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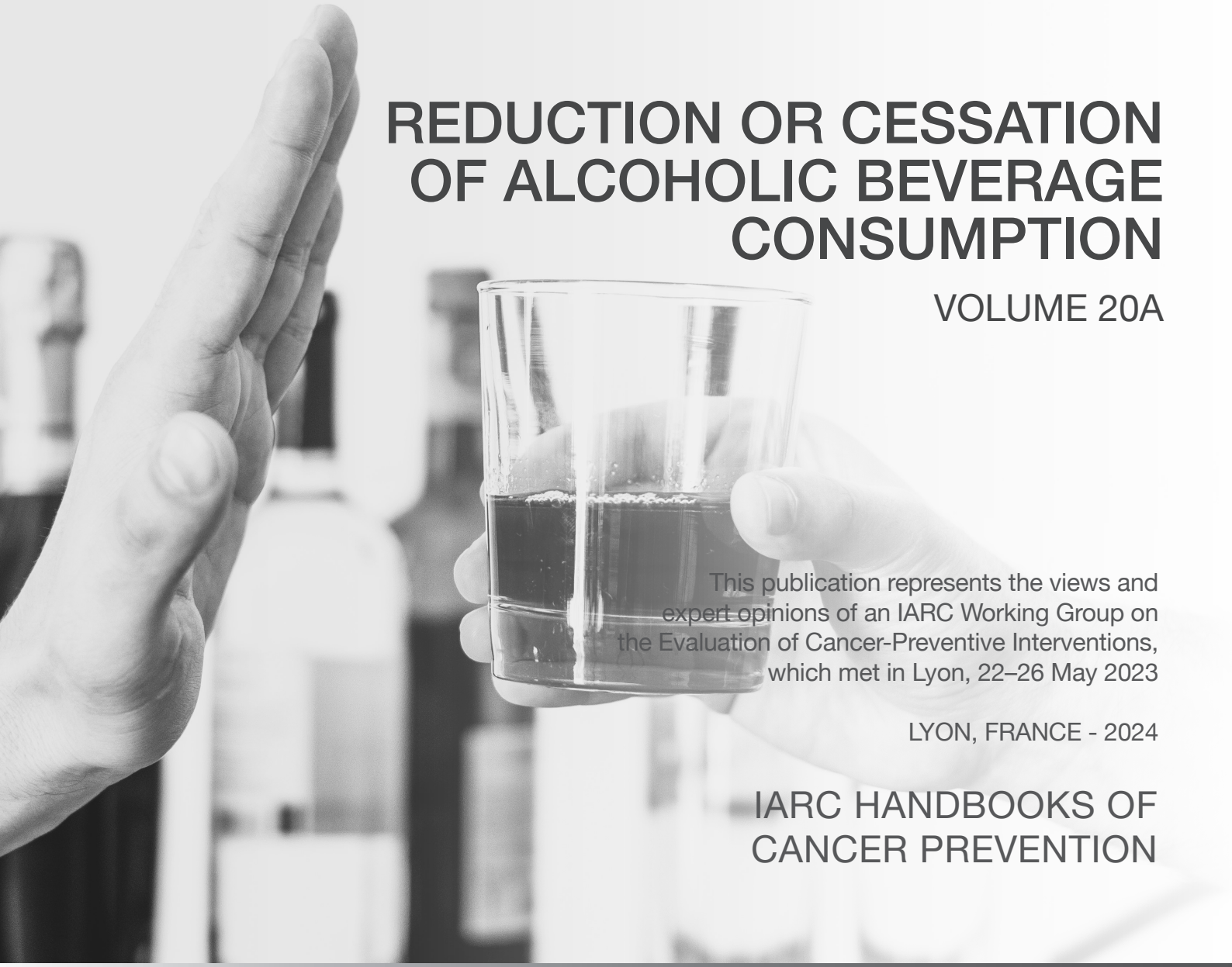

REDUCTION OR CESSATION OF ALCOHOLIC BEVERAGE CONSUMPTION

VOLUME 20A

This publication represents the views and expert opinions of an IARC Working Group on the Evaluation of Cancer-Preventive Interventions, which met in Lyon, 22–26 May 2023

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3. MECHANISTIC DATA

3.1 Overview of the mechanisms of alcohol-induced carcinogenesis among humans

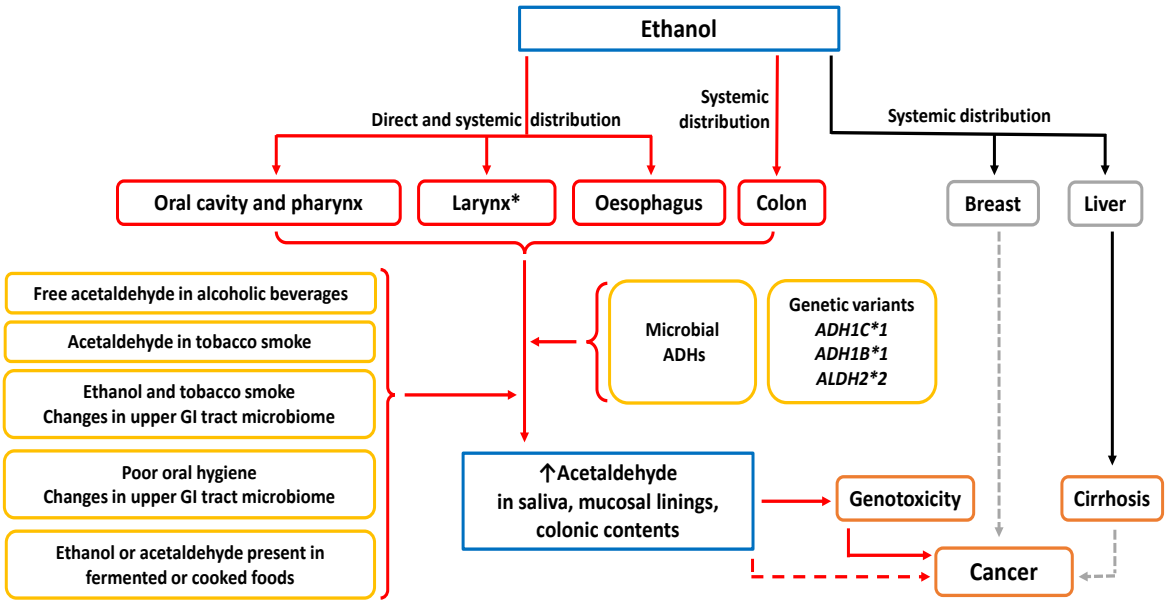
3.1.1 Absorption, distribution, and metabolism of ethanol

After a sip of an alcoholic beverage, ethanol is rapidly absorbed from the upper aerodigestive tract. Within 30 minutes of consumption, it is evenly distributed to the aqueous phase of the human body, including the saliva, sweat, gastric juices, colonic contents, blood, and urine. The bulk of ethanol elimination (~90%) takes place in the liver, where it is oxidized mainly by alcohol dehydrogenase (ADH) enzymes to acetaldehyde (Cederbaum, 2012; IARC, 2012). Oxidation of acetaldehyde to acetate by hepatic aldehyde dehydrogenase (ALDH) enzymes is so effective that among individuals with the active ALDH2 enzyme variant, acetaldehyde cannot be detected in the peripheral venous blood (DeMaster et al., 1983; Lindros, 1983). However, significantly elevated acetaldehyde concentrations may be detectable in the hepatic venous blood after alcohol ingestion, especially among individuals with alcohol use disorder (AUD) (DeMaster et al., 1983; Nuutinen et al., 1984). Acetate is oxidized in the peripheral tissues to carbon dioxide and water.

At high ethanol concentrations (> 10 mM), some ethanol is metabolized in the liver to acetaldehyde by the cytochrome P450-dependent ethanol-oxidizing system (cytochrome P450 2E1 [CYP2E1]) (Lieber, 1999; Cederbaum, 2012). Hepatic catalase is an insignificant (~2%) pathway for ethanol oxidation, as is excretion of unchanged ethanol in breath, sweat, or urine. Many microorganisms in the gastrointestinal tract can metabolize ethanol. In the oral cavity, oropharynx, and oesophagus, oxidation of ethanol is essentially mediated by the respective microbiomes, which may lead to high exposure of the local mucosa to acetaldehyde (Fig. 3.1). In addition, up to 10% of ingested ethanol is oxidized in the large intestine by the bacteriocolonic pathway (Salaspuro, 2003).

Chronic alcohol consumption increases the rate of ethanol elimination in both experimental animals and humans by attenuating the ethanol-induced change in the hepatic ratio of nicotinamide adenine dinucleotide, reduced form (NADH) to NAD and inducing the microsomal ethanol-oxidizing system CYP2E1 (Salaspuro and Kesänimi, 1973; Salaspuro et al., 1981; Lieber, 1999). The ethanol-induced CYP2E1 fades away within 8–15 days after the cessation of alcohol consumption (Oneta et al., 2002). The speed of ethanol-induced changes in the hepatic NADH/NAD ratio after cessation of or reduction in alcohol consumption is unknown.

Fig. 3.1 Major role of local acetaldehyde in alcohol-induced carcinogenesis



The red boxes indicate organs known to be exposed to high local acetaldehyde concentrations via saliva or colon contents. The red arrows indicate well-described mechanisms. The dotted red arrow indicates other mechanisms of carcinogenesis. The yellow boxes indicate additional factors that can increase local concentrations of acetaldehyde or duration of acetaldehyde exposure. The grey boxes indicate organs in which local levels of acetaldehyde are unlikely to be high. The dashed grey arrows indicate mechanisms of carcinogenesis that are currently unknown or hypothetical.
ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; GI, gastrointestinal.
* The magnitude of laryngeal exposure to acetaldehyde via saliva is unknown.
Prepared by the Working Group.

(a) Genetic polymorphisms

Several gene polymorphisms in the alcohol metabolic pathway modify the amount of ethanol or acetaldehyde to which an organ is exposed when an individual consumes alcohol. Genetic susceptibility has been demonstrated for ADH, polymorphic at the *ADH1B* and *ADH1C* loci, and for polymorphic variants of the gene that encodes mitochondrial ALDH2.

(i) ADH1B

ADH1B has three variants: *ADH1B*1*, *ADH1B*2*, and *ADH1B*3*. *ADH1B*1* is the most common allele among individuals of European ancestry, whereas *ADH1B*2* is frequent among individuals of East Asian ancestry (Eng et al., 2007; Li et al., 2007; Edenberg and McClintick, 2018). *ADH1B*3* is mainly found among individuals of African ancestry (McCarthy et al., 2010). The *ADH1B*2* gene encodes the ADH1B2 enzyme, which has a 40-fold higher in vitro activity than the ADH1B1 enzyme encoded by *ADH1B*1* (Yin et al., 1984). Individuals with the *ADH1B*2* alleles are more likely to abstain from alcohol or to consume less alcohol and have a reduced risk of developing AUD, although the exact mechanism of action is still unknown. Studies among individuals without AUD have failed to demonstrate any effects of the *ADH1B* genotype on the rate of ethanol elimination, blood acetaldehyde

concentrations, or psychological responses to ethanol (Mizoi et al., 1994; Peng and Yin, 2009; Chen et al., 2021); however, among individuals with *ADH1B*2* who develop AUD, the rate of ethanol elimination is greater than that among individuals with *ADH1B*1* who develop AUD (Yokoyama et al., 2016). Therefore, individuals with an *ADH1B*1* genotype have a comparatively higher risk of alcohol dependence and of alcohol-related upper aerodigestive tract cancer (Thomasson et al., 1991; Higuchi et al., 1996, 2004; Yang et al., 2010; Guo et al., 2012).

(ii) ADH1C

The *ADH1C*1* allele encodes a hepatic ADH enzyme that metabolizes ethanol 2.5 times as fast in vitro as the ADH enzyme encoded by the *ADH1C*2* allele (Bosron and Li, 1986). However, the rate of ethanol elimination is not correspondingly increased, because it presumably is limited in the liver by the reoxidation rate of NADH (Cederbaum, 2012).

(iii) ALDH2

A single point mutation in the *ALDH2*1* gene results in the replacement of glutamate at position 487 with lysine in the ALDH2 subunit protein, which is expressed predominantly in the liver (Yoshida et al., 1984). This *ALDH2*2* allele causes dominantly inherited ALDH2 enzyme deficiency, with zero in vitro activity among individuals who are homozygous for the allele and ~17% activity among individuals who are heterozygous for the allele (Lai et al., 2014). This allele is limited to individuals of East Asian ancestry.

Among individuals who are carriers of the *ALDH2*2* allele, consuming alcohol results in facial flushing, tachycardia, palpitation, and dose-dependent increases in blood acetaldehyde concentrations. These individuals are more likely to abstain from alcohol or to consume less alcohol and are less likely to develop AUD or alcohol-related health problems, especially if they are homozygous for this allele (Harada et al., 1983; Crabb et al., 1989; Higuchi et al., 1996; Peng et al., 1999; Koyanagi et al., 2020; Chen et al., 2021).

3.1.2 *Local exposure of target organs to acetaldehyde*

Exposure to high concentrations of acetaldehyde, a potent genotoxic metabolite of ethanol, is a major determinant of alcohol-related carcinogenesis, at least in the upper aerodigestive tract (Fig. 3.1; see Section 3.1.3). Exposure to acetaldehyde is markedly enhanced by genetic polymorphism of the genes that encode the ADH and ALDH2 enzymes; additional sources of exposure include acetaldehyde in tobacco smoke, ethanol or acetaldehyde present in fermented or cooked food, acetaldehyde present in alcoholic beverages, and changes in the oral microbiome induced by chronic smoking, chronic heavy alcohol consumption, or poor oral hygiene (Fig. 3.1; Salaspuro, 2017, 2020; Nieminen and Salaspuro, 2018). Sections (a)–(f) below review the available evidence on the role of local acetaldehyde in the development of cancer at those sites that have been identified as being linked to alcohol consumption, i.e. oral cavity, pharynx, larynx, oesophagus, colorectum, liver, and breast.

(a) Oral cavity and pharynx

The oral microbiome, present in the oral cavity and pharynx, contains many bacteria and yeasts that can effectively oxidize ethanol to acetaldehyde both in vitro and in vivo (Homann et al., 1997; Tillonen et al., 1999a; Muto et al., 2000; Kurkivuori et al., 2007; Uittamo et al., 2009; Nieminen et al., 2009; Moritani et al., 2015). This microbiome includes a variety of ADH enzymes with different activities and variations in their Michaelis constant (K_M) values; in vitro salivary formation of acetaldehyde has been shown to vary over a 30-fold range, depending on an individual's oral microbiome (Yokoyama et al., 2018). Acetaldehyde accumulates in the saliva because of the very low ALDH activity in both the oral

mucosa and the oral microbiome (Dong et al., 1996; Pavlova et al., 2013).

High salivary acetaldehyde concentrations produce dose-dependent acetaldehyde–DNA adducts in the oral mucosa of humans and rhesus monkeys (Balbo et al., 2012, 2016; Guidolin et al., 2021). [On the basis of the results from Homann et al. (1997), Väkeväinen et al. (2000), Salaspuro and Salaspuro (2004), and Balbo et al. (2012), the Working Group estimated that salivary acetaldehyde concentrations as low as 10 μM can lead to acetaldehyde–DNA adducts in the oral mucosa.] A positive linear correlation (r = 0.86) has been demonstrated between local exposure to acetaldehyde via the saliva and the risk of oropharyngeal cancer (Salaspuro and Lachenmeier, 2020).

Acetaldehyde production in the saliva after a sip of an alcoholic beverage can be described as an instant phase and a long-term phase, as shown in a schematic representation in Fig. 3.2.

(i) Instant phase of acetaldehyde formation in saliva

In the absence of alcohol consumption or exposure to tobacco smoke, endogenous salivary acetaldehyde concentrations are < 1 μM (Fig. 3.2). After each sip of 5 mL of 40% alcohol (v/v), ethanol remains in the saliva, gradually decreasing in concentration from ~800 mM at 30 seconds to 6 mM at 15 minutes (Helminen et al., 2013). The instant phase of microbial acetaldehyde formation starts immediately, and the concentration peaks at ~260 μM within 2 minutes. The instant phase (mean concentration, ~150 μM) lasts for ~10 minutes and represents ~70% of the total acetaldehyde exposure of the oropharynx. Acetaldehyde remains detectable in the saliva for up to 20 minutes after a single ethanol exposure (Nieminen and Salaspuro, 2018) (Fig. 3.2).

The strong positive correlation between ethanol and acetaldehyde concentrations in the saliva (Homann et al., 1997; Linderborg et al., 2011; Helminen et al., 2013; Tagaino et al., 2021) may explain the higher risk of head and neck cancer among individuals who consume liquor than among individuals who consume beer (Huang et al., 2017).

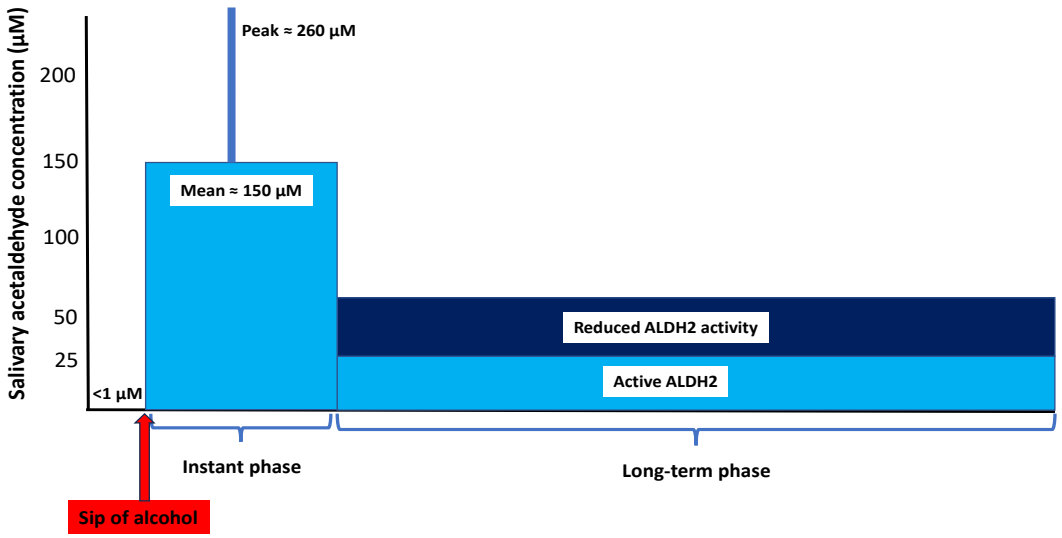
Alcoholic beverages may contain free acetaldehyde as a contaminant (see also Section 1.1.2). Ingestion of alcoholic beverages with high concentrations of free acetaldehyde (474–15 197 μM) has been found to result in a transitory peak in salivary acetaldehyde concentration up to > 1000 μM, which lasts for 1–2 minutes (Yokoyama et al., 2008; Lachenmeier and Monakhova, 2011; Linderborg et al., 2011).

