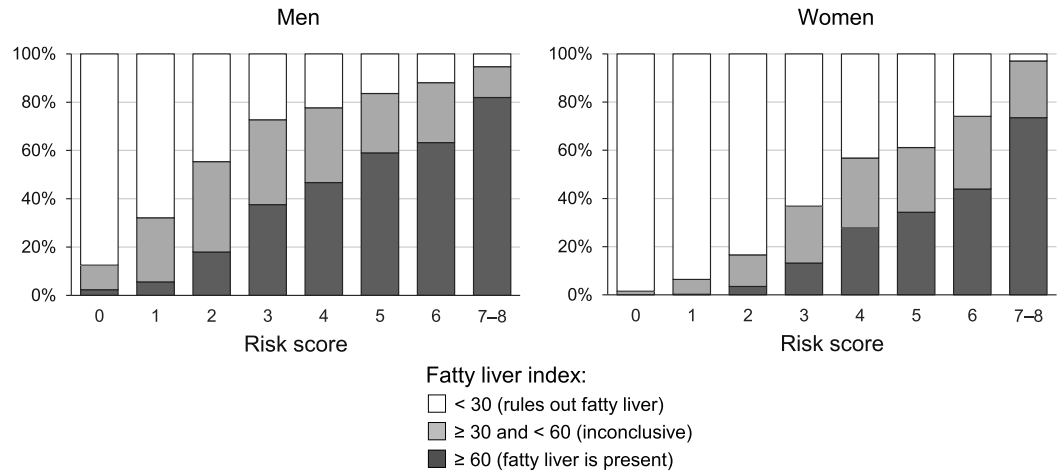

Fig. 1 The distributions of FLI scores below 30, ≥ 30 and < 60 and 60 or above in individuals classified to lifestyle risk factor scores as follows: Alcohol consumption, 0 = no consumption; 1 = alcohol consumption 1–14 (men) or 1–7 (women) drinks/week; 2 = alcohol consumption in amounts exceeding 14 drinks (men) or 7 drinks (women) per week Smoking, 0 = no smoking, 1 = 1–19 cigarettes/day, 2 = ≥ 20 cigarettes/day. BMI, 0 = BMI < 25; 1 = BMI ≥ 25 and < 30; 2 = ≥ 30. Physical activity, 0 = those with physical activity over 4 h/week; 1 = those with physical activity 0.5–4 h/week; 2 = those with physical activity less than 30 min/week. The sum of the scores yielded a total risk factor number, with higher scores indicating an unhealthier lifestyle (maximum = 8)

Alcohol drinking, cigarette smoking, and physical inactivity are currently the main modifiable high-risk determinants of lifestyle [1]. The present findings indicate that each of these components and especially their co-existence increase the risk of metabolic aberrations in the liver. In obese individuals or in smokers, regular alcohol drinking even in relatively modest amounts may increase the risk for abnormal liver enzyme activities [6, 15, 18, 32]. The combined triggers from multiple unfavorable lifestyle factors may also stimulate inflammation and lead to progression of fibrosis [6, 12, 15, 16, 33]. The present findings also lend support to the view that no safe limit of alcohol consumption in relation to the risk of progression of non-alcoholic fatty liver disease (NAFLD) can be defined. Thus, questioning such patients about