(ii) Long-term phase of acetaldehyde formation in saliva

Long-term salivary acetaldehyde is derived from the ethanol that diffuses back to the saliva from the blood after systemic distribution. The long-term phase of acetaldehyde formation lasts for as long as ethanol stays in the human body and therefore depends on the total dose of alcohol consumed. During this phase, the mean salivary acetaldehyde concentration among individuals with the active ALDH2 enzyme variant is ~25 μM (Fig. 3.2; Lachenmeier and Salaspuro, 2017).

Daily exposure of the mucosa to acetaldehyde among individuals with moderate alcohol consumption (defined as 3 doses of alcohol per day) is proportionately less than exposure among individuals with heavy alcohol consumption (defined as 7 doses of alcohol per day), because individuals with moderate alcohol consumption take fewer sips of alcohol (hence, fewer instant phases of acetaldehyde formation) and ethanol is present in their bloodstream for a shorter period of time (hence, shorter long-term phases of acetaldehyde formation) (Nieminen and Salaspuro, 2018).

Fig. 3.2 Schematic representation of acetaldehyde concentrations in the saliva after a dose of alcohol



In the absence of alcohol intake or tobacco smoking, salivary acetaldehyde concentrations are < 1 μM.
In the instant phase, after a sip of 40% alcohol (5 mL kept in the mouth for 5 seconds), ethanol is distributes rapidly to the aqueous phase of the oral cavity and remains there at high concentrations for up to 20 minutes. Simultaneously, microbial production of acetaldehyde from ethanol occurs, reaching high concentrations (with a peak at ~260 μM) and lasting for 15–20 minutes. The *ALDH2* genotype has no effect on this phase.
In the long-term phase, alcohol is distributed evenly to the water phase of the body, including saliva, within 30 minutes after its ingestion. Among individuals with the active ALDH2 enzyme variant, this results in average acetaldehyde concentrations of ~25 μM, whereas among individuals with the ALDH2 variant with reduced activity, Acetaldehyde concentrations are twice as high (mean, ~53 μM). The long-term phase lasts as long as ethanol is present in the body and depends on the total amount of alcohol ingested.
The light blue represents the acetaldehyde produced by the microbial oxidation of ethanol; the dark blue represents excess acetaldehyde derived from salivary glands.
ALDH, aldehyde dehydrogenase.

(iii) Effects of ADH *and* ALDH2 *gene polymorphisms*

Ethanol elimination is faster among individuals with the high-activity *ADH1B*2* allele than among individuals with the low-activity *ADH1B*1* genotype. As a result, individuals with the low-activity *ADH1B*1* genotype are exposed to higher salivary acetaldehyde concentrations for longer periods of time (Yokoyama et al., 2016), and these individuals have a higher risk of squamous cell carcinomas of the upper aerodigestive tract cancer compared with individuals with the high-activity ADH1B enzyme (Higuchi et al., 2004; Yang et al., 2010; Ji et al., 2011; Chang et al., 2012; Guo et al., 2012).

Although the *ADH1C*1* allele is not associated with faster alcohol elimination (see Section 3.1.1(a)(ii)), individuals who are homozygous for the *ADH1C*1* allele have significantly higher concentrations of acetaldehyde in their saliva in the presence of ethanol compared with individuals who are heterozygous or homozygous for the *ADH1C*2* allele (Visapää et al., 2004). As a result, individuals who are homozygous for *ADH1C*1* and have heavy alcohol consumption have a significantly higher risk of head and neck cancer compared with individuals with other

genotypes (Visapää et al., 2004; Homann et al., 2006).

Among individuals with reduced ALDH2 activity (individuals who are heterozygous for *ALDH2*2*), the mean salivary acetaldehyde concentration during the long-term phase of ethanol-derived acetaldehyde formation is 2.1 times that among individuals with active ALDH2 (Väkeväinen et al., 2000; Yokoyama et al., 2008). These individuals are unable to efficiently eliminate the excess acetaldehyde formed in their salivary glands (Väkeväinen et al., 2001). Among individuals with heavy alcohol consumption, the excess acetaldehyde exposure associated with the reduced ALDH2 activity has been associated with a 7-fold higher risk of head and neck cancer (Lachenmeier and Salaspuro, 2017).

Individuals who are heterozygous for *ALDH2*2* provide a good human cancer model for local acetaldehyde exposure, underscoring the positive correlation between the risk of alcohol-related upper aerodigestive tract cancer and the elevated acetaldehyde exposure via saliva during the long-term phase of acetaldehyde formation from systemic distribution of ethanol. It should be noted that the *ALDH2* genotype has no effect on salivary acetaldehyde concentrations if ethanol is not present in the systemic circulation (Helminen et al., 2013). Consequently, the low concentrations of alcohol that are present in many "non-alcoholic" beverages and foods do not result in greater local acetaldehyde exposure in the upper aerodigestive tracts of individuals who are deficient in ALDH2 than in those of individuals with active ALDH2. This provides a logical explanation for the absence of associations between ALDH2 genotype and cancer among individuals who do not consume alcohol (Wu et al., 2014; Im et al., 2022).

(iv) Tobacco and poor oral hygiene

Alcohol consumption, tobacco use, and poor oral hygiene are synergistic risk factors for head and neck cancer (Hashibe et al., 2009; IARC, 2012; Hsiao et al., 2018). Chronic smoking, heavy alcohol consumption, and poor oral hygiene modify the oral microbiome, which results in enhanced local acetaldehyde exposure from ethanol (Fig. 3.1). How quickly the upper aerodigestive tract microbiome may return to normal after cessation of heavy alcohol consumption and/or smoking is currently unknown. For more details about the mechanistic interactions between tobacco smoking and alcohol consumption, see Section 3.1.4.

(b) Larynx

The risk of alcohol-related laryngeal cancer has been shown to be highest among individuals with the low-activity *ADH1B*1* genotype and slow or non-functional *ALDH2* genotypes, which leads to exposure of the upper aerodigestive tract mucosa to elevated local acetaldehyde concentrations for extended periods of time (Huang et al., 2017). However, no data exist about local acetaldehyde concentrations in the larynx after alcohol consumption.

(c) Oesophagus

Consumption of alcoholic beverages is causally related to squamous cell carcinoma of the oesophagus; there is no or little association with adenocarcinoma of the oesophagus (IARC, 2010). The ADH activity of the oesophageal mucosa is 7–12 times that of the oropharyngeal mucosa (Yin et al., 1993; Dong et al., 1996). Individuals who are homozygous for the highly active *ADH1C*1* allele and have heavy alcohol consumption have a significantly higher risk of oesophageal squamous cell carcinoma compared with individuals with other *ADH1C* genotypes (Visapää et al., 2004; Homann et al., 2006). In addition, ALDH activity in the oesophageal mucosa is 1/35th of that in the liver (Yin et al., 1993; Yao et al., 1997). ALDH2 deficiency markedly increases the risk of oesophageal squamous cell carcinoma in a dose-dependent manner among individuals with heavy alcohol consumption (Yang et al., 2010).

Furthermore, CYP2E1 is induced in the oesophageal mucosa by alcohol consumption (Millonig et al., 2011). These data are consistent with a role for increased local exposure to acetaldehyde in the genesis of oesophageal cancer; however, the concentrations of ethanol and acetaldehyde in the oesophagus after alcohol consumption are unknown.

(d) Colorectum

Both the mucosal and microbial oxidation of ethanol to acetaldehyde provide potential mechanisms for ethanol-related colorectal carcinogenesis.

(i) Bacteriocolonic oxidation of ethanol

The human colon can be inhabited by > 400 species of bacteria and ~10^{14} individual bacteria (Luckey, 1977; Maier et al., 2014; Sender et al., 2016). The characteristics of these bacteria and their living environment determine their functions in ethanol and acetaldehyde metabolism (Fig. 3.3; Salaspuro, 2003). The ADH activity of colonic bacteria varies greatly, as do their K_M values, which range from 0.06 mM to 29.9 mM (Jokelainen et al., 1996a; Nosova et al., 1997). In anaerobic conditions, anaerobic and facultative anaerobic bacteria that have the ADH enzyme produce endogenous ethanol from glucose. In the aerobic or microaerobic conditions that prevail close to mucosal surfaces, the bacteria produce acetaldehyde from endogenous or exogenous ethanol (Salaspuro et al., 1999).

The human colonic microbiome also has catalase activity, which probably is of bacterial origin (anaerobic and facultative anaerobic bacteria) and suggests that acetaldehyde also can be produced by catalase from intracolonic ethanol (Tillonen et al., 1998).

The colonic mucosal and bacterial ALDH enzymes have a limited capacity to eliminate acetaldehyde. As a result, acetaldehyde accumulates in the colon at ethanol concentrations known to be present in the large intestine after normal alcohol consumption (Yin et al., 1994; Koivisto and Salaspuro, 1996; Nosova et al., 1996, 1998).

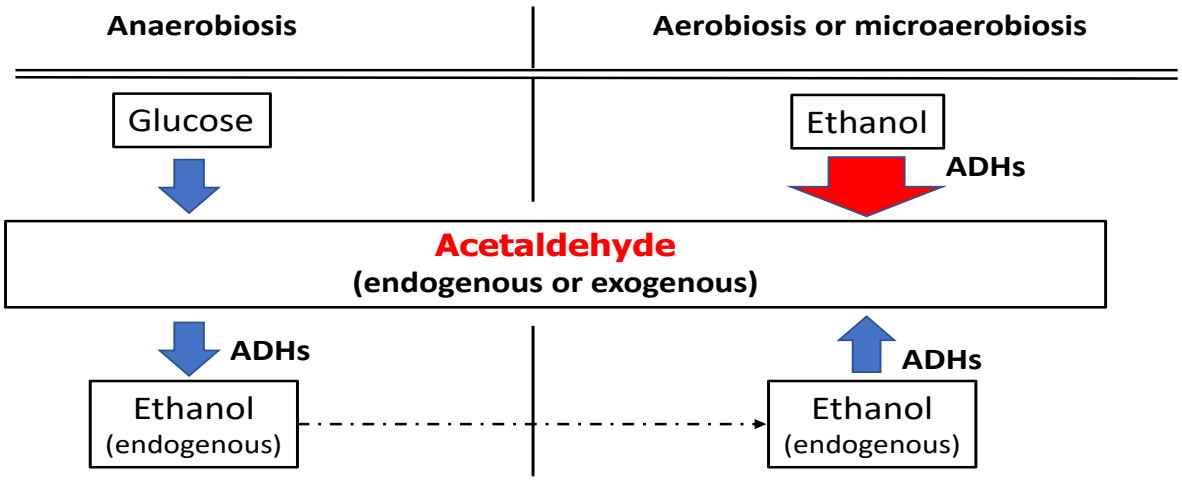
Alcohol administration results in significantly elevated acetaldehyde concentrations in the colonic mucosa of naive rats compared with germ-free animals (Seitz et al., 1990). In piglets, intracolonic acetaldehyde concentrations increased linearly (peak, 271 µM; $r = 0.85$) with increasing intracolonic ethanol levels (Jokelainen et al., 1996b). In rats, mean intracolonic acetaldehyde concentrations were 387 ± 185 µM after alcohol administration (intracolonic ethanol concentration, 28 ± 5 mM) (Homann et al., 2000b). Inhibition of ALDH2 in rats provokes a marked increase in intracolonic acetaldehyde concentration (8-fold increase vs blood concentrations) after an alcohol challenge (Visapää et al., 2002). [This supports the role of colonic mucosal ALDH2 in the regulation of intracolonic acetaldehyde levels.]

Reducing the colonic aerobic bacteria lowers the rate of ethanol elimination by ~9% in both rats and humans (Jokelainen et al., 1997; Nosova et al., 1999; Tillonen et al., 1999b). In rats, this results in almost total inhibition of the ethanol-induced increase in intracolonic acetaldehyde levels (Visapää et al., 1998). In contrast, reducing the colonic anaerobic bacteria induced a 5-fold increase in intracolonic acetaldehyde levels (Tillonen et al., 2000).

(ii) Genetic polymorphism

Human colonic mucosa expresses the ADH and ALDH enzymes (Yin et al., 1994; Seitz et al., 1996), and polymorphisms in these genes affect ethanol oxidation and elimination. ADH enzyme activity in rectal mucosa is 87% higher among individuals who are homozygous for *ADH1C*1* compared with individuals who are heterozygous (*ADH1C*1/*2* genotype); also, the activity of low-K_M enzymes (largely ALDH2) is 33% higher among individuals with the active *ALDH2* phenotype than among individuals

Fig. 3.3 Schematic representation of microbial production of acetaldehyde in the intestine



The characteristics of the microbiome and its living environment determine its functions regarding ethanol and acetaldehyde metabolism. Many bacteria and yeasts present in the normal intestinal microbiome contain alcohol dehydrogenase (ADH) enzymes with a variety of different values of the maximum velocity (V_{max}) and the Michaelis constant (K_M). Under anaerobic conditions, anaerobic and facultative anaerobic bacteria ferment glucose via pyruvate and acetaldehyde to ethanol. The second part of the reaction is catalysed by reversible ADH enzyme. Under the aerobic and microaerobic conditions that prevail in the mucosal surfaces, ethanol (endogenous or exogenous) is oxidized to acetaldehyde. The capacity of the microbiome and the mucosa to eliminate acetaldehyde is limited, which may lead to accumulation of acetaldehyde in the gastrointestinal tract.
Courtesy of Ville Salaspuro.

with the low-activity *ALDH2* phenotype (Chiang et al., 2012). Individuals with heavy alcohol consumption who are deficient in ALDH2 have a risk of colorectal cancer that is 3.4 times that among individuals with the active ALDH2 enzyme (Yokoyama et al., 1998; Murata et al., 1999; Matsuo et al., 2002). Also, the highly active *ADH1C*1/*1* genotype is more common (odds ratio, 1.67) in patients with colorectal neoplasia who have heavy alcohol consumption than in cancer-free controls (Homann et al., 2009). These observations support a causal role for acetaldehyde in alcohol-related colorectal carcinogenesis.

(e) Liver

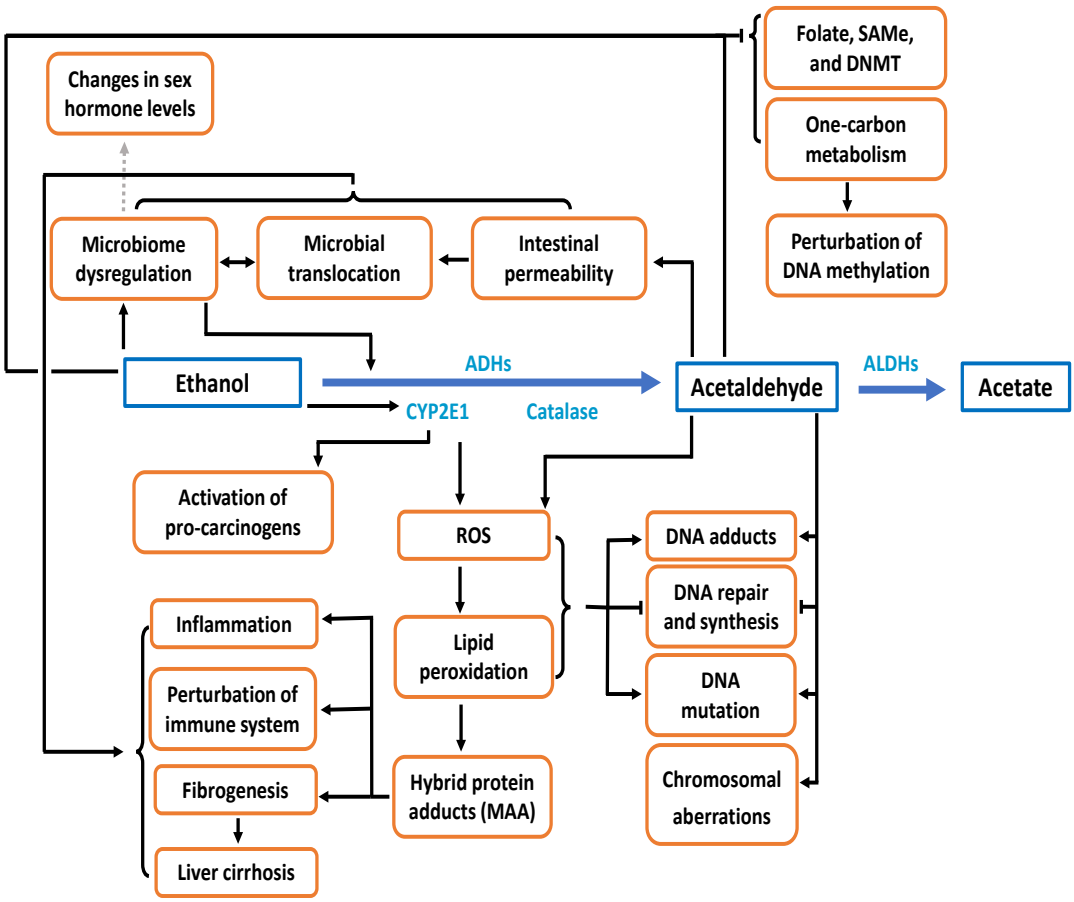
Chronic alcohol consumption is a risk factor for hepatocellular carcinoma (HCC), which is associated mainly with cirrhosis of the liver (IARC, 2012). Several studies have reported higher *ALDH2*1* allele frequency among individuals of Asian ancestry who have alcohol-related cirrhosis compared with healthy controls, suggesting that the inactive *ALDH2*2* allele does not predispose individuals with heavy alcohol consumption to HCC (Wang et al., 2020). Studies of the polymorphisms of *ALDH2* and *CYP2E1* indicate that acetaldehyde plays an insignificant role in hepatocellular carcinogenesis (Zhou et al., 2012; Chen et al., 2020). However, among individuals with hepatitis B virus-related cirrhosis and heavy alcohol consumption, those with the *ALDH2*2* allele have a significantly higher risk of HCC compared with individuals with the fully active ALDH2*1 phenotype (Tsai et al., 2022).

In addition to promoting cirrhosis, alcohol probably increases the risk of HCC by mechanisms that are not mediated by local levels of acetaldehyde (Fig. 3.4; Section 3.1.3).

(f) Breast

Although breast cancer is one of the most prevalent alcohol-related cancers, mechanisms of ethanol-induced breast cancer are hypothetical and largely unclear (Seitz et al., 2012; Castro and Castro, 2014; Ugai et al., 2019; Park et al., 2020; Mori et al., 2023). Human mammary tissue contains a class of ADH that has a limited potential to transform ethanol to acetaldehyde (Triano et al., 2003). There is no evidence that *ALDH2* is active in human breast tissue. Breast milk from women who are lactating does not contain measurable levels of acetaldehyde after they consume alcohol (Kesäniemi, 1974). Genetic polymorphism of ethanol- or acetaldehyde-metabolizing enzymes has not been shown to modify alcohol-related breast cancer risk (Freudenheim, 2020). An increased risk of breast cancer has been demonstrated among individuals who are homozygous for the *ALDH2*2* allele (Ugai et al., 2019); however, no evidence has been observed of

Fig. 3.4 Mechanisms for alcohol-induced carcinogenesis among humans



The grey dashed line indicates a hypothetical mechanism. For more details, see Section 3.1.3.
ADH, alcohol dehydrogenase; ALDH, aldehyde dehydrogenase; CYP2E1, cytochrome P450 2E1; DNMT, DNA methyltransferase; MAA, malondialdehyde–acetaldehyde–albumin adducts; ROS, reactive oxygen species; SAMe, *S*-adenosyl-L-methionine.
Prepared by the Working Group.

an interaction between the *ALDH2*2* genotype and alcohol consumption.

The CYP2E1 protein is expressed in both normal human breast and breast tumour tissues, and levels are higher in breast tumours (Kapucuoglu et al., 2003). However, no significant relationship has been found between the *CYP2E1* polymorphism and risk of breast cancer (Lu et al., 2017). Thus, the higher risk of breast cancer among women who consume alcohol does not appear to be mediated by local exposure to acetaldehyde.

Potential mechanisms of alcohol-related breast carcinogenesis possibly mediated by ethanol or acetaldehyde metabolism include oxidative stress, increased cell proliferation, effects on the intestinal microbiome, effects on sex and steroid hormones, and effects on one-carbon metabolism (see Section 3.1.3).

3.1.3 Alcohol-related mechanisms of carcinogenesis

Alcohol consumption leads to many disruptive changes in the human body, and several mechanisms have been described that could potentially be involved in alcohol-related carcinogenesis (Fig. 3.4). These have been discussed in many reviews (e.g. Rodriguez and Coveñas, 2021; Rumgay et al., 2021). A brief overview is presented here.

The genotoxic effects of alcohol have been comprehensively reviewed in several *IARC Monographs* volumes (IARC, 1988, 2010, 2012). The genotoxicity of ethanol is mediated mainly by its metabolism to acetaldehyde (discussed in Section 3.1.1). Acetaldehyde reacts with DNA, resulting in DNA damage that includes DNA adducts, chromosomal aberrations, and mutations (Guidolin et al., 2021; Hoes et al., 2021). The ethanol-inducible CYP2E1 enzyme (see Section 3.1.1) produces various reactive oxygen species, which lead to the formation of lipid peroxidation products such as malondialdehyde, and to oxidative stress, which can also lead to DNA damage and perturbation of DNA repair (Linhart et al., 2014). Moreover, acetaldehyde and malondialdehyde can react synergistically with proteins to form hybrid protein adducts, designated as malondialdehyde–acetaldehyde–albumin adducts, which are very immunogenic and have pro-inflammatory and fibrogenic properties. These adducts have been detected in patients with liver cirrhosis and hepatitis and may contribute to the development of alcohol-related liver damage (Rolla et al., 2000; Tuma, 2002). By inducing CYP2E1, alcohol also stimulates the metabolism of pro-carcinogens into carcinogens (Gao et al., 2018; Song et al., 2019).

Chronic alcohol consumption has a strong impact on both the oral microbiome and the intestinal microbiome. Alcohol consumption alters the composition of the intestinal microbiome, enhancing local levels of acetaldehyde production (discussed in Section 3.1.2(d)). High concentrations of acetaldehyde have a direct inhibitory effect on proteins involved in the formation of adherens junctions and tight junctions, which leads to epithelial barrier dysfunction and intestinal permeability. This results in increased translocation of microbiota and endotoxins (microbial products and lipopolysaccharide [LPS]) across the mucosa (Rao, 2009). The intestines and the liver are directly connected via the portal vein; microbial translocation from the intestines to the liver elicits chronic hepatic inflammation, severe hepatic injury such as cirrhosis, and eventually HCC (Giraud and Saleh, 2021; Ohtani and Hara, 2021; Petagine et al., 2021). Microbial translocation and endotoxaemia also trigger systemic inflammation, with increased risk of cancer through the effects of oxidative stress, changes in cytokine levels, and impaired anti-tumour immune systems (Greten and Grivennikov, 2019). In the oesophagus, chronic alcohol consumption could cause an inflammatory process known as pyroptosis, which may contribute to the development of

oesophageal cancer (Wang et al., 2018). Alcohol consumption also increases the permeability of the oral mucosa (Howie et al., 2001), possibly rendering the mouth more sensitive to the effects of other carcinogens, such as those found in tobacco smoke (Feller et al., 2013).

Additional mechanisms of alcohol-related carcinogenesis include reduction of folate concentrations in the colonic mucosa by local acetaldehyde generation. Heavy alcohol consumption reduces adsorption of folate, enhances urinary excretion of folate, and inhibits enzymes that are pivotal for one-carbon metabolism. Aberrant DNA methylation due to a deficiency in methyl donors is a common effect of alcohol-related folate deficiency (Sharma and Krupenko, 2020). Among women, alcohol consumption also increases the concentrations of estradiol, testosterone, and several other sex hormones in the circulation and decreases the concentration of sex hormone-binding globulin (SHBG), and these changes are hypothesized to be related to risk of breast cancer (Key et al., 2011; Freudenheim, 2020). Recently, an association between alterations in the intestinal microbiome and breast cancer has been reported, suggesting that the microbiome may play a role in regulating estrogen levels (Kwa et al., 2016; Parida and Sharma, 2019). In addition, consumption of alcoholic beverages leads to concentrations of estradiol that are 3-fold higher among women who are taking oral estrogen and progestin as postmenopausal hormone therapy (Ginsburg et al., 1996). Furthermore, in the Women's Alcohol Study, a controlled feeding trial conducted among healthy non-smoking postmenopausal women in the USA, direct assessments were performed of the impact of 1 or 2 drinks per day versus no drinks per day, and the participants served as their own controls in this feeding study. Changes in several end-points, including biomarkers of estrogen metabolism, oxidative stress, and inflammation, were measured, providing valuable insights into the effects of ethanol on mechanistic pathways relevant to cancer (Dorgan et al., 2001; Laufer et al., 2004; Mahabir et al., 2004, 2017; Hartman et al., 2005; Stote et al., 2016).

3.1.4 Mechanistic interactions between alcohol consumption and tobacco smoking

Epidemiological studies have provided consistent evidence for a synergistic interaction between alcohol consumption, tobacco smoking, and risk of cancers at several sites, including squamous cancers of the head and neck and the oesophagus (Hashibe et al., 2009; Anantharaman et al., 2011; Radoï et al., 2013). Data suggest several possible mechanisms associated with more risk than the effects of the two carcinogenic exposures combined:

(i) Alcohol may have a local permeabilizing effect on penetration of the oral mucosa by tobacco carcinogens (Du et al., 2000).

(ii) Induction of CYP2E1 by ethanol increases metabolic activation of tobacco carcinogens, leading to enhanced formation of reactive chemical species at target sites (IARC, 2012).

(iii) Ethanol also acts as a competitive inhibitor of CYP enzymes (e.g. CYP2E1, CYP1A1, 2B6, and 2C19). Direct inhibition of CYPs by ethanol in target tissues may increase exposure to genotoxic tobacco carcinogens that are substrates for these CYP enzymes (IARC, 2012).

(iv) Chronic smoking combined with chronic heavy alcohol consumption induces changes in the oral microbiome, especially in microbial strains that have high acetaldehyde formation activity (Homann et al., 2000a). Salaspuro and Salaspuro (2004) demonstrated that tobacco use modifies the efficiency of conversion of ethanol to acetaldehyde in the oral cavity, by measuring salivary acetaldehyde concentrations among individuals during controlled

exposures to ethanol and cigarette smoke (Fig. 3.5). Compared with individuals who do not smoke, individuals who smoke had an approximately 2-fold higher level of acetaldehyde in their saliva (~25 µM vs ~50 µM), even when they were not currently smoking, and this difference persisted for hours (Fig. 3.5A). After an ethanol challenge, salivary acetaldehyde concentrations during concomitant smoking among individuals who smoke were 7-fold higher than those among individuals who did not smoke, reaching 350–400 µM (Fig. 3.5B). Acetaldehyde is also a component of tobacco smoke, and smoking a single cigarette – without concomitant alcohol consumption – results in a rapid increase in salivary acetaldehyde concentrations (up to 250 µM), followed by a rapid decrease within 5–10 minutes (Salaspuro and Salaspuro, 2004). These data imply that in organs that have direct contact with saliva, exposure to tobacco and alcohol together results in more local acetaldehyde than the sum of the two exposures alone.

Fig. 3.5 Synergistic effect of alcohol consumption and tobacco smoking on salivary acetaldehyde concentration

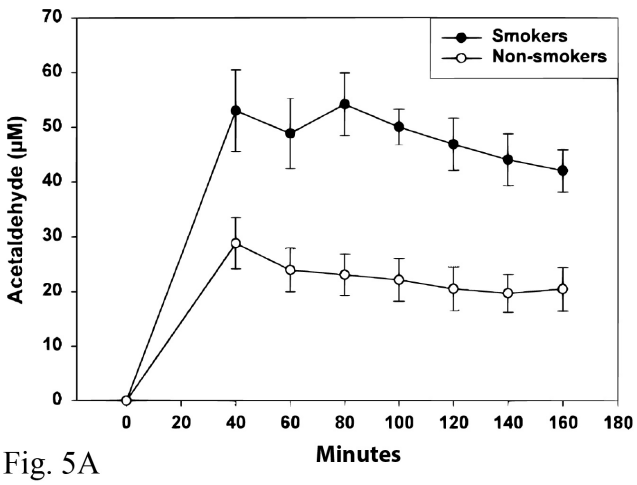


Fig. 5A

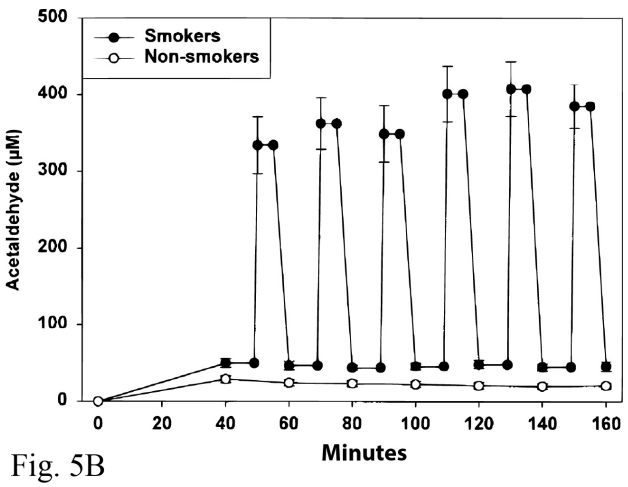


Fig. 5B

Acetaldehyde is present in tobacco smoke; without concomitant ethanol intake, salivary acetaldehyde concentration immediately increases on tobacco smoking to ~260 µM, and decreases within 10 minutes.
(A) After ethanol ingestion but without concomitant smoking, salivary acetaldehyde concentrations among individuals who smoke (smokers) are 2 times those among individuals who do not smoke (non-smokers). Differences between acetaldehyde concentrations were significant at all time points ($P < 0.05$).
(B) After an ethanol challenge (0.8 g of ethanol per kg of body weight), salivary acetaldehyde levels (area under the curve) among participants who were actively smoking (smokers) and who concomitantly smoked (i.e. 1 cigarette every 20 minutes) were 7 times those among individuals who did not smoke (non-smokers). Each peak corresponds to one cigarette smoked.
In both (A) and (B), the peak that would correspond to the instant phase of alcohol consumption alone (as shown in Fig. 3.2) does not appear, because in these experiments acetaldehyde was first measured 40 minutes after ethanol intake.
Adapted from Salaspuro and Salaspuro (2004).

3.2 Cancer-related mechanistic changes after cessation of alcohol consumption

3.2.1 Study designs and limitations of the available studies

Section 3.2 reviews and assesses mechanistic data related to the effect of alcohol cessation on risk of cancer. No mechanistic studies of reduction in alcohol consumption (rather than cessation) were available to the Working Group. Several limitations of the available studies pertain to the different sections and are summarized here. First, several studies evaluated a relatively small number of individuals. A common research design was to examine changes in biomarkers among individuals at entry into a treatment programme for AUD and at intervals after cessation of alcohol consumption, and a comparison may have been made with healthy individuals in a control group or with clinically normal ranges for values, if available. In other studies, the comparison was between individuals with AUD who had become abstinent years before and individuals in a control group, meaning that the comparisons were between different groups of individuals. A small number of studies tested the effect of short-term alcohol exposure in a controlled setting.

In many studies, reporting of duration and intensity of previous alcohol consumption was lacking and participants were categorized as entering rehabilitation treatment or having AUD. It is worth noting that alcohol dependence and AUD were distinctly used in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV; American Psychiatric Association, 1994), whereas the 5th edition (DSM-5; American Psychiatric Association, 2013) conserved only the AUD terminology, which encompasses both alcohol dependence and AUD of DSM-IV (NIAAA, 2021). When information on previous alcohol consumption was provided, classification of levels of alcohol consumption varied (e.g. excessive vs non-excessive; mild, moderate, or heavy based on units of alcohol consumed per day); proof of abstinence in long-term studies was not always provided, and often the level of alcohol consumption among participants in the control group was not stated (e.g. not consuming alcohol vs consuming alcohol at a low level).

The demographic makeup of the studies was in many cases predominantly men and often only people of European ancestry. Sometimes, potential confounders (other medical illnesses, especially the presence of subclinical liver disease, smoking, nutritional status, and obesity) were not controlled for. It was difficult to appreciate the effect of the stress of alcohol withdrawal itself on the outcome measures, and the potential effects of pharmacological treatments used during rehabilitation or of compensatory behaviours (e.g. increase in cigarette smoking). Given the critical role of the alcohol–tobacco interaction in cancer etiology, lack of control for tobacco use and changes in use over the time course of these studies is a major limitation. Studies of inpatients in rehabilitation treatment centres did not take into account the change, and often improvement, in their dietary intake while they were hospitalized.

There were few studies on the target tissues of alcohol-related carcinogenesis, and surrogates such as circulating white blood cells may not reflect changes in the target organ. Given the known long period of persisting increased risk of cancer after cessation of alcohol consumption, few studies were of sufficient duration to evaluate for full resolution of the effects under consideration.

3.2.2 Genotoxicity

Studies investigating the genotoxic effects of alcohol consumption have focused mainly on measuring and quantifying chromosomal

aberrations and micronuclei. Several studies have focused more specifically on measuring covalent modification of DNA (DNA adducts). A few studies have investigated mitochondrial DNA deletions. Most of the studies measured DNA damage in peripheral blood mononuclear cells [a surrogate sample with cells characterized by type of exposure and turnover quite different from those characterizing the target tissues].

The body of literature that addressed the reversibility of ethanol-related DNA-damaging effects is summarized below. Detailed information about each study is given in Table 3.1. When assessing long periods of abstinence, most of the studies included groups of individuals with AUD, individuals who ceased consuming alcohol, and controls. Studies assessing the change in DNA damage among individuals with AUD who cease consuming alcohol are limited to observations during a period of at most 1 year. Some studies investigated the effects of alcohol ingestion by comparing the DNA damage before and after consumption of a specific dose of alcohol.

A first set of studies compared DNA damage among individuals who currently consume alcohol and among individuals who formerly consumed alcohol and had abstained for a certain period of time.

Castelli et al. (1999) compared the frequency of peripheral blood cells with chromosomal aberrations (percentage of aberrant cells) and of micronuclei (number of micronuclei per 1000 binucleated cells) among 3 groups composed of 11 participants with AUD, 9 participants with AUD who abstained from alcohol consumption for ≥ 1 year, and 10 healthy controls. All study participants, except for 3 individuals among the group with AUD and 4 individuals among the group that abstained, smoked heavily. The group with AUD had a significantly higher frequency of chromosomal aberrations (mean ± standard deviation [SD]), 4.00% ± 2.27%) than the group that abstained (no frequency provided) and the healthy controls (0.90% ± 0.74%) ($P < 0.01$). Similarly, the group with AUD had a higher frequency of micronuclei (11.00% ± 4.11%) than the group that abstained (no frequency provided) and the healthy controls (5.11% ± 2.60%) ($P < 0.05$). [The frequencies for the group that abstained were not reported; there was a lower percentage of individuals who smoked heavily in this group (5 of 9 individuals who abstained vs 8 of 11 individuals with AUD).]

Maffei et al. (2002) analysed the same markers in peripheral blood lymphocytes from 20 people with AUD, 20 people with AUD who abstained (for ≥ 1 year), and 20 controls who did not consume alcohol, with comparable composition for sex, age, and smoking status. The group with AUD had a significantly higher frequency of structural chromosomal aberrations (chromatid breaks and exchanges and chromosome breaks and exchanges) (mean ± SD, 4.35% ± 2.06%) than the group that abstained (2.00% ± 1.21%; $P = 0.001$) and the controls (1.45% ± 0.83%; $P = 0.001$). The group with AUD had a significantly higher frequency of binucleated cells with micronuclei (mean ± SD, 12.05% ± 5.43%) than the group that abstained (7.15% ± 2.64%; $P = 0.001$) and the controls (7.60% ± 1.57%; $P = 0.001$). The frequencies for the group that abstained were similar to those for the controls. A multiple regression analysis was performed to investigate whether the duration of AUD or of abstinence was correlated with either the frequency of chromosomal aberrations or the frequency of micronuclei, but none of these analyses resulted in significant findings.

Another set of studies monitored changes in DNA damage among individuals with AUD who abstained from consuming alcohol for various durations of time.