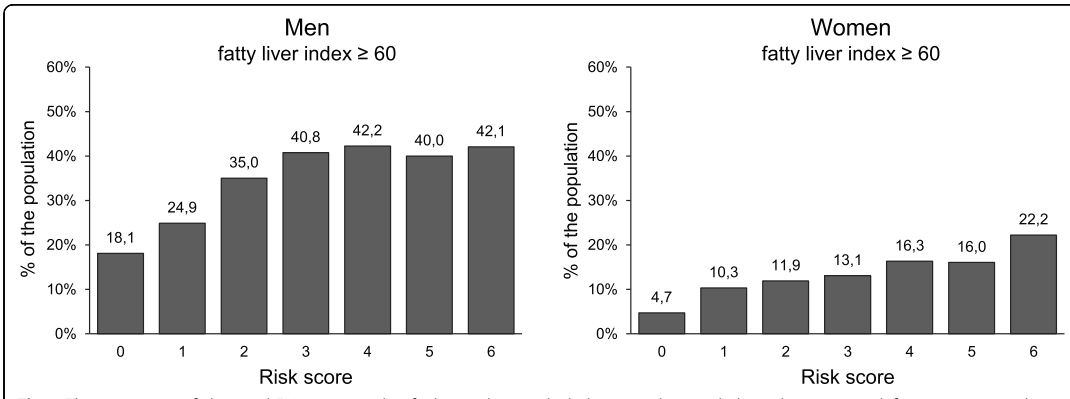

Fig. 2 The occurrence of abnormal FLI in groups classified according to alcohol use, smoking and physical activity as risk factors in men and women as follows: Alcohol consumption, 0 = no consumption; 1 = alcohol consumption 1–14 (men) or 1–7 (women) drinks/week; 2 = alcohol consumption exceeding 14 drinks (men) or 7 drinks (women) per week Smoking, 0 = no smoking, 1 = 1–19 cigarettes/day, 2 = ≥ 20 cigarettes/day. BMI, 0 = BMI < 25; 1 = BMI ≥ 25 and < 30; 2 = ≥ 30. Physical activity, 0 = physical activity more than 4 h/week; 1 = physical activity 0.5–4 h/week; 2 = physical activity less than 30 min/week. The sum of the scores yielded a total risk factor number, with higher scores indicating an unhealthier lifestyle (maximum = 6)

Table 3 Odds ratios for abnormal FLI according to the individual number of lifestyle risk factor scores, as derived from multinomial logistic regression analysis, adjusted for BMI, age and coffee consumption

	Men		Women	
	FLI ≥ 30 and < 60	FLI ≥ 60.0	FLI ≥ 30 and < 60	FLI ≥ 60.0
Risk score	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
0	1.0	1.0	1.0	1.0
1	1.8 (1.3–2.6)	1.7 (1.0–2.8)	0.8 (0.6–1.2)	2.2 (1.1–4.5)
2	2.3 (1.7–3.3)	3.6 (2.2–5.9)	1.2 (0.8–1.7)	3.5 (1.7–7.2)
3	2.9 (2.0–4.1)	6.6 (4.0–11.0)	1.8 (1.2–2.7)	6.4 (3.0–13.5)
4	4.6 (3.0–6.9)	13.5 (7.7–23.7)	2.1 (1.3–3.4)	9.6 (4.1–22.9)
5	7.4 (4.5–12.1)	29.7 (15.5–57.1)	3.5 (1.9–6.6)	26.3 (8.9–77.8)
6	6.2 (3.0–12.7)	28.1 (11.2–70.9)	9.3 (2.3–37.6)	32.8 (11.6–92.3)

FLI fatty liver index, *OR* odds ratio

alcohol intake and other factors of lifestyle warrants further attention. Previous findings have indicated that there may be common pathogenic features in lifestyle-related disease manifestations, including systemic inflammatory response, oxidative stress and altered fatty acid metabolism [9, 34–36]. Therefore, use of FLI together with biomarkers reflecting the above mentioned pathophysiological pathways could also help in elucidating the primary mechanisms of fatty deposition in various behavioral phenotypes. Recently, a link between hepatic and extrahepatic manifestations of fatty liver have been proposed based on findings indicating that LDL oxidation in coronary atherosclerotic plaques can be boosted by the action of GGT enzyme, which is also a key mediator of oxidative stress [37, 38]. There may also be an interplay between oxidative stress and inflammation [13, 39–41]. In line with this view, current data shows that abnormalities in serum CRP, a biomarker and important regulator of inflammation also coincide with the burden of high-risk lifestyle factors and abnormalities in FLI.