In a study in Japan, Matsushima (1987) compared the frequencies of chromosomal aberrations in lymphocytes from 25 participants who formerly consumed alcohol and reported varying periods of abstinence (< 5 years, 5–10 years, > 10 years) with those among 17 participants

Table 3.1 Effects of cessation of alcohol consumption on genotoxicity

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Matsushima (1987) Japan	17 individuals with chronic AUD (ages, 22–74 years) 25 individuals with AUD who abstained (ages, 39–69 years), divided into 3 groups according to duration of abstinence: < 5 years, 5–10 years, and > 10 years Controls: 10 healthy volunteers, all women (ages, 19–21 years)	Controls were not matched All women, younger, non-drinkers, and non-smokers	All individuals with chronic AUD except 1 had been drinking heavily for > 10 years Individuals with AUD who abstained had been drinking heavily for > 20 years	Peripheral lymphocytes Chromosomal aberrations	Values are mean ± SD **Chromosome profiles (%)** ***Abnormal metaphases*** Individuals with chronic AUD: 20.6 ± 6.5 Individuals with AUD who abstained: 11.1±3.9*** Controls: 10.2 ± 3.1*** ***Gaps*** Individuals with chronic AUD: 14.4 ± 6.0 Individuals with AUD who abstained: 8.0 ± 3.4*** Controls: 6.9 ± 2.7*** ***Breaks*** Individuals with chronic AUD: 6.1 ± 3.2 Individuals with AUD who abstained: 2.7 ± 2.4*** Controls: 3.8 ± 2.2 ***Dicentric chromosomes*** Individuals with chronic AUD: 1.2 ± 1.0 Individuals with AUD who abstained: 0.4 ± 0.5*** Controls: 0.2 ± 0.6** ***Rings*** Individuals with chronic AUD: 1.1 ± 1.2 Individuals with AUD who abstained: 0.2 ± 0.5*** Controls: 0.2 ± 0.6* ***Interchanges*** Individuals with chronic AUD: 0.2 ± 0.5 Individuals with AUD who abstained: 0.0 Controls: 0.0 *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs individuals with chronic AUD

Table 3.1 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Gattás and Saldanha (1997) Brazil	55 individuals with AUD who abstained (45 men; ages, 24–70 years; 10 women; ages, 29–63 years); mostly heavy smokers Controls: 55 healthy volunteers (31 men; ages, 23–56 years; 24 women; ages, 19–47 years)	Sex and age Controls were not screened for alcohol consumption or smoking Smoking, drug use, and sex were considered in the statistical analysis	Individuals with AUD had been drinking heavily for > 10 years before abstinence Duration of abstinence: range, 1 month to 32 years (average, 46 months)	Peripheral lymphocytes Chromosomal aberrations	**Cells with structural aberrations (%)** Individuals with AUD who abstained: 7.1 Controls: 2.4 $P < 0.0001$
Castelli et al. (1999) Italy	11 individuals with chronic AUD (4 women, 7 men; ages 29–63 years) 9 individuals with AUD who abstained (2 women, 7 men; ages 31–69 years) Controls: 10 healthy individuals (4 women, 6 men; ages 30–60 years) All individuals were in a fair state of general nutrition	All individuals with AUD except 3 and all individuals with AUD who abstained except 4 were heavy smokers (> 20 cigarettes per day); all controls were heavy smokers Sex and age	Alcohol consumption: > 120 g per day Duration of alcohol consumption: average, 19 years (range, 3–30 years) Duration of abstinence: ≥ 1 year	Peripheral lymphocytes Chromosomal aberrations and micronuclei score	Values are mean ± SD **Aberrant cells (%)** Individuals with chronic AUD: 4.00 ± 2.27 Controls: 0.90 ± 0.74 Individuals with AUD who abstained: similar to controls $P_{\text{AUD vs controls}} < 0.01$ **Micronuclei/1000 binucleated cells** Individuals with chronic AUD: 11.00 ± 4.11 Controls: 5.11 ± 2.60 Individuals with AUD who abstained: similar to controls $P_{\text{AUD vs controls}} < 0.05$
Hüttner et al. (1999) Germany	31 individuals with chronic AUD (26 men; ages, 23–59 years; 5 women; ages, 36–48 years) Controls: 31 healthy non-drinking volunteers (26 men, 5 women; ages, 24–60 years)	Drinking status, smoking status, sex, and age	Alcohol consumption: 120–400 g per day Duration of AUD: 5–37 years	Peripheral lymphocytes Samples collected after abstinence for 1 week (31 participants), 3 months (8 participants), and 1 year (14 participants) Chromosomal aberrations	Values are mean ± SD (range) **Aberrant cells (%)** Controls: 1.28 Individuals with chronic AUD First week of abstinence: 3.01 ± 1.17 (0.50–5.50) $P_{\text{first week vs controls}} \leq 0.001$ After 3 months of abstinence: 3.81 ± 1.16 (2.50–6.00) After 1 year of abstinence: 4.61 ± 2.14 (1.50–9.00) $P_{\text{first week vs 1 year of abstinence}} < 0.001$

Table 3.1 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Tsuchishima et al. (2000) Japan	4 healthy volunteers 56 individuals with ALD who abstained (mean age ± SD, 57 ± 11 years) Controls: 106 healthy individuals without chronic AUD (mean age ± SD, 54 ± 18 years)	Age	Healthy volunteers abstained from alcohol for ≥ 1 month and were then administered 23 g of ethanol every night until white blood cell mitochondrial DNA heteroplasmy was detected Individuals with ALD had consumed > 80 g of ethanol per day for > 5 years	White blood cells For individuals with ALD who abstained, samples collected after abstinence for 3 days (56 individuals) and 4 weeks (18 individuals) Mitochondrial DNA heteroplasmy within the ATPase region (PCR and fluorography)	**Number of individuals with heteroplasmy/total number of individuals (%)** Controls: 0/106 (0%) Healthy volunteers 4 days after start of alcohol intake: 4/4 (100%) 7 days after alcohol cessation: 0/4 (0%) Individuals with ALD who abstained After 3 days of abstinence: 38/56 (68%) After 4 weeks of abstinence: 8/18 (44%)
Maffei et al. (2002) Italy	20 individuals with chronic AUD (mean age ± SD, 49.9 ± 9.9 years) 20 individuals with AUD who abstained (mean age ± SD, 52.2 ± 10.6 years) Controls: 20 (mean age ± SD, 47.5 ± 10.2 years) 13 men and 7 women in each group	Sex, age, and smoking status	Alcohol consumption for 4–40 years, > 120 g per day. Abstainers had the same consumption for ≥ 5 years (range, 12–60 years), were abstinent for ≥ 1 year (range, 12–60 years)	Peripheral lymphocytes Chromosomal aberrations and micronuclei	Values are mean ± SD **Aberrant cells (%)** Individuals with chronic AUD: 4.10 ± 1.94 Individuals with AUD who abstained: 1.95 ± 1.10 Controls: 1.45 ± 0.83 $P_{\text{AUD vs abstaining AUD and controls}} = 0.001$ **Structural chromosomal aberrations (%)** Individuals with chronic AUD: 4.35 ± 2.06 Individuals with AUD who abstained: 2.00 ± 1.21 Controls: 1.45 ± 0.83 PAUD vs abstaining AUD and controls = 0.001 **Binucleated cells with micronuclei (%)** Individuals with chronic AUD: 12.05 ± 5.43 Individuals with AUD who abstained: 7.15 ± 2.64 Controls: 7.60 ± 1.57 $P_{\text{AUD vs abstaining AUD and controls}} = 0.001$

Table 3.1 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Maffei et al. (2002) (cont.)					**NDI** Individuals with chronic AUD: 1.38 ± 0.16 Individuals with AUD who abstained: 1.44 ± 0.13 Controls: 1.37 ± 0.05 Mean NDI values were similar in the 3 groups
Burim et al. (2004) Brazil	29 individuals with chronic AUD (23 men, 6 women; 20 smokers, 9 non-smokers) 11 individuals with AUD who abstained (9 men, 2 women; 4 smokers, 7 non-smokers) Controls: 10 healthy volunteers (9 men, 1 woman; 5 smokers, 5 non-smokers)	Not reported	Individuals with chronic AUD: > 60 g of alcohol consumption per day for ≥ 3 years Individuals with AUD who abstained: 3 months to 4 years of abstinence	Peripheral lymphocytes Chromosomal aberrations (mitotic indexes, proliferation indexes); genomic translocation (FISH)	**Chromosomal aberrations (per 100 cells), mean ± SEM** Individuals with chronic AUD: 5.15 ± 0.37* Individuals with AUD who abstained: 3.87 ± 0.34* Controls: 1.72 ± 0.52 *Significantly different from controls, $P < 0.001$ **Genomic frequency of translocations (F_G/100)** Individuals with chronic AUD: 0.790 Individuals with AUD who abstained: 0.577 Controls: 0.198 $P_{\text{AUD vs controls}} < 0.05$ Chromosomal aberration frequency and duration of abstinence: direct association Chromosomal aberration frequency and increased periods of dependence: no association
Balbo et al. (2012) USA	10 healthy volunteers with moderate alcohol consumption (5 men, 5 women; ages 21–31 years) with no history of AUD Controls: baseline samples from the same participants serve as their own controls	Sex and age; all non-smokers	Increasing doses of alcohol to target blood alcohol levels of 0.03% (~1 drink) (week 1), 0.05% (~2 drinks) (week 2), and 0.07% (~3 drinks) (week 3), based on body weight and sex	Oral cell DNA Samples collected 1 week before consumption of the first dose, and also before and 2, 4, 6, 24, 48, and 120 hours after each dose of alcohol Acetaldehyde-derived DNA adduct N^2-ethylidene-dGuo (LC/MS)	**N^2-ethylidene-dGuo** Increased up to 100-fold above baseline within 4 hours after each dose in a dose-dependent manner Returned to baseline concentrations within 24 hours after alcohol intake $P = 0.001$

ALD, alcohol-related liver disease; ATPase, adenosine triphosphatase; AUD, alcohol use disorder; F_G, genomic frequency of translocations; FISH, fluorescence in situ hybridization; LC, liquid chromatography; MS, mass spectrometry; N^2-ethylidene-dGuo, N^2-ethylidenedeoxyguanosine; NDI, nuclear division index; PCR, polymerase chain reaction; SD, standard deviation; SEM, standard error of the mean.

with AUD and 10 healthy controls. The individuals with AUD had a significantly higher frequency of abnormal metaphases (mean ± SD, 20.6% ± 6.5%) than the individuals who formerly consumed alcohol (11.1% ± 3.9%; $P < 0.001$) and the controls (10.2% ± 3.1%; $P < 0.001$). No significant correlation was found between the number of years of abstinence and the frequency of chromosomal aberrations. The frequency of chromosomal aberrations in the three groups with varying durations of abstinence was comparable to that observed among the controls. [The control group included only women, of much younger age and non-smoking, whereas among the groups of individuals who currently or formerly consumed alcohol, all participants except for one were men and all smoked.]

Gattás and Saldanha (1997) compared the frequencies of structural or numerical chromosomal aberrations in lymphocytes from 55 participants with AUD who had been abstinent for from 1 month to 32 years with those among 55 healthy controls. The participants with AUD who abstained had an almost 3-fold higher frequency of structural chromosomal aberrations in peripheral blood lymphocytes compared with the controls ($P < 0.0001$), and aberrations such as breaks, gaps, and rearrangements were more prevalent among the participants with AUD. The frequency of structural chromosomal aberrations did not change with increased duration of abstinence; the frequency (mean ± SD) was 10.23% ± 1.5% for the individuals with AUD who had abstained for > 5 years and 9.17% ± 1.7% for those who had abstained for < 5 years ($P = 0.7$). [Controls were not screened for alcohol consumption, and comparisons were not made with a group of participants who currently consume alcohol, resulting in some limitations in this study. As the period of abstinence gets longer, other factors and exposures could contribute to these aberrations. Specifically, they could be affected by smoking (in particular, when smoking intensity increases to make up for the abstinence from alcohol consumption) and ageing.]

Hüttner et al. (1999) investigated the frequency of structural chromosomal aberrations in peripheral blood lymphocytes from 31 individuals with chronic AUD at the beginning of a treatment programme and then repeated the analysis on a subset of participants at 3 months and 12 months after the start of the sobriety programme. Most individuals recruited into the study smoked. The mean frequency of chromosomal aberrations among the individuals with AUD during the first week of abstinence (3.01%) was significantly higher than that among the 31 controls who did not consume alcohol (1.28%; $P \leq 0.001$). The mean frequency of chromosomal aberrations was 3.81% among the group of 8 individuals with AUD who were re-analysed after 3 months of abstinence, and it increased to 4.61% after 12 months of abstinence. This increase from the first sample to the third was significant ($P < 0.001$). [The increase over time was attributed to an increase in smoking (documented by measurement of carboxyhaemoglobin) in compensation for alcohol abstinence.]

Burim et al. (2004) compared the frequency of chromosomal aberrations in lymphocytes from 29 individuals with chronic AUD, 11 individuals with AUD who abstained (over a duration of 3 months to 4 years), and 10 controls. The frequencies of chromosomal aberrations among the individuals with chronic AUD (5.15%) and among the individuals who abstained (3.87%) were higher than those among the controls (1.72%). Chromosomal translocations for chromosomes 1, 3, and 6 were analysed in a subset of samples from 6 individuals with AUD, 6 individuals with AUD who abstained, and 6 controls, using a fluorescence in situ hybridization method. The calculated genomic translocation frequencies were not significantly different between individuals with AUD and individuals with AUD who abstained, suggesting that DNA damage may persist for a long time. [The

measurement of translocation frequency was performed on samples from 6 individuals per group only. The group with AUD who abstained was older, on average, than the group with AUD who did not abstain.]

Another set of studies focused on quantifying acetaldehyde-derived DNA adducts in genomic DNA isolated from samples collected from individuals with AUD and compared with controls (Fang and Vaca, 1995; Matsuda et al., 2006).

A specific investigation of the effects of alcohol abstinence on the levels of these adducts is currently missing from the literature. However, Balbo et al. (2012) measured the levels of the major acetaldehyde-derived DNA adduct, N^2-ethylidenedeoxyguanosine (N^2-ethylidene-dGuo), in oral cell DNA isolated from healthy participants who were exposed to three increasing doses of alcohol administered once a week for 3 weeks in a controlled clinical setting and resulting in a blood alcohol level of 0.03% ± 0.01% for week 1, 0.05% ± 0.01% for week 2, and 0.07% ± 0.01% for week 3. Oral cell samples were collected 1 week before ingestion of the first dose and 2, 4, 6, 24, 48, and 120 hours after each dose. A significant increase in the levels of N^2-ethylidene-dGuo was detected after ingestion of the lowest dose (comparable to ~1 standard alcoholic drink). The adduct levels increased significantly, as much as 100-fold from baseline, within 4 hours after each dose among all the participants and in a dose-dependent manner. The adduct levels returned to baseline within 24 hours after each dose was administered.

Tsuchishima et al. (2000) investigated the DNA-damaging effects of alcohol consumption on mitochondrial DNA. Heteroplasmy (the presence of ≥ 2 mitochondrial DNA variants within the same cell, usually due to de novo mutations in the germline or somatic tissues) in peripheral blood mitochondrial DNA was assessed by using polymerase chain reaction (PCR) to amplify the adenosine triphosphatase region with a 491-base pair deletion. Healthy volunteers (n = 4) were exposed to alcohol for several days until heteroplasmy was detected (shortly before the beginning of day 4); then participants abstained from alcohol and were followed up for several days by collecting daily samples from them. Mitochondrial DNA heteroplasmy was no longer detected in any of the participants 7 days after they became abstinent. [Only 4 volunteers were included in the alcohol dosing part of the study.] The same assessment was performed on blood collected from patients with alcohol-related liver disease (ALD) who had abstained for 4 weeks. Among 10 of the 18 patients tested, the mitochondrial DNA heteroplasmy disappeared within 4 weeks of abstinence.

3.2.3 Epigenetics

Alcohol-induced epigenetic modifications have been implicated in variations in ethanol consumption (Wolstenholme et al., 2011), addiction (Berkel and Pandey, 2017), and mediation of physiological responses to alcohol exposure, and they may serve as biomarkers of exposure (Liu et al., 2018). Mechanistic studies of alcohol withdrawal have reported a range of effects of epigenetic modifications, with most studies examining methylation, including global DNA hypomethylation and hypermethylation of individual gene promoters. Chronic alcohol exposure can reduce folate availability, which deprives enzymes of the methyl groups that process methylation changes to DNA. Ethanol and acetaldehyde alter the activity of methionine synthase, methionine adenosyltransferase, and DNA methyltransferase (Varela-Rey et al., 2013). Detailed studies have catalogued the epigenetic modifications associated with alcohol exposure in cancers of the upper aerodigestive tract, liver, colorectum, and breast (Varela-Rey et al., 2013). A few studies have examined how a reduction in alcohol exposure modulates these mechanisms with or without abstinence (Table 3.2).

Table 3.2 Effects of cessation of alcohol consumption on epigenetic modifications

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)	Results[a]
Heberlein et al. (2015) Germany	99 men with AD (mean age ± SD, 42.90 ± 9.01 years) admitted for detoxification treatment Controls: 33 healthy men (mean age ± SD, 42.2 ± 10.32 years) All individuals who participated in this study were active smokers Exclusion criteria: psychiatric illness, substance abuse other than alcohol or nicotine, severe somatic illnesses, known autoimmune diseases, known HPA axis deregulations, and history of cerebral damage	Age Adjusted for carbamazepine and clomethiazole dose and for thrombocyte count for *BDNF*	Consumption (mean ± SD) of 195.43 ± 81.61 g of alcohol per day for a duration (mean ± SD) of 9.79 ± 7.67 years	Blood DNA and serum Samples collected at abstinence and on days 1, 7, and 14 CpG methylation within the promoter regions of *BDNF* exon IV (bisulfite sequencing); serum BDNF concentration (ELISA)	Significant change in mean overall and *BDNF* promoter methylation during alcohol withdrawal ($P < 0.001$) Significant association between *BDNF* promoter methylation and duration of abstinence on day 14 ($P < 0.001$) *BDNF* serum concentrations were not correlated with mean methylation ($P = 0.170$) or methylation of individual CpG dinucleotides ($P = 0.322$)
Witt et al. (2020) Germany	99 men with AUD (mean age ± SD, 47.6 ± 9.1 years), of which 80% were smokers, with severe withdrawal symptoms upon abstinence Controls: 95 healthy men (mean age ± SD, 47.4 ± 8.9 years), of which 19% were smokers Exclusion criteria: mental illness, severe physical illness, other dependence syndromes according to DSM-IV	Age Comparison adjusted for technical quality and batch effects, cell type distribution, and smoking	NR	Blood DNA For individuals with AUD, samples collected 1–3 days after admission (time point 1) and after 2 weeks (time point 2). For controls, samples collected within first week Epigenome-wide methylation analysis at 710 944 CpG sites; single-site analysis as well as an analysis of differentially methylated regions and gene ontology analysis	**Number of differentially methylated CpG sites:** 2876 in participants with AUD at time point 1 vs after 2 weeks, FDR < 0.05 9845 in participants with AUD at time point 1 vs controls, FDR < 0.05 6094 at time point 2 vs controls, FDR < 0.05 Most significant single-site difference at *SCAP* Top differentially methylated region at *TRIM39*

Table 3.2 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)	Results[a]
Dugué et al. (2021) Australia	5606 people (68% men; median age, 61 years; IQR, 54–65 years) selected for inclusion in 1 of 7 previously conducted nested case–control studies of DNA methylation At follow-up, participants who reported weekly or current consumption > 200 g per day	Age, sex, smoking status, BMI, country of birth, sample type and white blood cell composition, and batch effects	Alcohol use questionnaire at baseline in 5606 participants, and 11 years later in 1088 participants	DNA from blood spots stored on Guthrie cards or from frozen buffy coats Epigenome-wide methylation analysis to detect the methylation status of 485 577 CpGs; single-site analysis as well as an analysis of differentially methylated regions	**Number of differentially methylated CpG sites:** 1414 CpGs associated with alcohol consumption at $P < 10^{-7}$, with 1078 replicated in 2 independent data sets 530 of the 1414 CpGs were differentially methylated for former vs current drinking using nominal *P* values ($P < 0.05$) 513 of the 1414 CpGs were differentially methylated with a change in alcohol consumption, some of which were replicated in 1 independent data set
Proskynitopoulos et al. (2021) Germany	34 men with AUD (mean age ± SD, 53 ± 8.8 years) Controls: 43 healthy men (mean age ± SD, 36 ± 17.0 years) Exclusion criteria: psychiatric illness, substance abuse other than alcohol or nicotine, cerebral ischaemia, cerebral haemorrhage, epilepsy, cardiovascular disease, and renal disease	None Measurements adjusted for multiple comparisons	Alcohol use severity (assessed by questionnaire): mean ± SD: 2.3 ± 1.3	Blood DNA For individuals with AUD, samples collected 1, 2, 3, 4, and 7–10 days after alcohol withdrawal. For controls, samples collected at baseline *ANP* and *VP* promoter region methylation (bisulfite DNA sequencing)	No significant difference in mean methylation for *VP* or *ANP* across time points for AUD Methylation of *ANP* at CpG 114 site: lower in AUD at baseline vs controls ($P = 0.000$) Methylation of *VP* at 5 CpG sites (CpG 033, CpG 064, CpG 103, CpG 118, and CpG 194): higher in AUD at baseline vs controls ($P < 0.05$) Methylation of *VP* at 3 CpG sites (CpG 053, CpG 060, and CpG 214): lower in AUD at baseline vs controls ($P < 0.05$)

Table 3.2 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)	Results[a]
Soundararajan et al. (2021) India	52 men with AUD (mean age ± SD, 33.98 ± 4.3 years) Controls: 52 healthy men (mean age ± SD, 32.17 ± 4.9 years) Exclusion criteria: use of any substance other than nicotine or any major psychiatric and medical disorders, based on clinical history and diagnosis	Age Ruled out the need to adjust for white blood cell count, age, BMI, FTND, medication, and dose of medication used during treatment	Duration of AUD (mean ± SD): 8.27 ± 5.4 years Alcohol consumption (mean ± SD): 12.87 ± 6.2 units per day 43 individuals were smokers Abstinence was defined as alcohol-free during ≥ 80% of the follow-up period	Blood (leukocyte) DNA and RNA Samples collected at baseline (T1), after detoxification (T2; mean ± SD of 7.81 ± 2.0 days), and after 3 months (T3) CpG-site DNA methylation in the 5′ regions of the *ALDH2* and *MTHFR* genes, and global *LINE-1* methylation	Participants with AUD vs controls: At baseline (T1): Significantly higher in *ALDH2* ($P < 0.001$) Significantly higher in *MTHFR* ($P = 0.001$) Significantly lower in *LINE-1* ($P = 0.004$) The significant differences persisted at T2 and T3 for *ALDH2*, *MTHFR*, and *LINE-1*

AD, alcohol dependence; *ALDH2*, aldehyde dehydrogenase gene; *ANP*, atrial natriuretic peptide gene; AUD, alcohol use disorder; *BDNF*, brain-derived neurotrophic factor gene; BMI, body mass index; DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; ELISA, enzyme-linked immunosorbent assay; FDR, false discovery rate; FTND, Fagerström Test for Nicotine Dependence; HPA, hypothalamic–pituitary–adrenal; IQR, interquartile range; *LINE-1*, long interspersed element 1 gene; *MTHFR*, methylenetetrahydrofolate reductase gene; NR, not reported; SCAP, sterol regulatory element-binding protein cleavage-activating protein gene; SD, standard deviation; *TRIM39*, tripartite motif-containing 39 gene; *VP*, vasopressin gene.