Lack of physical activity has recently been recognized as an increasingly important lifestyle-associated contributor to poor health [42, 43]. Spending more time in sedentary behaviors associates with a wide variety of adverse health outcomes, including cardiovascular diseases, diabetes and carcinogenesis [1, 44–47]. The present data shows that physical inactivity is also a major independent contributor of abnormal FLI. Those with moderate and vigorous physical activity show markedly lower odds for fatty liver than those with sedentary activity. Sufficient doses of physical exercise could also have a major impact in reducing the adverse metabolic effects of unfavorable lifestyle. Regular physical activity may also be expected to lead to significant long-term health benefits in reducing hepatic steatosis and insulin resistance

Table 4 Individual impacts of lifestyle factors on fatty liver index in multivariate binary logistic regression analysis

	Men		Women	
	Adjusted OR (95% CI)	p^*	Adjusted OR (95% CI)	p^*
Physical activity per week		< 0.0005		< 0.0005
> 4 h	1.0		1.0	
0.5–4 h	2.54 (2.21–2.92)		2.53 (1.99–3.22)	
< 30 min	2.78 (2.35–3.28)		3.82 (2.94–4.96)	
Standard drinks per week		< 0.0005		0.086
none	1.0		1.0	
1–14 (men) or 1–7 (women)	1.01 (0.88–1.15)		0.84 (0.72–0.99)	
> 14 (men) or > 7 (women)	1.81 (1.53–2.15)		1.02 (0.80–1.30)	
Cigarettes per day		0.047		0.185
none	1.0		1.0	
1–19	0.84 (0.73–0.98)		0.88 (0.7–1.08)	
≥ 20	0.88 (0.75–1.04)		1.25 (0.88–1.78)	

*likelihood ratio test

[35, 45, 48–50]. In accordance with this view, moderate or vigorous physical activity were recently shown to reduce fat, inflammation and oxidative stress in the liver even in cases without any notable changes in BMI status [35].

Previous studies have shown that Western diet characterized by high fat, high carbohydrate and insufficient vitamin intake may provide triggers for insulin resistance and associated hepatotoxicity [14, 46, 51–56]. On the other hand, adherence to a healthy diet has recently been emphasized among the first-line treatment options for NAFLD [52, 57]. Unfortunately, in this work information on the exact compositions of the diet were not available. A large body of evidence has supported the view that nutrients rich in antioxidants show an inverse association with the risk of mortality due to NAFLD [52]. Interestingly, consumption of coffee, which is a rich source of antioxidants, has been previously associated with a reduced risk for liver cirrhosis and liver enzyme elevations in alcohol consumers [58, 59]. Coffee intake has also been suggested to be inversely related with the risk of NAFLD possibly by modulating pathways of the gut-liver axis [60]. In the present population, the lifestyle risk factor score was found to correlate positively with coffee intake, which was explained by a high prevalence of coffee drinking among smokers [61]. The question whether and how coffee consumption could exert protective effects towards the oxidative stress induced by combined lifestyle associated risk factors remains, however, unknown.

A major strength of this study is the large sample size of over 12,000 participants with a comprehensive assessment of the relationships between FLI, other laboratory markers and lifestyle-related risk factors. Although the present material was collected from different geographical areas in Finland, the population represents a Caucasian population with a high degree of environmental and genetic homogeneity. Based on previous evidence indicating profound gender-related differences in susceptibilities for liver disease, we have also included separate analyses for men and women. In accordance with recent findings from an animal model for NAFLD [62], our data suggests that alterations in liver enzymes and lipid status among men may occur relatively early in the sequence of events leading abnormal FLI. However, the changes in CRP, a biomarker of inflammation, in response to combined life style risk factors appeared to be more pronounced among women.

The main limitation of the study is the cross-sectional setting and lack of follow-up data to address possible causal relationships. The data on lifestyle determinants were based on self-reports and therefore we cannot rule out the possibility of recall bias or underreporting especially concerning the data reflecting socially less desirable behaviors, such as alcohol intake. Lack of detailed information on the patterns of diet may also be kept as a limitation of the study. Therefore, future longitudinal studies are needed to examine causal relationships between combinations of life style risk factors and fatty change in the liver. The possible role of FLI as a clinical tool for supporting behavior changes in NAFLD patients also warrant future studies in large materials. It should further be emphasized that although elevated blood glucose levels is known to be an important determinant of metabolic health in both normal weight and obese subjects [63], in this study data on simultaneous measurements of fasting blood glucose levels were not available. The occurrence of abnormal blood glucose status is, however, unlikely to create a significant confounding factor in the present analyses since we excluded all subjects who had been previously diagnosed with diabetes or had shown abnormal results in oral glucose tolerance tests.

Conclusions

Taken together, current data demonstrates distinct relationships of lifestyle-related risk factors and fatty liver, which should be implicated in recommendations aimed at promoting liver health. The data also emphasizes the possibility of using FLI algorithm as a non-invasive clinical tool for providing feedback in approaches to reduce the number of unfavorable lifestyle risk factors and to prevent morbidity and mortality resulting from fatty liver disease and associated metabolic comorbidities. Interestingly, recent studies have indicated that FLI could also serve as a risk predictor for extrahepatic complications, such as chronic kidney disease [64].

Abbreviations

ALT: Alanine aminotransferase; BMI: Body mass index; CRP: C-reactive protein; FLI: Fatty liver index; GGT: Gamma-glutamyl transferase; HDL: High-density lipoproteins; LDL: Low-density lipoprotein; NAFLD: Non-alcoholic fatty liver disease; NASH: Non-alcoholic steatohepatitis

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Authors' contributions

Study design: ON, MN, MA, TL; Data analysis: UN, ON, MN, AB, RB, MA, TL; Project administration and supervision: ON, MA, TL; All authors read and approved the manuscript.

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Availability of data and materials

THL Biobank administrates and grants access to the FINRISK data to research projects that are of high scientific quality and impact, are ethically

conducted, and that correspond with the research areas of THL Biobank. All data are available for application at https://thl.fi/en/web/thl-biobank/for-researchers/sample-collections/the-national-finrisk-study-1992-2012. The name of dataset is the National FINRISK Study 1992–2012.