[a] https://www.genecards.org/

Heberlein et al. (2015) performed hypothesis-driven analyses of targeted epigenomic modifications that may affect continuing ethanol consumption, among 99 men with alcohol dependence and 33 age-matched healthy men as controls, and measured promoter methylation in the brain-derived neurotrophic factor (*BDNF*) gene using DNA extracted from whole blood. *BDNF*, a gene that encodes a neurotrophic growth factor that has previously been linked to addictive behaviour, has significantly higher methylation in its promoter region among individuals with alcohol dependence than among controls. The level of methylation decreased significantly ($P < 0.001$) from day 1 to day 7 after the individuals with alcohol dependence underwent withdrawal treatment, and was similar to that among controls on day 14. Compared with controls, the difference in the means was 0.063 on day 1, 0.033 on day 7, and 0.005 on day 14. The return of methylation state to control levels after the 14-day abstinence was not reflected in the serum concentration of the BDNF protein.

Witt et al. (2020) conducted an epigenome-wide analysis of blood DNA methylation among 99 men with alcohol dependence, when they were admitted for treatment and 2 weeks after they stopped consuming alcohol, with additional comparisons with 95 age-matched healthy men as controls. Abstinence resulted in widespread changes in patterns of methylation at individual CpG dinucleotides and in differentially methylated regions. Increases and decreases in methylation were observed at many individual sites and differentially methylated regions across many chromosomes. These included changes in promoters of withdrawal-associated genes (e.g. *SLC29A1*, *FYN*). The most significant single-site difference (false discovery rate [FDR], $P = 2.0 \times 10^{-19}$) in the longitudinal comparison was in sterol regulatory element-binding protein chaperone (SCAP), an escort protein required for cholesterol synthesis and lipid homeostasis. Analysis of differentially methylated regions showed the largest effect at tripartite motif containing 39 (*TRIM39*), an E3 ubiquitin ligase gene in the major histocompatibility complex (MHC) class I region, which is involved in inflammatory processes. [The study was undersized for a full genomic analysis, but many findings remained significant using FDR *P* values.]

Dugué et al. (2021) conducted an epigenome-wide analysis of the effect of alcohol exposure on blood DNA methylation among 5606 participants in the Melbourne Collaborative Cohort Study (MCCS) overall, which included 1088 participants who had alcohol consumption recorded at baseline and 11 years later. The longitudinal analysis focused on the subset of CpG sites ($n = 1414$) that were significantly associated ($P < 10^{-7}$) with alcohol consumption at baseline, and the authors extracted similar results from an available data set from the Cooperative Health Research in the Augsburg Region (KORA) study, in which alcohol consumption was measured 7 years apart (Wilson et al., 2017). A change in alcohol consumption was associated with changes in methylation at 267 CpGs in the MCCS study cohort and at 331 CpGs in the KORA study cohort, and 92 CpGs were different in both study cohorts. [Only 88 individuals in the MCCS became abstinent during the 11-year interval between measurements, limiting the strength of the results when accounting for multiple comparisons. Also, residual confounding by other key personal exposures, such as tobacco smoking and body mass index, could not be ruled out.]

Proskynitopoulos et al. (2021) studied 34 men with AUD and 43 healthy men as controls. They studied promoter methylation changes in atrial natriuretic peptide (*ANP*) and vasopressin (*VP*), two genes associated with alcohol cravings and withdrawal symptoms. Although there were significant differences in methylation patterns at baseline, there were no changes over the first 7–10 days after treatment started.

Soundararajan et al. (2021) studied promoter methylation in *ALDH2* and methylenetetrahydrofolate reductase (*MTHFR*), two genes relevant to alcohol-induced carcinogenesis, as well as long interspersed element 1 (*LINE-1*) repetitive element methylation, a proxy for global DNA methylation. They recruited 52 men with AUD and 52 age-matched healthy men as controls and measured blood DNA methylation at baseline and after the men with AUD had stopped consuming alcohol for 3 months. There were significant differences at baseline between men with AUD and controls, and none of the measures changed after 3 months of abstinence.

Several groups have investigated the effect of alcohol withdrawal on epigenetic changes in genes associated with alcohol cravings. [Although these hypotheses have not been directly linked to mechanisms associated with cancer in alcohol target organs, this work is included to show the breadth of studies investigating the impact of alcohol cessation on epigenetic modifications.]

3.2.4 Endocrine system

Mechanistic studies of the physiological impact of alcohol withdrawal on the endocrine system are confined to the effects of ethanol exposure or to the effects of withdrawal among individuals with alcohol dependence. The clearest link that has been described between alcohol modulation of the endocrine system and cancer is for breast cancer.

(a) Sex hormones

(i) Among humans

Endocrine mechanisms linking alcohol to breast cancer include induction of higher serum estrogen and dehydroepiandrosterone sulfate concentrations (Liu et al., 2015) and enhanced estrogen receptor activity (Dumitrescu and Shields, 2005); modulation of SHBG also may be involved (Assi et al., 2020). Among women, alcohol consumption increases the concentrations of estradiol, testosterone, and several other sex hormones in the circulation and decreases the concentration of SHBG, and these changes are hypothesized to be related to risk of breast cancer (Key et al., 2011). Alcohol consumption increases the risk of both premenopausal and postmenopausal breast cancer, and the risk is higher for estrogen receptor-positive tumours than for estrogen receptor-negative tumours (Sun et al., 2020). Studies on reduction or cessation of alcohol consumption are presented below, and detailed data among humans are given in Table 3.3.

Välimäki et al. (1982) studied 29 men with chronic AUD, including 13 with cirrhosis and 16 without cirrhosis. Blood samples were collected after 1–2 weeks of abstinence. Compared with the participants without cirrhosis, those with cirrhosis had significantly lower levels of serum testosterone and significantly higher levels of luteinizing hormone, prolactin, and estrone, but there were no differences in levels of estradiol or SHBG. [The effects of alcohol withdrawal were not ascertained, because the measurements were at the end of the observation period. However, these findings suggest that liver damage modulates the effects of alcohol withdrawal on circulating levels of sex hormones.]

Välimäki et al. (1984) studied 32 men with AUD and without cirrhosis at admission and after 1–2 weeks of alcohol withdrawal. The mean serum testosterone concentration increased by 19%, and among 4 men who had low testosterone concentrations at admission, the values returned to the normal range.

Iturriaga et al. (1995) studied 30 men with AUD at admission and at discharge after an average of 11 days in the treatment unit. They also recruited 15 healthy volunteers as controls. They measured levels of testosterone, estradiol, follicle-stimulating hormone, luteinizing hormone, and SHBG at both time points among the participants being treated and once among the controls. At discharge among the treatment group, levels of testosterone, estradiol, and

Table 3.3 Effects of cessation of alcohol consumption on the endocrine system

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Välimäki et al. (1982) Finland	29 men with chronic AUD (ages, 28–58 years), 13 with cirrhosis and 16 without cirrhosis 15 healthy controls (ages, 25–53 years) after ≥ 1 week of abstinence Exclusion criteria: use of certain pharmaceuticals	Drinking history	NR	Blood Samples collected after abstinence for 7–14 days Hormonal status Reference values correspond to the normal range of concentrations in men aged 20–60 years	Values are mean ± SEM **Testosterone (nmol/L)** Reference values: 14–38 With cirrhosis: 11.7 ± 2.2*** Without cirrhosis: 25.2 ± 1.5 **LH (IU/L)** Reference values: 10–20 With cirrhosis: 31.2 ± 6.0* Without cirrhosis: 17.0 ± 2.7 **Prolactin (mU/L)** Reference values: 120–220 With cirrhosis: 478 ± 72** Without cirrhosis: 251 ± 31 **Estrone (pmol/L)** Reference values 110–210 With cirrhosis: 464 ± 59*** Without cirrhosis: 229 ± 25 *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$
Loosen et al. (1983) USA	29 participants with chronic AUD (men aged 30–66 years) who had abstained for ≥ 2 years 17 healthy controls (men aged 37–55 years) None of the participants were taking medication	Age	Duration of abstinence: range, 2–29 years before baseline	Blood Samples collected at baseline and after injection of 0.5 mg of TRH TSH, T3, T4, prolactin, cortisol, testosterone, thyroid-binding globulin The differences (Δ) in T3, T4, TSH, and prolactin levels were calculated between value after injection and baseline value	Values are mean ± SE **ΔTSH (μU/mL)** Chronic AUD during abstinence: 8.3 ± 0.9 Controls: 12.3 ± 1.5 $P < 0.02$ **ΔT3 (ng/dL)** AUD during abstinence: 51 ± 6 Controls: 36 ± 9 *P*: NS **ΔTotal T4 (μg/dL)** AUD during abstinence: 1.2 ± 0.2 Controls: 1.0 ± 0.2 *P*: NS **ΔProlactin (ng/mL)** AUD during abstinence: 24.9 ± 3.6 Controls: 25.8 ± 2.6 *P*: NS

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Loosen et al. (1983) (cont.)					**Cortisol (µg/dL)** AUD during abstinence: 10.6 ± 0.7 Controls: 10.7 ± 1.2 *P*: NS **Testosterone (ng/mL)** AUD during abstinence: 5.2 ± 0.4 Controls: 5.1 ± 0.4 *P*: NS **Thyroid-binding globulin (µg/mL)** AUD during abstinence: 17.7 ± 0.6 Controls: 15.7 ± 0.5 $P < 0.01$
Välimäki et al. (1984) Finland	32 men (ages, 28–51 years) with AUD and without cirrhosis who volunteered for withdrawal therapy Exclusion criteria: use of certain pharmaceuticals	Drinking history	7–30 years of drinking history before admission	Blood Samples collected at admission and after 1 week and 2 weeks of abstinence Plasma testosterone and serum cortisol and ACTH	Values are mean ± SEM **Testosterone (nmol/L)** Reference range: 14–38 1 day of abstinence: 21.6 ± 1.3 8 days of abstinence: 25.8 ± 1.6** 15 days of abstinence: 24.0 ± 1.8 **Serum cortisol (nmol/L) (morning)** Reference range: 200–800 1 day of abstinence: 590 ± 27 8 days of abstinence: 481 ± 25** 15 days of abstinence: 479 ± 34** **Serum cortisol (nmol/L) (evening)** Reference range: 100–400 1 day of abstinence: 224 ± 18 8 days of abstinence: 185 ± 16 15 days of abstinence: 152 ± 21*** **ACTH (ng/L)** Reference range: 10–80 1 day of abstinence: 148 ± 32 8 days of abstinence: 86 ± 24** 15 days of abstinence: 82 ± 27* *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs day 1

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Marchesi et al. (1992) Italy	11 individuals with AUD (ages, 29–51 years) who had been abstinent for 4 weeks 9 men (ages, 31–66 years) with AUD who had been abstinent for ≥ 1 year 9 healthy controls (ages, 28–58 years)	Age	Among the participants with AUD: Duration of AUD (mean ± SD): 22.7 ± 10.3 years Duration of abstinence (mean ± SD): 3.7 ± 0.8 years before baseline	Blood Samples collected just before (time 0) and 10, 20, 30, 45, and 60 minutes after injection of TRH TSH and prolactin levels after TRH stimulation were evaluated with a specific double-antibody radioimmunoassay The differences (Δ) in TSH and prolactin levels were calculated between value after injection and baseline value	Values are mean ± SD **ΔTSH (mU/L)** Abstinence (4 weeks): 10.4 ± 17.31 Abstinence (≥ 1 year): 8.05 ± 5.14 Controls: 7.83 ± 3.58 $P_{ANOVA} = 0.54$ **ΔProlactin (ng/mL)** Abstinence (4 weeks): 50.60 ± 26.78 Abstinence (≥ 1 year): 33.75 ± 19.55 Controls: 27.46 ± 7.4 $P_{ANOVA} = 0.043$ AUD with 4-week abstinence had significantly higher prolactin response to TRH vs other 2 groups (at time 10 minutes, $P_{ANOVA} < 0.01$)
Iturriaga et al. (1995) Chile	30 men with chronic AUD (ages, 24–51 years) and without liver failure and without severe systemic illness Controls: 15 healthy volunteers	NR	Alcohol consumption > 150 g per day for 2–33 years Duration of abstinence (mean ± SD): 1.9 ± 1.7 days	Blood For participants with AUD, samples collected at admission and at discharge (mean ± SD, 11.1 ± 4.7 days after admission). For controls, samples collected at baseline Hormonal status	Values are mean ± SD **Testosterone (ng/mL)** Controls: 8.2 ± 1.4 AUD at admission: 6.9 ± 2.5 AUD at discharge: 6.2 ± 2.6† **Estradiol (pg/mL)** Controls: [ND] AUD at admission: 10.5 ± 6.8 AUD at discharge: 10.1 ± 5.6 **FSH (mIU/mL)** Controls: 10.8 ± 2.7 AUD at admission: 7.7 ± 4.0† AUD at discharge: 8.0 ± 4.3 **LH (mIU/mL)** Controls: 13.5 ± 4.1 AUD at admission: 12.9 ± 4.1 AUD at discharge: 8.2 ± 5.3††*

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Iturriaga et al. (1995) (cont.)					**SHBG (mmol/L)** Controls: 32.5 ± 39.0 AUD at admission: 117.2 ± 33.3††† AUD at discharge: 65.4 ± 21.6†††** *P < 0.001, **P < 0.0001 for discharge vs admission †P < 0.01, ††P < 0.02, †††P < 0.0001 vs controls
Rajzer et al. (1997) Poland	50 men (ages, 25–45 years) who met the DSM-III-R criteria for alcohol abuse and volunteered to stop drinking and undergo detoxification	NR	Alcohol consumption: 500–1500 mL (mean, 860 mL) of 40% ethanol per day for a duration (mean ± SD) of 12.4 ± 3.3 years	Blood samples collected 2–7 days and 4 weeks after abstinence Serum glucose and insulin measurement after an overnight fast and during a standard oral glucose tolerance test	Values are mean ± SD **Natural log of sum of secreted insulin (mU/L per minute)** After 2–7 days: 8.957 ± 0.474 After 4 weeks: 8.558 ± 0.651 P < 0.0001 Serum glucose levels not significantly different between 4 weeks and 2–7 days
Ozsoy et al. (2006) Türkiye	39 men (ages, 20–55 years) treated as inpatients for AD and alcohol withdrawal, divided into subgroups by aggression level, age at onset of AUD, and family history Controls: 28 healthy men (ages, 20–55 years) Exclusion criteria: any psychiatric disease, substance abuse other than alcohol or cigarette, any significant medical and endocrine disorder, and liver disease	Age and smoking status	Duration of alcohol consumption: 9–35 years Amount of alcohol consumed: 150–630 g per day	Blood Samples collected for early withdrawal (1 day after cessation) and late withdrawal (day 28 of cessation). For controls, samples collected at baseline Levels of fT4, fT3, and TSH	Values are mean ± SD **fT3 and fT4 (pg/mL)** $fT3_{controls}$: 3.32 ± 0.41 $fT4_{controls}$: 11.95 ± 1.49 $fT3_{1\text{-day cessation}}$: 3.18 ± 0.72 $fT4_{1\text{-day cessation}}$: 12.68 ± 2.50 $fT3_{28\text{-day cessation}}$: 2.71 ± 0.56* $fT4_{28\text{-day cessation}}$: 10.80 ± 1.86* **TSH (uIU/mL)** $TSH_{controls}$: 1.48 ± 0.71 $TSH_{1\text{-day cessation}}$: 1.93 ± 1.83 $TSH_{28\text{-day cessation}}$: 1.61 ± 0.86 *$P$ < 0.05 vs controls and early withdrawal (1-day cessation)

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Ozsoy et al. (2007) Türkiye	22 men (ages, 25–55 years) treated as inpatients for AD, divided into subgroups based on their withdrawal symptom severity scores on day 21 of alcohol cessation: continuing withdrawal symptoms (n = 8) and no withdrawal symptoms (recovered withdrawal group; n = 14) Exclusion criteria: some medical and endocrinological disorders Controls: 23 healthy men (ages, 25–55 years)	Age, BMI, and smoking status Individuals received diazepam and multivitamins for up to 3 weeks	NR	Blood Samples collected on day 21 of abstinence from individuals with AD and from controls GH level (baclofen challenge test); 20 mg of baclofen was given orally to the participants, and blood samples were collected every 30 minutes for the next 150 minutes	Values are mean ± SD **Basal GH (mIU/mL)** Controls: 0.06 ± 0.03 Participants who abstained: 0.18 ± 0.15* **ΔGH (μIU/mL)** Controls: 0.53 ± 0.84 Recovered withdrawal group: 0.80 ± 1.78 Continuing withdrawal group: 0.11 ± 0.27* *P < 0.05 vs controls
Alvisa-Negrín et al. (2009) Spain	48 participants with AUD (3 women), 28 who abstained and 20 who did not abstain 28 healthy controls (3 women) who did not consume alcohol heavily	Age and BMI	Alcohol consumption: mean ± SD, 204 ± 82 g per day Duration of consumption: mean ± SD, 28.4 ± 11.4 years Controls were people who consumed < 10 g of ethanol per day, as assessed at baseline	Bone and blood Bone mineral content, bone mineral density (dual-energy X-ray absorptiometry of lumbar spine and hip, and whole body) Serum osteocalcin (immunometric chemiluminescence assay) Serum telopeptide (1-step ELISA)	AUD with continuing alcohol consumption after 6 months: Loss of bone mass Decrease in osteocalcin Increase in telopeptide AUD after 6 months of abstinence: No change or increase in bone mass Increase in osteocalcin Increase in telopeptide