Ethics approval and consent to participate

The investigation was performed with the understanding and written informed consent of each individual and was approved by the Coordinating Ethics Committee of the Helsinki and Uusimaa Hospital District. All surveys were conducted in accordance with the Declaration of Helsinki according to the ethical rules of the National Public Health Institute.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Li Y, Pan A, Wang DD, Liu X, Dhana K, Franco OH, et al. Impact of healthy lifestyle factors on life expectancies in the US population. Circulation. 2018; 138:345–55.
2. Lim SS, Vos T, Flaxman AD, Danaei G, Shibuya K, Adair-Rohani H, et al. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. Lancet. 2012;380:2224–60.
3. Manuel DG, Perez R, Sanmartin C, Taljaard M, Hennessy D, Wilson K, et al. Measuring burden of unhealthy behaviours using a multivariable predictive approach: life expectancy lost in Canada attributable to smoking, alcohol, physical inactivity, and diet. PLoS Med. 2016;13:e1002082.
4. Rutten-Jacobs LC, Larsson SC, Malik R, Rannikmae K, Sudlow CL, Dichgans M, et al. Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: cohort study of 306 473 UK biobank participants. BMJ. 2018;363:k4168.
5. McGinnis M, Williams-Russo P, Knickman JR. The case for more active policy attention to health promotion. Health Aff. 2002;21:78–93.
6. Niemelä O, Niemelä M, Bloigu R, Aalto M, Laatikainen T. Where should the safe limits of alcohol consumption stand in light of liver enzyme abnormalities in alcohol consumers? PLoS One. 2017;12:e0188574.
7. Tamakoshi A, Tamakoshi K, Lin Y, Yagyu K, Kikuchi S, JACC Study Group. Healthy lifestyle and preventable death: findings from the Japan collaborative cohort (JACC) study. Prev Med. 2009;48:486–92.
8. Teeriniemi AM, Salonurmi T, Jokelainen T, Vähänikkilä H, Alahäivälä T, Karppinen P, et al. A randomized clinical trial of the effectiveness of a web-based health behaviour change support system and group lifestyle counselling on body weight loss in overweight and obese subjects: 2-year outcomes. J Intern Med. 2018;284:534–45.
9. Danielsson J, Kangastupa P, Laatikainen T, Aalto M, Niemelä O. Impacts of common factors of life style on serum liver enzymes. World J Gastroenterol. 2014;20:11743–52.
10. Kim WR, Flamm SL, Di Bisceglie AM, Bodenheimer HC. Serum activity of alanine aminotransferase (ALT) as an indicator of health and disease. Hepatology. 2008;47:1363–70.
11. Koehler EM, Schouten JN, Hansen BE, Hofman A, Stricker BH, Janssen HL. External validation of the fatty liver index for identifying nonalcoholic fatty liver disease in a population-based study. Clin Gastroenterol Hepatol. 2013; 11:1201–4.
12. Lau K, Baumeister SE, Lieb W, Meffert PJ, Lerch MM, Mayerle J, et al. The combined effects of alcohol consumption and body mass index on hepatic steatosis in a general population sample of European men and women. Aliment Pharmacol Ther. 2015;41:467–76.
13. Nivukoski U, Niemelä M, Bloigu A, Bloigu R, Aalto M, Laatikainen T, et al. Impacts of unfavourable lifestyle factors on biomarkers of liver function, inflammation and lipid status. PLoS One. 2019;14:e0218463.