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Mehta et al. (2018) United Kingdom	94 participants who abstained (43 men, 51 women; mean age ± SD, 45.5 ± 1.2 years) who completed 1 month with no alcohol consumption Controls: 47 participants (22 men, 25 women; mean age ± SD, 48.7 ± 1.8) who continued their usual alcohol consumption Individuals were not randomized to these groups Exclusion criteria: > 3 days of abstinence from alcohol before study start, presence of known liver disease, AD, and diabetes requiring treatment	Abstinence outcome models were controlled for diet and exercise	Previous alcohol consumption was > 64 g per week (men) or > 48 g per week (women)	Blood Samples collected at baseline and after 1 month from abstinence group and from controls Insulin resistance as HOMA score	**HOMA score, median (IQR)** Baseline: 1.4 (1.0–2.1) 1-month abstinence: 1.0 (0.7–1.4); mean decrease of 25% $P < 0.001$ No significant change in controls A multivariate model including changes in diet and exercise over the month showed that abstinence was associated with a significant improvement in HOMA score ($P = 0.002$) but changes in lifestyle were not
Uribe et al. (2018) USA	25 Latino adults (60% men; ages, 25–65 years), 17 without and 8 with hepatitis C virus infection Exclusion criteria: previous diagnosis of diabetes or use of antidiabetic agents, presence of cirrhosis, HIV, or chronic hepatitis B virus	Drinking history	NR	Blood Samples collected at baseline and 6 weeks after alcohol discontinuation Peripheral insulin resistance (steady-state plasma glucose); hepatic insulin resistance (2-step, 240-minute insulin suppression test); insulin secretion rate (graded glucose infusion test)	**Hepatic insulin resistance, mean ± SD** Baseline: 13.9 ± 5.7 Follow-up: 16.5 ± 5.8 $P = 0.014$ Peripheral insulin resistance and insulin secretion rates remained unchanged

Table 3.3 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Price and Nixon (2021) USA	12 men (mean age ± SD, 43.42 ± 10.54 years) and 7 women (mean age ± SD, 39.57 ± 15.12 years) with moderate to severe AUD who sought treatment Exclusion criteria: use of steroidal inhalers or injections	Age, education level, monthly alcohol consumption, < 6 weeks of abstinence, and smoking habits	NR	Hair Samples collected at ~6 weeks after start of treatment (alcohol abstinence) Segment (proximal, mid-segment, and distal) hair cortisol concentrations (testing of neuroendocrine hormones) Proximal: representing sustained alcohol abstinence Mid-segment: representing the previous month, in which abstinence was attained Distal: representing the previous 2 months of active drinking	**Mean difference in cortisol (pg/mg)** Distal vs mid-segment: Not provided $P = 0.51$ Proximal vs distal: 0.200 (95% CI, 0.076–0.325) $P = 0.004$ Proximal vs mid-segment: 0.175 (95% CI, 0.100–0.249) $P < 0.001$

ACTH, adrenocorticotropic hormone; AD, alcohol dependence; ANOVA, analysis of variance; AUD, alcohol use disorder; BMI, body mass index; DSM-III-R, Diagnostic and Statistical Manual of Mental Disorders, 3rd revised edition; ELISA, enzyme-linked immunosorbent assay; FSH, follicle-stimulating hormone; fT3, free triiodothyronine; fT4, free thyroxine; GH, growth hormone; HOMA, homeostatic model assessment; IQR, interquartile range; LH, luteinizing hormone; ND, not detected; NR, not reported; NS, not significant; SD, standard deviation; SEM, standard error of the mean; SHBG, sex hormone-binding globulin; T3, triiodothyronine; T4, thyroxine; TRH, thyrotropin-releasing hormone; TSH, thyroid-stimulating hormone.

follicle-stimulating hormone were unchanged, but levels of luteinizing hormone and SHBG were significantly lower. The SHBG levels remained elevated, twice as high as among the controls.

(ii) Among experimental animals

Forquer et al. (2011) studied changes in concentrations of testosterone and 17β-estradiol during ethanol intoxication and ethanol withdrawal among male and female WSR and WSP mice. Among the WSP strain, compared with controls, ethanol intoxication led to significantly higher testosterone concentrations among females and significantly lower testosterone concentrations among males. Compared with controls, neither male nor female WSR mice had a significant change in total testosterone concentrations after chronic intoxication. Changes after ethanol withdrawal were strain-specific among both male and female mice. Among male WSP mice, testosterone concentrations began to normalize 24 hours after withdrawal and had returned to normal after 21 days; among male WSR mice, there was a similar but amplified pattern, with higher testosterone levels after 21 days compared with controls. In both strains, among female mice there was a reduction in testosterone concentrations during the first 24 hours after ethanol withdrawal. After 21 days, among female WSP mice testosterone concentrations had returned to normal levels, whereas in female WSR mice testosterone concentrations were significantly increased compared with controls. [The strain-specific effects make direct extrapolation to humans difficult, but the findings among female mice showed that altered testosterone concentrations persist for ≥ 3 weeks after alcohol exposure ends.] Concentrations of 17β-estradiol were only modestly changed by ethanol withdrawal among both strains and sexes of mice.

(b) Insulin

Insulin, insulin resistance, and diabetes have been widely studied for their relevance to cancer in many organs (Pearson-Stuttard et al., 2021). Peripheral insulin concentrations may be elevated during chronic heavy alcohol consumption (Piccardo et al., 1994). In contrast, moderate alcohol consumption has been linked to reduced fasting insulin concentrations and insulin resistance among postmenopausal women (Davies et al., 2002). Only one study examined the effects of abstinence on these end-points.

Rajzer et al. (1997) recruited 50 men who met the Diagnostic and Statistical Manual of Mental Disorders, third revised edition (DSM-III-R) criteria for alcohol abuse (American Psychiatric Association, 1987) and who volunteered to stop consuming alcohol. Blood was collected 2–7 days and again 4 weeks after the men became abstinent, which was verified by blood analysis and self-reporting. The authors measured serum glucose and insulin after fasting and during standard oral glucose tolerance tests and calculated a variety of insulin-resistance indices based on these measures. They reported a significant reduction in the sum of the insulin secreted during the test, 8.558 ± 0.651 mU/L per minute versus 8.957 ± 0.47 mU/L per minute ($P < 0.0001$), which is an improvement in insulin resistance but not a normalization. They reported no differences in the other markers and indices, including serum insulin, glucose, the sum of glucose measured during the test, and the ratio of glucose to insulin.

Uribe et al. (2018) studied 25 Latino adults (10 women and 15 men) without diabetes or cirrhosis and with a history of moderate alcohol consumption, which was defined among the women as no more than 3 drinks in any day or 7 drinks in a week and among the men as no more than 4 drinks in a day or 14 drinks in a week. In addition to assessing body size, the authors performed a variety of standard serum measurements (e.g.

alanine transaminase, aspartate aminotransferase) and assessed the insulin secretion rate and peripheral and hepatic insulin resistance at baseline and after a median of 7 weeks of abstinence. Body mass index, alanine transaminase, aspartate aminotransferase, fasting glucose, and fasting insulin levels and peripheral insulin resistance and insulin secretion rates remained unchanged. In contrast, hepatic insulin resistance was significantly higher among the individuals after they abstained from alcohol.

Mehta et al. (2018) recruited healthy individuals with regular alcohol consumption of > 64 g per week for men or > 48 g per week for women and asked them to indicate whether they would abstain from alcohol for 1 month or continue their usual consumption. A total of 77 participants completed the abstinence protocol and returned for the second visit, and 40 continued their usual consumption and returned for the second visit. The primary aim was to measure insulin resistance using homeostatic model assessment (HOMA). After 1 month, the HOMA score was reduced by a mean of 25% in the group that abstained from alcohol ($P < 0.001$), whereas there was no significant change ($P = 0.42$) in the group that continued their usual consumption. To account for other changes in lifestyle that the participants may have undertaken, the authors measured changes in diet and exercise using a questionnaire. In a multivariate model, only abstinence was associated with improvement in the HOMA score. [Participants who abstained from alcohol also showed improvement in blood pressure, weight, cholesterol, alanine transaminase and aspartate aminotransferase levels, and other markers, whereas among the controls, the only significant difference was higher aspartate aminotransferase levels.]

(c) Other hormones

The human hormonal system includes a wide array of messengers and targets and may contribute to many health outcomes associated with alcohol exposure, but much of the current body of research has been directed towards understanding human craving, addiction, and withdrawal symptoms, with little information directly relevant to cancer target organs. Alcohol consumption has been shown to affect the activity of the hypothalamic–pituitary–adrenal axis, including levels of adrenocorticotropic hormone (ACTH) and cortisol and other related hormones (Gianoulakis et al., 2003). Thyroid hormones also have been investigated alone or in combination with ACTH. Many of the hormonal responses that have been studied were in pathways that may not yet be tied directly to cancer in a specific organ. In many instances, the studies have focused on how the hormonal responses are associated with alcohol cravings and other aspects of addiction. Hormone studies also have included vitamin D, a fat-soluble secosteroid hormone that has been studied extensively with regard to risk of breast cancer (Visvanathan et al., 2023), colorectal cancer (McCullough et al., 2019), and other cancer types.

(i) Among humans

Thyroid hormones are reactive to illness and have been studied in the earliest days (Melander et al., 1982) and weeks (Välimäki et al., 1984) after individuals become abstinent, but these short-term changes probably do not reflect their hormonal status after the initial treatment period.

Loosen et al. (1983) studied 29 men who had been abstinent for ≥ 2 years and 17 healthy men as controls. They compared levels of triiodothyronine (T3), thyroxine (T4), thyrotropin (or thyroid-stimulating hormone [TSH]), and several other related hormones and calculated indices at study baseline and after injection with thyrotropin-releasing hormone (TRH). Several parameters, such as T3, T3:T4 ratio, TSH at baseline, and TSH and T4 after administration of TRH, were significantly different. Basal cortisol, basal testosterone, and prolactin levels at baseline and after TRH treatment did not differ. There was

also a significant increase in thyroid-binding globulin among participants who were abstinent.

Marchesi et al. (1992) studied 11 individuals with AUD who had abstained for 4 weeks, 9 men with AUD who had abstained for ≥ 1 year, and 9 age-matched healthy controls. There was no difference in the TSH levels among the groups after administration of TRH, but there was a marginally significant increase in prolactin response in the group that had abstained for 4 weeks.

Ozsoy et al. (2006) studied 39 men who were being treated for AUD and 28 healthy men as controls. Concentrations of free thyroxine, free triiodothyronine, and TSH were measured once among the controls and on day 2 and day 28 of withdrawal among the individuals with AUD. On day 2, the concentrations of the three thyroid hormones did not differ from those among the controls. On day 28, the levels of both free thyroxine and free triiodothyronine were significantly lower among the individuals being treated for AUD than among the controls (measured at the first time point). Subanalyses by age at onset of AUD, family history, and aggressiveness showed that all factors modified these changes.

Ozsoy et al. (2007) assessed gamma-aminobutyric acid (GABA) dysfunction by measuring growth hormone responses to oral baclofen treatment among 22 men with alcohol dependence after they had abstained for 21 days compared with 23 healthy men as controls. The participants with AUD were divided into two groups on the basis of their withdrawal symptom severity scores on day 21 of alcohol cessation: those with continuing withdrawal symptoms, and those with no withdrawal symptoms (recovered withdrawal group). As expected, baclofen treatment significantly increased growth hormone responsiveness among the participants in the control group, but not among the men with AUD who had abstained. This impairment was evident only among the men with continuing withdrawal symptoms, whereas responsiveness had been restored among those in the recovered withdrawal group.

Alvisa-Negrín et al. (2009) studied levels of osteocalcin, vitamin D, and other bone health markers among individuals with AUD before and after alcohol withdrawal. They enrolled 77 participants with AUD (68 men and 9 women) at baseline; 48 (including 3 women) were evaluated 6 months later, when 28 were abstinent and 20 were not abstinent. After 6 months, the individuals who continued to consume alcohol had lost bone mass, whereas those who abstained had either no change or an increase in bone mass. The participants who abstained had a significant increase in osteocalcin, whereas there was a decrease among those who continued to consume alcohol. Changes in serum telopeptide levels were similar among the two groups. In a subset of participants, serum levels of insulin-like growth factor 1 (IGF-1), vitamin D, and parathyroid hormone were measured at entry and after 6 months. IGF-1 concentrations were unchanged, vitamin D levels increased, and parathyroid hormone levels increased non-significantly [quantitative data for these changes were not reported].

Price and Nixon (2021) used segmental hair analysis among individuals (12 men and 7 women) with AUD ~6 weeks after alcohol withdrawal to study changes in cortisol concentrations over time. Cortisol accumulated in hair during alcohol consumption, and abstinence led to significantly lower cortisol concentrations; there were no differences between men and women.

(ii) Among experimental animals

Although their relevance to humans and to carcinogenesis is currently unknown, the few available studies in experimental animals about the effects of alcohol withdrawal on other hormones are summarized below.

Rasmussen et al. (2000) examined the effects of ethanol withdrawal on the hypothalamic–

pituitary–adrenal axis in male Sprague-Dawley rats and pair-fed and ad libitum-fed controls. The animals were administered ethanol over a 3-week gradual introduction to a 5% weight by volume diet, then 4 weeks of continuing administration, and then ethanol withdrawal over a 3-week period. After the 3-week withdrawal period, anterior pituitary pro-opiomelanocortin (POMC) messenger RNA (mRNA) concentrations were significantly suppressed among the exposed group compared with the pair-fed and ad libitum-fed control groups; thymus and spleen weights were also higher among the exposed rats.

Li et al. (2010) examined whether alcohol withdrawal could reverse alcohol-induced exocrine pancreatic insufficiency, which is more common among individuals with alcohol dependence. They used male Wistar rats, among which ethanol alone cannot induce chronic pancreatitis but is known to alter markers of sufficiency. A total of 48 rats were divided evenly into 4 groups: a group exposed at final exposure (incremental exposure over 4 weeks) to 25% ethanol (v/v) for 6 months; a group exposed to 25% ethanol for 6 months, followed by 3 months of enforced abstinence; a 6-month distilled water control group; and a 9-month distilled water control group. Ethanol exposure for 6 months led to significantly reduced levels of amylase and lipase, and the levels in the group with ethanol exposure followed by enforced abstinence were not restored to those among the paired control group. A similar irreversible effect was noted for cholecystokinin, measured by radioimmunoassay in pancreatic acinar cells and small intestinal cells. Ethanol administration had no effect on cholecystokinin A receptors.

Allen et al. (2018) assessed ACTH and cortisol levels in a study in which adult male experimentally naive rhesus macaques were exposed to ethanol for 14 months, followed by periods of enforced abstinence that lasted ~3 months. Exposed animals (n = 8) had ethanol and water available from two different bottles, and they developed a wide array of patterns of voluntary ingestion. Yoked-control animals (n = 4) had water available from both bottles. Serum cortisol and ACTH concentrations were measured during the three periods of enforced abstinence. During the first period of abstinence, a sharp increase and subsequent decrease in cortisol concentrations were seen in all groups. During the second period, cortisol concentrations remained higher among the animals with heavy ethanol consumption. During the third period, cortisol concentrations increased, and they remained elevated among all three groups. ACTH concentrations were more variable than those of cortisol and were not correlated to the alternating phases of exposure and enforced abstinence.

3.2.5 Microbiome

The human microbiome may play roles in alcohol-induced cancer. The oral microbiome plays an important role in the metabolism of alcohol in the mouth, and alcohol consumption can induce changes in the composition and abundance of the oral microbiome (see Section 3.1). Chronic alcohol consumption also has an impact on the intestinal microbiome; this may act through metabolism, inflammation, and intestinal permeability, including translocation of microbes (see Section 3.2.6). Studies assessing the effects of alcohol cessation on the oral and intestinal microbiomes are described in Table 3.4.

(a) Oral microbiome

Heavy alcohol consumption has been shown to decrease secretion of saliva, change the electrolyte concentration in saliva, and decrease protein synthesis in the salivary glands; this suggests an impact on the oral microbiome (Inenaga et al., 2017). Furthermore, it has been suggested that alcohol influences the inflammatory effect of the oral microbiota or facilitates enhanced pathogenicity of commensal

Table 3.4 Effects of cessation of alcohol consumption on the oral and intestinal microbiome

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Oral microbiome					
Yokoyama et al. (2007) Japan	80 men with AUD who were admitted to a 3-week treatment programme for alcoholism. Among the 34 individuals tested after 3 weeks, 12 were heterozygous for *ALDH2*	67 smokers and 13 non-smokers All participants continued drinking until the day before admission to the hospital, had never used alcohol-aversive drugs, and had no signs or symptoms of liver cirrhosis	NR	Saliva Acetaldehyde production capacity measured at admission and after 3 weeks (n = 34); bacteria and yeast counts	Values are median (Q1, Q3) **Acetaldehyde production (μM)** AUD at baseline: 270 (179, 397) AUD after abstinence (3 weeks): 132 (82, 195) $P_{\text{abstinence vs baseline}} < 0.0001$ **Salivary bacteria and yeast counts ($\log_{10}$ CFU/mL)** Total salivary bacteria and yeast counts AUD at baseline: 7.45 (7.32, 7.78) AUD after abstinence (3 weeks): 7.04 (6.81, 7.30) $P_{\text{abstinence vs baseline}} = 0.0002$ Correlated decreases in total salivary bacteria and yeast counts and acetaldehyde production (r = 0.35; P = 0.042) *Stomatococcus* species AUD at baseline: 5.60 (< DL, 6.26) AUD after abstinence (3 weeks): 5.30 (< DL, 6.15) $P_{\text{abstinence vs baseline}} = 0.96$ *Corynebacterium* spp. AUD at baseline: 6.00 (< DL, 6.53) AUD after abstinence (3 weeks): 5.26 (< DL, 6.26) $P_{\text{abstinence vs baseline}} = 0.58$ α-Haemolytic *Streptococci* AUD at baseline: 7.20 (6.96, 7.62) AUD after abstinence (3 weeks): 6.70 (6.38, 7.04) $P_{\text{abstinence vs baseline}} < 0.0001$

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
van Zyl and Joubert (2015) South Africa	30 men with AUD (median age, 42 years; IQR, 29–48 years) who were admitted to a 21-day inpatient rehabilitation programme followed by 9 weeks of observation (total of 12 weeks of observation) Racial distribution: 15 Black, 11 White, and 4 mixed race A smaller pilot study was conducted among 7 men from the treatment centre who were compared with 7 men from the general population Exclusion criteria: antibiotic use during the previous month and dependence on drugs other than alcohol, nicotine, or marijuana	Sex	The 30 study participants included 10 who consumed > 60 units per week, 4 who consumed 40–59 units per week, 13 who consumed 15–39 units per week, and 3 who consumed < 15 units per week During the 12 weeks, 16 participants remained abstinent and 13 participants resumed drinking	Saliva Acetaldehyde production capacity measured 2, 4, 11, and 18 days after admission to the programme	Values are median (Q1, Q3) **Salivary acetaldehyde production capacity (µmol/L)** AUD after abstinence (18 days): 178 (172, 188) AUD resumed drinking (18 days): 168 (161, 180) $P_{\text{abstinence vs drinking}} = 0.02$ The difference between the 2 groups was not statistically significant on days 2, 4, and 11
Intestinal microbiome					
Leclercq et al. (2014) Belgium	60 participants with AUD (47 men, 13 women) entering an inpatient rehabilitation programme for 19 days. Participants were tested on the day after admission and divided into 2 groups: with high IP and with low IP. Among the 44 individuals who remained abstinent during the 19 days, 13 (8 men, 5 women) were tested for intestinal microbiota composition and functionality before and after abstinence 15 healthy controls who consumed < 20 g of alcohol per day	Age, sex, and BMI	AUD was diagnosed according to DSM-IV	Faecal samples Intestinal microbiota analysis (pyrosequencing and qPCR of 16S rDNA in faecal samples) Intestinal permeability (^{51}Cr-EDTA)	**Microbial composition** ***$AUD_{high\text{-}IP}$ at baseline*** Family level Decreased *Ruminococcaceae* and *Incertae Sedis XIII* vs $AUD_{low\text{-}IP}$ and controls $P < 0.05$ Increased *Lachnospiraceae* and *Incertae Sedis XIV* vs $AUD_{low\text{-}IP}$ and controls $P < 0.05$ Genus level Decreased *Ruminococcus*, *Faecalibacterium*, *Subdoligranulum*, *Clostridia*, and *Oscillibacter* vs $AUD_{low\text{-}IP}$ and controls $P < 0.05$ Decreased *Anaerofilum* vs $AUD_{low\text{-}IP}$ and controls *P*: NR

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Leclercq et al. (2014) (cont.)	Exclusion criteria: BMI > 30 kg/m^2, diabetes, inflammatory bowel disease, other chronic inflammatory diseases such as rheumatoid arthritis, cancer, or use of antibiotics, probiotics, glucocorticoids, or non-steroidal anti-inflammatory drugs in the 2 months before admission				Increased *Dorea*, *Blautia*, and *Megasphaera* vs $AUD_{low\text{-}IP}$ and controls $P < 0.05$ ***$AUD_{high\text{-}IP}$ after abstinence (19 days)*** Increased *Ruminococcaceae* $P_{19\text{-day abstinence vs baseline}} < 0.05$ Decreased family *Erysipelotrichaceae* and genus *Holdemania* $P_{19\text{-day abstinence vs baseline}} < 0.05$ Increased genera *Ruminococcus* and *Subdoligranulum* $P_{19\text{-day abstinence vs baseline}} = 0.11$ (NS) ***$AUD_{low\text{-}IP}$ after abstinence (19 days)*** Decreased family *Erysipelotrichaceae* and genus *Holdemania* $P_{19\text{-day abstinence vs baseline}} < 0.05$ **Microbial abundance, log_{10} (bacterial cells/g faeces)** ***$AUD_{high\text{-}IP}$ at baseline*** Decreased total amount of bacteria vs $AUD_{low\text{-}IP}$ and controls $P < 0.01$ Decreased *Faecalibacterium prausnitzii* and *Bifidobacterium* spp. vs $AUD_{low\text{-}IP}$ and controls $P < 0.05$ Decreased *Lactobacillus* spp. vs $AUD_{low\text{-}IP}$ and controls $P > 0.05$, NS ***$AUD_{high\text{-}IP}$ after abstinence (19 days)*** Increased total amount of bacteria, *Bifidobacterium* spp., and *Lactobacillus* spp. $P_{19\text{-day abstinence vs baseline}} < 0.05$ *Faecalibacterium prausnitzii:* no change

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Ames et al. (2020) USA	22 participants with AUD entering a 4-week inpatient detoxification programme (14 men, 8 women; average age ± SD, 45.82 ± 13.0 years) Racial distribution: 13 White, 6 Black, 2 mixed race, 1 of unknown race	16/22 participants smoked, 8/22 used illicit substances, and 10/22 tested positive for cannabinoids in urine. None of the participants had cirrhosis or liver failure. 86% of the participants were diagnosed with periodontal disease. 3 participants were taking antibiotics	Participants were divided into 2 groups: VHD (≥ 10 drinks per day) and LHD (< 10 drinks per day) They were followed up during 3 weeks of abstinence	Stool homogenization and oral tongue brushings analysed with a microbiome sequencing kit to examine 6 of the 9 hypervariable regions of the *16S* gene	**Microbial composition, average relative abundance (%)** *Erysipelotrichaceae* LHD at baseline: 13 VHD at baseline: 0.01–0.05 *P*: NR *Lachnospiraceae* LHD at baseline: 13 VHD at baseline: 0.01–0.05 *P*: NR After abstinence (3 weeks), greater changes in VHD group than in LHD group **Shannon diversity index** No significant difference between LHD and VHD groups at baseline or after abstinence (3 weeks) 1/8 LHD and 4/14 VHD participants: significant linear diversity changes from baseline to after abstinence (3 weeks) *P*: NR **BSDD** Average BSDD values across the 2 groups were analysed using pairwise comparisons between day 1 and day 5 ($P = 0.03$) and day 1 and week 3 ($P = 0.02$) Between-group differences were significant: values 0.10 higher in VHD group than in LHD group Average BSDD range after abstinence (3 weeks) LHD: 0.1–0.2 VHD: 0.13–0.43 $P_{\text{VHD vs LHD}} = 0.02$

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Gao et al. (2020) Belgium	30 participants with AUD (23 men, 7 women; median age, 42 years; range, 27–59 years) entering a 3-week detoxification programme Participants were divided into 2 groups: with high CAP (> 300 dB/m) and with low CAP (< 300 dB/m) Controls: 8 healthy volunteers (6 men, 2 women; median age, 52 years; range, 37–71 years) who consumed < 20 g of alcohol per day, defined as social drinking	Age, sex, and BMI	Participants with AUD had consumed 60 g of alcohol per day for ≥ 1 year Detoxification programme for participants with AUD: 1 week of inpatient detoxification, followed by 1 week of outpatient care and 1 week of inpatient treatment	Stool samples (collected from first bowel movement after each hospitalization) Microbial composition and microbial pathways (shotgun metagenomic sequencing) Liver steatosis (transient elastography combined with CAP measurements) LDA effect size used to identify the features most likely to account for between-group differences	**LDA score (log_{10})** (LDA threshold: > 2.0) ***All participants with AUD after abstinence (2 weeks)*** Isoprene biosynthesis I $P_{abstinence\ vs\ baseline} < 0.05$ $P_{abstinence\ vs\ controls} < 0.01$ Phytol degradation $P_{abstinence\ vs\ baseline} < 0.05$ $P_{abstinence\ vs\ controls} < 0.05$ L-isoleucine biosynthesis II $P_{abstinence\ vs\ baseline} < 0.05$ $P_{abstinence\ vs\ controls}$: NS Superpathway of geranylgeranyl diphosphate biosynthesis II $P_{abstinence\ vs\ baseline} < 0.01$ $P_{abstinence\ vs\ controls}$: NS NAD salvage pathway II $P_{abstinence\ vs\ baseline} < 0.01$ $P_{abstinence\ vs\ controls}$: NS Glutaryl-coenzyme A degradation $P_{abstinence\ vs\ baseline} < 0.05$ $P_{baseline\ vs\ controls} < 0.05$ $P_{abstinence\ vs\ controls} < 0.05$ Superpathway of geranylgeranyl diphosphate biosynthesis I $P_{abstinence\ vs\ baseline} < 0.05$ $P_{abstinence\ vs\ controls} < 0.05$ ***$AUD_{high\text{-}CAP}$ after abstinence (2 weeks)*** L-isoleucine biosynthesis II, superpathway of β-D-glucuronosides degradation, superpathway of hexuronide and hexuronate degradation, superpathway of geranylgeranyl diphosphate biosynthesis II (via MEP), and glutaryl-coenzyme A degradation: LDA > 2.0 Heterolactic fermentation: LDA < –2.0

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Gao et al. (2020) (cont.)					***$AUD_{low\text{-}CAP}$ after abstinence (2 weeks)*** Adenosine nucleotides degradation II, guanosine nucleotides degradation III, superpathway of pyrimidine ribonucleosides degradation, purine nucleotides degradation II (aerobic), and ppGpp biosynthesis: LDA > 2.0
Maccioni et al. (2020) Belgium	106 individuals with AUD (78 men; mean age ± SEM, 46 ± 9.2 years) entering a 3-week detoxification programme Participants were divided into 2 groups, with high IP and with low IP Controls: 24 healthy volunteers (14 men, 10 women; mean age ± SEM, 42 ± 11 years) who consumed < 20 g of alcohol per day, defined as social drinking	Age, sex, and BMI (1 to 4 ratio)	Participants with AUD who had consumed > 60 g of alcohol per day for ≥ 1 year Participants with AUD admitted to a detoxification programme consisting of 1 week of inpatient detoxification followed by 1 week of outpatient care and 1 week of inpatient treatment	Blood, urine, and faecal samples collected at the beginning of the programme and after 2 weeks of abstinence Microbial translocation (measuring Gram-negative and Gram-positive serum markers by ELISA); microbiota (16S rRNA sequencing) IP (urinary excretion of ^{51}Cr-EDTA), faecal albumin content, and immunohisto-chemistry in distal duodenal biopsies	**sCD14** AUD after abstinence (2 weeks): decrease $P_{\text{abstinence vs baseline}} = 0.0001$ **Lipopolysaccharide binding protein** AUD after abstinence (2 weeks): no change **Peptidoglycan recognition proteins** AUD after abstinence (2 weeks): no change **α-diversity (Shannon diversity index)** $AUD_{\text{high-IP}}$ after abstinence (2 weeks): increase $P_{\text{abstinence vs baseline}} = 0.076$ $AUD_{\text{low-IP}}$ after abstinence (2 weeks): no change $P_{\text{abstinence vs baseline}} = 0.6$ $AUD_{\text{progressive ALD}}$ after abstinence (2 weeks): no change $P_{\text{abstinence vs baseline}} = 0.8$ $AUD_{\text{non-progressive ALD}}$ after abstinence (2 weeks): increase $P_{\text{abstinence vs baseline}} = 0.15$ **α-diversity (Simpson diversity index)** $AUD_{\text{high-IP}}$ after abstinence (2 weeks): increase $P_{\text{abstinence vs baseline}} = 0.065$ $AUD_{\text{low-IP}}$ after abstinence (2 weeks): no change $P_{\text{abstinence vs baseline}} = 0.8$ $AUD_{\text{progressive ALD}}$ after abstinence (2 weeks): no change $P_{\text{abstinence vs baseline}} = 0.8$ $AUD_{\text{non-progressive ALD}}$ after abstinence (2 weeks): increase $P_{\text{abstinence vs baseline}} = 0.15$ **β-diversity** AUD after abstinence (2 weeks): no change

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Hartmann et al. (2021) Belgium	66 participants with AUD (47 men; average age ± SD, 45 ± 12 years) entering a 3-week detoxification programme Controls: 18 healthy volunteers (14 men, 4 women; average age ± SD, 41 ± 12 years) who consumed < 20 g of alcohol per day, defined as social drinking	Age, sex, and BMI 80% of participants with AUD and 20% of controls were smokers	Participants with AUD had consumed > 60 g of alcohol per day for ≥ 1 year Participants with AUD admitted to a detoxification programme consisting of 1 week of inpatient detoxification, followed by 1 week of outpatient care and 1 week of inpatient treatment	Blood and stool samples (collected from first bowel movement after each hospitalization) DNA from faecal samples characterized using fungal metagenomic sequencing and internal transcribed spacer 2 amplicon Principal coordinate analyses performed to summarize outcomes of the relative abundance of all fungal genera between the different groups LDA effect size used to identify the features most likely to account for between-group differences	**Principal coordinate analyses** $P_{\text{baseline vs controls}} = 0.001$ $P_{\text{abstinence vs baseline}} = 0.001$ **LDA score ($\log_{10}$)** Participants with AUD after abstinence (2 weeks) vs participants with AUD at baseline Genus level *Candida*, *Malassezia*, *Pichia*, *Kluyveromyces*, *Issatchenkia*, *Claviceps*, *Cyberlindnera*, and *Hanseniaspora*: LDA < –2 *Trichosporon*: LDA > 2 Species level *C. albicans*, *C. zeylanoides*, *I. orientalis*, and *Cyberlindnera jadinii*: LDA < –2 Family level *Saccaromycodaceae*, *Malasseziaceae*, *Cystostereaceae*, *Didymellaceae*, and *Clavicipitiaceae*: LDA < –2 *Metschnikowiaceae* and *Trichosporonaceae*: LDA > 2

Table 3.4 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point (test used)[a]	Results
Hsu et al. (2022) Belgium	62 participants with AUD (44 men; average age ± SD, 44.4 ± 11.9 years) entering a 3-week detoxification programme Controls: 16 healthy volunteers (13 men, 3 women; average age ± SD, 40.8 ± 12.3 years) who consumed < 20 g of alcohol per day, defined as social drinking	Age, sex, and BMI	Participants with AUD who had consumed > 60 g of alcohol per day for ≥ 1 year Detoxification programme consisting of 1 week of inpatient detoxification, followed by 1 week of outpatient care and 1 week of inpatient treatment	Blood and stool samples (collected from first bowel movement after each hospitalization) Viral nucleic acid analysis (metagenomic sequencing) Principal coordinate analyses performed to identify differences in the relative abundance of all phages grouped according to their hosts LDA effect size used to identify the features most likely to account for between-group differences	**Principal coordinate analyses** Participants with AUD after abstinence (2 weeks) vs controls and participants with AUD at baseline: $P = 0.027$ **LDA effect size** Participants with AUD at baseline Decreased % of individuals with bacteriophages targeting *Propionibacterium* vs controls: $P_{\text{baseline vs controls}} < 0.001$ Participants with AUD after abstinence (2 weeks): $P_{\text{abstinence vs baseline}}$ Increased phages targeting *Lactococcus*: $P = 0.020$ Increased phages targeting *Leuconostoc*: $P = 0.016$ Increased phages targeting *Streptococcus*: $P = 0.077$ Increased phages targeting *Propionibacterium*: $P = 0.030$ Increased phages targeting *Lactobacillus*: $P = 0.007$ Increased % of individuals with bacteriophages targeting *Lactobacillus*: $P < 0.001$ Increased % of individuals with bacteriophages targeting *Propionibacterium*: $P = 0.005$ Relative abundance of *Propionibacterium* phages in participants with AUD at baseline and after abstinence vs controls: $P_{\text{Kruskal–Wallis}} = 0.002$

[a] % ^{51}Cr-EDTA is the percentage of the ingested dose of ^{51}Cr-EDTA found in urine, normalized for creatinine.

16S rDNA, 16S ribosomal DNA subunit; ALD, alcohol-related liver disease; *ALDH2*, aldehyde dehydrogenase gene; AUD, alcohol use disorder; BMI, body mass index; BSDD, binary Sørensen–Dice dissimilarity coefficient; CAP, controlled attenuation parameter; CFU, colony-forming unit; DL, detection level (1.30 $\log_{10}$ CFU/mL for bacteria); DSM-IV, Diagnostic and Statistical Manual of Mental Disorders, 4th edition; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; IP, intestinal permeability; IQR, interquartile range; LDA, linear discriminant analysis; LHD, less heavy drinking; MEP, methyl-D-erythritol 4-phosphate; NAD, nicotinamide adenine dinucleotide; NR, not reported; NS, not significant; ppGpp, guanosine 3′,5′-bis(diphosphate); Q1, first quartile; Q3, third quartile; qPCR, quantitative polymerase chain reaction; sCD14, soluble CD14; SD, standard deviation; SEM, standard error of the mean; VHD, very heavy drinking.

microorganisms. A potential interaction between alcohol and the oral microbiome, leading to a malignant transformative event, also has been suggested, which supports the hypothesis that the microbiome could influence the level of acetaldehyde, mediating its genotoxic effects. The interaction between the oral microbiome and ethanol metabolism is a complex and widely unexplored process, with numerous possible genus- or species-related outcomes. One of the main links between alcohol consumption and the oral microbiome is the enzyme ADH. It is found in several species of commensal bacteria (e.g. *Neisseria mucosa* and *Streptococcus* species) and the fungus *Candida albicans*, which have the potential to produce genotoxic levels of acetaldehyde (Nieminen and Salaspuro, 2018; Yokoyama et al., 2018; O'Grady et al., 2020). In addition, alcohol consumption (along with other exogenous factors, e.g. tobacco smoking) can alter the composition of the oral microbiome and change its metabolic contributions (Hsiao et al., 2018). Details about the role of the microbiome in ethanol metabolism are provided in Section 3.1. Host genetics also was found to have an impact on the microbiome in a comparison of data from the Human Microbiome Project in hosts for which a genome-wide association study (GWAS) analysis was performed (Blekhman et al., 2015). This could imply that different genetic backgrounds may lead to variations in the oral microbiome that could ultimately affect its influence on acetaldehyde production and metabolism (see Section 3.1).

To characterize changes in the oral microbiome associated with abstinence, studies have measured the impact of abstinence on the capacity of salivary microbes to metabolize ethanol to acetaldehyde, by collecting saliva samples and exposing them ex vivo to ethanol (Homann et al., 2001). These studies are described below and in Table 3.4.

In a study in Japan, Yokoyama et al. (2007) measured the salivary microbiome as bacteria and yeast counts in saliva samples collected from 80 men with AUD who entered a rehabilitation treatment programme. Saliva specimens were collected immediately after admission and 3 weeks later from 34 participants. Acetaldehyde production capacity was significantly correlated with the number of microorganisms measured in the saliva. The number of salivary bacteria and yeasts decreased after 3 weeks of abstinence and correlated with a decrease in acetaldehyde production in saliva ($r = 0.35$; $P = 0.042$). Specifically, the prevalence and number of α-haemolytic *Streptococci*, *Stomatococcus* sp., and *Corynebacterium* sp. were very high at admission, and the number of α-haemolytic *Streptococci*, which are known to be associated with increased salivary acetaldehyde production, was significantly reduced after 3 weeks of abstinence ($P < 0.001$).

van Zyl and Joubert (2015) investigated acetaldehyde production capacity in saliva samples collected from 30 individuals 2, 4, 11, and 18 days after they were admitted to a rehabilitation programme. Among the selected participants, 16 remained abstinent during the 12 weeks of observation, and 13 resumed alcohol consumption. No differences in the levels of acetaldehyde production were detected between the samples collected at baseline and those collected at the later time points. Statistically significant differences were found in the acetaldehyde production after 12 weeks between the group that remained abstinent and the group that resumed alcohol consumption. [These results are of limited significance because acetaldehyde production capacity is an indirect measurement of bacterial function, which is potentially influenced by an individual's endogenous acetaldehyde production and is also likely to be affected by other major sources of acetaldehyde exposure, such as tobacco smoking.]