14. Ruhl CE, Everhart JE. Elevated serum alanine aminotransferase and gamma-glutamyltransferase and mortality in the United States population. Gastroenterology. 2009;136:477–85.
15. Breitling LP, Raum E, Müller H, Rothenbacher D, Brenner H. Synergism between smoking and alcohol consumption with respect to serum gamma-glutamyltransferase. Hepatology. 2009;49:802–8.
16. Park EY, Lim MK, Oh JK, Cho H, Bae MJ, Yun EH, et al. Independent and supra-additive effects of alcohol consumption, cigarette smoking, and metabolic syndrome on the elevation of serum liver enzyme levels. PLoS One. 2013;8:e63439.
17. Lee TH, Kim WR, Benson JT, Therneau TM, Melton LJ III. Serum aminotransferase activity and mortality risk in a United States community. Hepatology. 2008;47:880–7.
18. Niemelä O, Nivukoski U, Bloigu A, Bloigu R, Aalto M, Laatikainen T. Laboratory test based assessment of WHO alcohol risk drinking levels. Scand J Clin Lab Invest. 2019;79:58–64.
19. Ruttmann E, Brant LJ, Concin H, Diem G, Rapp K, Ulmer H, et al. Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality: an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation. 2005;112:2130–7.
20. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population. BMC Gastroenterol. 2006;6:33.
21. The World Health Organization MONICA Project (Monitoring trends and determinants in cardiovascular disease): a major international collaboration. WHO MONICA Project Principal Investigators. J Clin Epidemiol. 1988;41:105–14.
22. Kuulasmaa K, Tolonen H, Cepaitis Z, Laatikainen T, Nissinen A, Vartiainen E, et al. European health risk monitoring project. Helsinki: Finnish National Public Health Institute (KTL); 2006. www.thl.fi/ehrm (11.9.2018).
23. Byrne CD, Patel J, Scorletti E, Targher G. Tests for diagnosing and monitoring non-alcoholic fatty liver disease in adults. BMJ. 2018;362:k2734.
24. Iqbal U, Perumpail BJ, Akhtar D, Kim D, Ahmed A. The epidemiology, risk profiling and diagnostic challenges of nonalcoholic fatty liver disease. Medicines (Basel). 2019;6(1):41.
25. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15:11–20.
26. Kazemi-Shirazi L, Endler G, Winkler S, Schickbauer T, Wagner O, Marsik C. Gamma glutamyltransferase and long-term survival: is it just the liver? Clin Chem. 2007;53:940–6.
27. Niemelä O. Biomarker-based approaches for assessing alcohol use disorders. Int J Environ Res Public Health. 2016;13:166.
28. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol. 2014;2: 901–10.
29. Huang X, Xu M, Chen Y, Peng K, Huang Y, Wang P, et al. Validation of the Fatty Liver Index for Nonalcoholic Fatty Liver Disease in Middle-Aged and Elderly Chinese. Medicine (Baltimore). 2015;94:e1682.
30. Jäger S, Jacobs S, Kröger J, Stefan N, Fritsche A, Weikert C, et al. Association between the fatty liver index and risk of type 2 diabetes in the EPIC-Potsdam study. PLoS One. 2015;10:e0124749.
31. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and nutrition examination survey. Aliment Pharmacol Ther. 2015;41:65–76.
32. Wood AM, Kaptoge S, Butterworth AS, Willeit P, Warnakula S, Bolton T, et al. Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies. Lancet. 2018;391:1513–23.
33. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999-2016: observational study. BMJ. 2018;362:k2817.

34. Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med. 2018;378:1096–106.
35. Oh S, Shida T, Yamagishi K, Tanaka K, So R, Tsujimoto T, et al. Moderate to vigorous physical activity volume is an important factor for managing nonalcoholic fatty liver disease: a retrospective study. Hepatology. 2015;61:1205–15.
36. Soldavini J, Kaunitz JD. Pathobiology and potential therapeutic value of intestinal short-chain fatty acids in gut inflammation and obesity. Dig Dis Sci. 2013;58:2756–66.
37. Kozakova M, Palombo C, Eng MP, Dekker J, Flyvbjerg A, Mitrakou A, et al. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. Hepatology. 2012;55:1406–15.
38. Pais R, Giral P, Khan JF, Rosenbaum D, Housset C, Poynard T, et al. Fatty liver is an independent predictor of early carotid atherosclerosis. J Hepatol. 2016;65:95–102.
39. Koenig W. C-reactive protein and cardiovascular risk: will the controversy end after CANTOS? Clin Chem. 2017;63:1897–8.
40. Libby P, Ridker PM, Hansson GK. Leducq transatlantic network on Atherothrombosis. Inflammation in atherosclerosis: from pathophysiology to practice. J Am Coll Cardiol. 2009;54:2129–38.
41. Moradi H, Streja E, Kalantar-Zadeh K. Serum high density lipoprotein cholesterol level and risk of death: let's avoid the extremes. J Thorac Dis. 2017;9:4849–52.
42. Sundberg CJ. Physical activity: what is already being done and how we can avert 1 million deaths annually in future. Br J Sports Med. 2016;50:319.
43. Warburton DE, Bredin SS. Reflections on physical activity and health: what should we recommend? Can J Cardiol. 2016;32:495–504.
44. Chomistek AK, Manson JE, Stefanick ML, Lu B, Sands-Lincoln M, Going SB, et al. Relationship of sedentary behavior and physical activity to incident cardiovascular disease: results from the Women's Health Initiative. J Am Coll Cardiol. 2013;61:2346–54.
45. Kyu HH, Bachman VF, Alexander LT, Mumford JE, Afshin A, Estep K, et al. Physical activity and risk of breast cancer, colon cancer, diabetes, ischemic heart disease, and ischemic stroke events: systematic review and dose-response meta-analysis for the global burden of disease study 2013. BMJ. 2016;354:i3857.
46. Romero-Gómez M, Zelber-Sagi S, Trenell M. Treatment of NAFLD with diet, physical activity and exercise. J Hepatol. 2017;67:829–46.
47. Smith AD, Crippa A, Woodcock J, Brage S. Physical activity and incident type 2 diabetes mellitus: a systematic review and dose-response meta-analysis of prospective cohort studies. Diabetologia. 2016;59:2527–45.
48. Lawlor DA, Sattar N, Smith GD, Ebrahim S. The associations of physical activity and adiposity with alanine aminotransferase and gamma-glutamyltransferase. Am J Epidemiol. 2005;161:1081–8.
49. St George A, Bauman A, Johnston A, Farrell G, Chey T, George J. Independent effects of physical activity in patients with nonalcoholic fatty liver disease. Hepatology. 2009;50:68–76.
50. van der Windt DJ, Sud V, Zhang H, Tsung A, Huang H. The effects of physical exercise on fatty liver disease. Gene Expr. 2018;18:89–101.
51. Berná G, Romero-Gómez M. The role of nutrition in non-alcoholic fatty liver disease: pathophysiology and management. Liver Int. 2020;40(Suppl 1):102–8.
52. Abenavoli L, Boccuto L, Federico A, Dallio M, Loguercio C, Di Renzo L, et al. Diet and non-alcoholic fatty liver disease: the Mediterranean way. Int J Environ Res Public Health. 2019;16:3011.
53. Ghouri N, Preiss D, Sattar N. Liver enzymes, nonalcoholic fatty liver disease, and incident cardiovascular disease: a narrative review and clinical perspective of prospective data. Hepatology. 2010;52:1156–61.
54. Liu B, Balkwill A, Reeves G, Beral V. Million women study collaborators. Body mass index and risk of liver cirrhosis in middle aged UK women: prospective study. BMJ. 2010;340:c912.
55. Abenavoli L, Milic N, Di Renzo L, Preveden T, Medic-Stojanoska M, De Lorenzo A. Metabolic aspects of adult patients with nonalcoholic fatty liver disease. World J Gastroenterol. 2016;22(31):7006–16.
56. Tsukamoto H, Horne W, Kamimura S, Niemelä O, Parkkila S, Ylä-Herttuala S, et al. Experimental liver cirrhosis induced by alcohol and iron. J Clin Invest. 1995;96:620–30.
57. Kanerva N, Kaartinen NE, Schwab U, Lahti-Koski M, Männistö S. Adherence to the Baltic Sea diet consumed in the Nordic countries is associated with lower abdominal obesity. Br J Nutr. 2013;109:520–8.
58. Danielsson J, Kangastupa P, Laatikainen T, Aalto M, Niemelä O. Dose- and gender-dependent interactions between coffee consumption and serum GGT activity in alcohol consumers. Alcohol Alcohol. 2013;48:303–7.
59. Gunter MJ, Murphy N, Cross AJ, Dossus L, Dartois L, Fagherazzi G, et al. Coffee drinking and mortality in 10 European countries: a multinational cohort study. Ann Intern Med. 2017;167:236–47.
60. Vitaglione P, Mazzone G, Lembo V, D'Argenio G, Rossi A, Guido M, et al. Coffee prevents fatty liver disease induced by a high-fat diet by modulating pathways of the gut-liver axis. J Nutr Sci. 2019;8:e15.
61. Bjørngaard JH, Nordestgaard AT, Taylor AE, Treur JL, Gabrielsen ME, Munafò MR, et al. Heavier smoking increases coffee consumption: findings from a Mendelian randomization analysis. Int J Epidemiol. 2017;46:1958–67.
62. Marin V, Rosso N, Dal Pen M, Raseni A, Boschelle M, Degrassi C, et al. An animal model for the juvenile non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. PLoS One. 2016;11(7):e0158817.
63. Stefan S, Schick F, Häring H-U. Causes, characteristics, and consequences of metabolically unhealthy normal weight in humans. Cell Metab. 2017;26:292–300.
64. Huh JH, Kim JY, Choi E, Kim JS, Chang Y, Sung KC. The fatty liver index as a predictor of incident chronic kidney disease in a 10-year prospective cohort study. PLoS One. 2017;12(7):e0180951.

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