[These studies underscore the complexity of the influence of alcohol on the oral microbiome. Consumption of alcohol influences the composition and abundance of the oral microbiome,

which in turn may affect local metabolism of ethanol and, consequently, the effects of alcohol mediated by acetaldehyde. These particular interactions in the oral cavity support the need to consider the oral microbiome separately from the intestinal microbiome.]

(b) Intestinal microbiome

Chronic alcohol consumption changes the composition and abundance of the intestinal microbiome, which results in an insult to the intestinal mucosal barrier, compromising intestinal homeostasis and leading to an imbalance in the microbiota with loss of beneficial microbes and expansion of pathogenic ones, also known as intestinal dysbiosis. AUD further increases intestinal mucosal damage and intestinal permeability, causing an increase in the translocation of microbial products into peripheral circulation (Donnadieu-Rigole et al., 2018; see Section 3.2.6). Recently, an association between alterations in the intestinal microbiome and breast cancer has been reported, suggesting that the microbiome may play a role in regulating estrogen levels (Parida and Sharma, 2019). However, this area of research is only in its infancy. Changes in the intestinal microbiome are considered a risk factor for the progression of ALD, which ultimately may develop into HCC (Hsu et al., 2022).

Bajaj et al. (2014) compared stool samples from individuals with alcohol-related cirrhosis with those from individuals with cirrhosis with etiologies other than solely alcohol. Among the individuals with alcohol-related cirrhosis, the beneficial taxa of the phyla *Firmicutes*, such as *Lachnospiraceae* and *Ruminococcaceae*, were less abundant, whereas organisms in the phyla *Proteobacteria,* including *Enterobacteriaceae*, were drastically increased in abundance (all variations were significant at $P < 0.05$). In another study, an even greater significance ($P < 0.0001$) for differences in abundance was reported when comparing individuals with cirrhosis with healthy controls (Qin et al., 2014). These studies are described below and in Table 3.4.

Leclercq et al. (2014) assessed the effects of AUD on intestinal permeability and microbiome composition among individuals entering a detoxification programme at baseline and 19 days after abstinence, and among individuals in a control group. (Results for intestinal permeability are presented in Section 3.2.6 and Table 3.4.) Among a subset of 13 participants, intestinal microbiome composition and functionality were assessed. Non-metric multidimensional scaling revealed that the bacterial profiles of the participants with AUD who had high intestinal permeability differed from those of the participants with low intestinal permeability and the controls. Specifically, at the family level, bacteria from *Ruminococcaceae* and *Incertae Sedis XIII* were less abundant ($P < 0.05$), whereas bacteria from *Lachnospiraceae* and *Incertae Sedis XIV* were more abundant ($P < 0.05$) among individuals with high intestinal permeability than among those with low intestinal permeability and the controls. When the effects of abstinence were considered, a significant increase in *Ruminococcaceae* was observed among participants with high intestinal permeability ($P < 0.05$). In addition, bacteria from the family *Erysipelotrichaceae* and the genus *Holdemania* decreased significantly among all participants with AUD ($P < 0.05$) after they abstained. Alcohol withdrawal had no impact on the abundance of the other families or genera that were found to be modified among the participants with high intestinal permeability at entry into the programme. However, quantitative PCR analysis revealed that the total amount of bacteria, as well as the levels of *Bifidobacterium* spp. and *Lactobacillus* spp., increased significantly during alcohol withdrawal among individuals with high intestinal permeability and returned to the levels of controls ($P < 0.05$). [The number of participants with AUD was very small, with < 7 per

group when investigating the effects of alcohol withdrawal on the microbiome.]

Ames et al. (2020) collected samples from 22 individuals with AUD who were classified as having "less heavy drinking" (LHD) (< 10 drinks per day, $n = 8$) or "very heavy drinking" (VHD) (≥ 10 drinks per day, $n = 14$) and entered an inpatient treatment programme for 28 days. Homogenized whole stool was analysed using a microbiome sequencing kit to examine 6 of the 9 hypervariable regions of the *16S* gene. The average relative microbial abundance for each time point showed some genera specificity for each group (LHD vs VHD). *Erysipelotrichaceae* and *Lachnospiraceae* were significantly more abundant at the first time point in the LHD group than in the VHD group. Changes in the intestinal microbiota among the LHD and VHD groups differed significantly from day 1 to day 5 ($P = 0.03$) and from day 1 to week 3 ($P = 0.02$). The VHD group had a greater change from baseline than the LHD group. The Shannon diversity index of the intestinal microbiome changed significantly during abstinence among 5 participants, and among 4 individuals there was a significant increase in diversity over time. [The analyses over time of abstinence were performed on an extremely small number of samples, in some cases only 2 or 3 samples.]

A series of studies performed with participants from a cohort in Belgium reported on changes in the intestinal microbiome after abstinence (Gao et al., 2020; Maccioni et al., 2020; Hartmann et al., 2021; Hsu et al., 2022). In these studies, participants with AUD entered a 3-week detoxification and rehabilitation programme in which 2 weeks of inpatient treatment (week 1 and week 3) were separated by 1 week of outpatient care. Stool samples were collected from each participant's first bowel movement after hospital admission, i.e. at the beginning of week 1 and week 3 (reflecting 2 weeks of abstinence). [Abstinence during the week of outpatient treatment (week 2) was not confirmed with any specific testing.]

In the first study (Gao et al., 2020), shotgun metagenomic sequencing was used to assess the reversibility of functional alterations in the intestinal microbiota among individuals with AUD when they were abstinent for 2 weeks. Samples from 30 participants with AUD and 8 controls without AUD were compared. Seven microbial pathways were found to be sensitive to abstinence, with a linear discriminant analysis score > 2.0; all of them were enriched in the sample at week 3 [after 2 weeks of abstinence]. These pathways were isoprene biosynthesis I, phytol degradation, L-isoleucine biosynthesis II, superpathway of geranylgeranyl diphosphate biosynthesis II (via methyl-D-erythritol 4-phosphate), NAD salvage pathway II, glutaryl-coenzyme A degradation, and superpathway of geranylgeranyl diphosphate biosynthesis I (via mevalonate). Among these, four pathways were different when comparing the participants with AUD with the controls at week 1 or week 3. In particular, the relative abundance of the glutaryl-coenzyme A degradation gene was lower among the participants with AUD at week 1 compared with the controls, and it was higher at week 3 than at week 1 but remained lower at week 3 compared with the controls. Furthermore, the link between functional alterations in the intestinal microbiota and alcohol-associated steatosis was investigated. The microbial functional responses were assessed by characterizing microbial composition and controlled attenuation parameter (CAP). Participants with AUD were divided into two groups: high CAP (> 300 dB/m) and low CAP (< 300 dB/m). Functional microbial responses to abstinence were found to vary among individuals with AUD depending on the degree of hepatic steatosis. Among the high-CAP group, five microbial pathways were enriched at week 3: L-isoleucine biosynthesis II, the superpathway of β-D-glucuronosides degradation, the superpathway of hexuronide and hexuronate degradation, the

superpathway of geranylgeranyl diphosphate biosynthesis II (via methyl-D-erythritol 4-phosphate), and glutaryl-coenzyme A degradation. One microbial pathway was enriched at week 1: heterolactic fermentation. Among the low-CAP group, five microbial pathways were enriched at week 1: adenosine nucleotides degradation II, guanosine nucleotides degradation III, the superpathway of pyrimidine ribonucleosides degradation, purine nucleotides degradation II (aerobic), and guanosine 3′,5′-bis(diphosphate) (ppGpp) biosynthesis.

In the second study (Maccioni et al., 2020), samples were collected from 106 individuals with AUD and 24 healthy controls. Intestinal permeability was measured among 86 individuals. Among the participants who had both high urinary excretion of ^{51}Cr-labelled ethylenediaminetetraacetic acid (^{51}Cr-EDTA) and high faecal albumin content, values at week 3 had returned to those observed among the controls, whereas levels of intestinal permeability remained low among the participants with AUD who had normal levels at admission (see Section 3.2.6). Microbial translocation was assessed with serum levels of three markers: the Gram-negative markers soluble CD14 (sCD14) and LPS binding protein (LBP) and the Gram-positive marker peptidoglycan recognition proteins (PGRPs). Serum sCD14 levels decreased significantly upon abstinence ($P = 0.0001$). In contrast, neither LBP nor PGRP levels were modified by alcohol cessation, supporting the observations that increased intestinal permeability is not an absolute requirement for microbial translocation. The microbial composition in the stool was also assessed at the end of the 2-week detoxification programme among participants with AUD, who were subdivided according to both intestinal permeability and stage of ALD. The authors found an increased evenness among species, as expressed by the Shannon and Simpson diversity indexes, only among the participants with AUD who had high intestinal permeability; the number of observed species remained the same. In contrast, α-diversity indexes (richness and evenness) did not change among participants with AUD who had normal intestinal permeability. In addition, a minor change in α-diversity indexes was observed among participants with AUD who had non-progressive ALD but not among those who had progressive ALD. [However, these results were not significant.] Overall, microbial profile (β-diversity) did not change with abstinence. [These results support the concept of a possible link between faecal microbiota dysbiosis and leaky intestines but not with ALD progression among humans.]

The third study (Hartmann et al., 2021) focused on investigating changes in the fungal microbiome in faecal samples from 66 participants with AUD and 18 healthy controls. Principal coordinate analysis of the mycobiome among participants with AUD who were consuming alcohol ($n = 63$) and paired samples from individuals after 2 weeks of abstinence showed a significant difference between the two groups ($P = 0.001$). Specifically, at the genus level, the relative abundance of *Candida, Malassezia*, *Pichia*, *Kluyveromyces*, *Issatchenkia*, *Claviceps*, *Cyberlindnera*, and *Hanseniaspora* was significantly reduced after abstinence, whereas *Trichosporon* was significantly enriched after abstinence compared with during alcohol consumption. Abstinence among participants with AUD was associated with significantly lower proportions of the species *C. albicans, C. zeylanoides, I. orientalis,* and *Cyberlindnera jadinii* than before abstinence. In addition, the relative abundance of the families *Saccaromycodaceae, Malasseziaceae, Cystostereaceae*, *Didymellaceae*, and *Clavicipitiaceae* was significantly more depressed among participants with AUD after abstinence compared with during alcohol consumption; participants with AUD who abstained had significantly higher levels of the families *Metschnikowiaceae* and *Trichosporonaceae*. The specific anti-*C. albicans* immunoglobulin G

(IgG) and IgM serum levels were significantly higher among participants with AUD compared with controls, whereas anti-*C. albicans* IgA levels were similar between the groups. Abstinence resulted in a significant decrease in anti-*C. albicans* IgG levels, whereas the anti-*C. albicans* IgM and IgA levels were not significantly different. [Significance was defined as $P < 0.05$.]

Finally, the effects of abstinence on the intestinal virome were investigated (Hsu et al., 2022). Stool samples from 62 participants with AUD and 16 healthy controls were analysed. Significant differences in the faecal virome, specifically in the composition of bacteriophage species, were observed when comparing the controls with the participants with AUD, regardless of alcohol consumption status. The faecal virome was significantly different among participants with AUD after 2 weeks of abstinence. Phages targeting specific *Lactococcus*, *Leuconostoc*, and *Streptococcus* species and those targeting *Propionibacterium* and *Lactobacillus* species as a whole were more abundant. Significantly fewer individuals who were actively consuming alcohol than who were abstinent had bacteriophages targeting *Lactobacillus* bacteria. Furthermore, in a set of samples collected from participants with AUD entering the programme at week 1, the proportion of samples with *Propionibacterium* phages was significantly smaller than among the samples collected from the controls and from participants after 2 weeks of abstinence. The relative abundance of *Propionibacterium* phages also differed significantly across these three groups ($P = 0.002$, Kruskal–Wallis test), with significantly lower abundance among individuals with AUD who were actively consuming alcohol compared with the controls ($P < 0.001$) and participants with AUD who abstained ($P = 0.005$).

[All these studies are characterized by small sample sizes and unclear descriptions of whether the results obtained are from sample sets from the same participants with AUD and controls, because they all originate from the same cohort. Also, the Working Group noted that the relatively short duration of the abstinence period and the parallel drastic change in dietary and lifestyle habits due to the inpatient nature of the rehabilitation programme limit the strength of the findings about the beneficial effects of abstinence.]

3.2.6 Inflammatory and immune responses

The tumour microenvironment comprises stromal cells (e.g. fibroblasts and endothelial cells) and immune cells (e.g. resident macrophages and lymphocytes), blood vessels, and the extracellular matrix (Anderson and Simon, 2020). Inflammation in the microenvironment contributes to oncogenesis through the production of reactive chemical species, which in turn contribute to DNA mutations (see Sections 3.1.3 and 3.2.1). Stromal cells may also provide survival signals that promote tumour cell growth in primary or metastatic cancer sites (Inamura et al., 2022).

Alcohol consumption can alter the microenvironment of the gastrointestinal tract by increasing microbial translocation. This can result from generation of acetaldehyde (which affects paracellular permeability), direct epithelial damage by high concentrations of alcohol (as encountered in oropharyngeal and oesophageal mucosa, causing increased transcellular permeability), and other mechanisms that are less well characterized (Maccioni et al., 2020). Alcohol consumption alters the intestinal microbiome (see Section 3.2.5) and the mucosal immune system, inducing a local inflammatory state and altering the mucosal-associated invariant T (MAIT) cells (Li et al., 2019). Microbial translocation to the portal vein activates hepatic inflammation, a critical step in the development of alcohol-related hepatitis (ARH) and cirrhosis, which is a well-known precursor of HCC development. Levels of other microbial products, such as LPS, also are increased in peripheral blood (Liangpunsakul et al., 2017), and inflammatory

changes have been found in the adipose tissue of people with liver disease who have heavy alcohol consumption (Voican et al., 2015). As a result, the blood levels of numerous cytokines and circulating immune cells are altered among individuals with heavy alcohol consumption, contributing to a chronic inflammatory state and potentially impairing tumour immune surveillance (Greten and Grivennikov, 2019). The time course of reversal of alcohol-initiated activation of the innate immune system varies, and it may be prolonged among individuals with ARH.

The available studies assessing the effects of alcohol cessation on intestinal permeability and levels of translocation markers and on changes in cytokines are described below, and details are given in Table 3.5.

(a) Intestinal permeability and microbial translocation in the gastrointestinal microenvironment

The upper gastrointestinal tract is exposed to very high molar concentrations of alcohol, which may have effects not seen in tissues exposed to systemic alcohol concentrations; these tissues are also exposed to high concentrations of locally generated acetaldehyde or acetaldehyde present in beverages or tobacco smoke (see Section 3.1).

A study among 40 individuals with alcohol dependence (Leclercq et al., 2012) found increases in intestinal permeability (measured by absorption of ^{51}Cr-EDTA from the intestines) and associated increases in plasma LPS levels, which resolved by 19 days of abstinence (consisting of hospitalization for 1 week, outpatient treatment for 1 week, and inpatient care for 1 week). Direct assessment of intestinal permeability among 60 individuals with alcohol dependence showed that 26 (43%) of them exhibited increased intestinal permeability (Leclercq et al., 2014). The change in intestinal permeability was more marked in the small intestine than in the colon and was trending towards normal by 19 days of abstinence, although it did not normalize in all participants.

In a group of individuals entering alcohol treatment (Maccioni et al., 2020), intestinal permeability was measured among 86 individuals using urinary excretion of ^{51}Cr-EDTA, and among 78 individuals using faecal albumin content (a measure of capillary leakiness). At admission, two thirds of the participants with AUD had intestinal permeability measurements close to those of the controls, whereas 36–40% had high intestinal permeability. This led to a separation of participants into two categories: high and low intestinal permeability. Among individuals with both high urinary excretion of ^{51}Cr-EDTA ($P = 0.0036$ vs controls) and high faecal albumin content ($P = 0.0025$ vs controls), the intestinal permeability returned to values observed among the controls over the 3-week period of abstinence. However, the two measures of intestinal barrier function were not correlated with each other. Intestinal injury, assessed by measuring serum levels of intestinal fatty acid binding protein (I-FABP), a marker of enterocyte death, was not seen (Leclercq et al., 2014; Maccioni et al., 2020).

In a study in Brazil, Varella Morandi Junqueira-Franco et al. (2006) reported improvement in intestinal permeability measured using urinary excretion of ^{51}Cr-EDTA [and oxidative stress (see Section 3.2.7)] among 10 individuals with alcohol-related pellagra who had heavy alcohol consumption (> 90 g per day) after hospitalization for 27 days for abstinence and treatment with the antioxidant niacin (100 mg per day). The percentage of urinary excretion of ^{51}Cr-EDTA (mean ± SD) was 4.29% ± 1.92% before abstinence and 1.90% ± 1.19% after abstinence ($P < 0.05$). [This study did not have any comparison with control samples, and any positive effects resulting from abstinence remain unclear because participants had daily administration of niacin. The participants probably also benefited from an improved diet. Because alcohol is

Table 3.5 Effects of cessation of alcohol consumption on the immune system

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Maier et al. (1999) Germany	21 participants with AUD who were abstinent for < 5 days (group A1); 6 participants with AUD who were abstinent for 5–10 days (group A2) 25 healthy controls	Unknown	Participants who consumed ≥ 80 g of alcohol per day for > 2 years	Endoscopic biopsy of the duodenum Quantitative immunohisto-chemistry	Group A1 vs controls: 37% increase in B lymphocytes $P < 0.005$ 50% decrease in CLA-positive interepithelial leukocytes $P < 0.05$ 54% decrease in macrophages $P < 0.025$ These differences were not seen in group A2
González-Quintela et al. (2000) Spain	29 participants (6 women, 23 men; median age, 47 years) who were admitted for AWS after abstaining from drinking for 24–48 hours. Many participants had liver disease Controls: 5 healthy men	None	Average alcohol consumption in participants: > 120 g per day for ≥ 5 years	Serum samples collected at admission and after a median of 6 days (range, 2–15 days) of hospital stay and subsequent alcohol abstinence Enzyme immunoassay	At baseline: participants with AWS vs controls IL-6 and IL-10 increased $P \leq 0.0007$ IL-8 and IL-12 increased $P = 0.1$, NS After a median of 6 days of abstinence IL-6 and IL-10 decreased $P_{\text{abstinence vs baseline}} \leq 0.004$ IL-8 and IL-12 did not change significantly
Varella Morandi Junqueira-Franco et al. (2006) Brazil	10 men (average age, 35 years) with alcohol-related pellagra who had heavy alcohol consumption and were entering a 27-day inpatient detoxification programme Exclusion criteria: previously treated with vitamins	No information reported about age, BMI, or smoking status	Alcohol consumption: > 90 g of ethanol per day Participants were given 100 mg of niacin per day	Urine IP (^{51}Cr-EDTA)	**IP (% ^{51}Cr-EDTA)**, mean ± SD Baseline: 4.29 ± 1.92 After 27-day abstinence: 1.90 ± 1.19 $P_{\text{abstinence vs baseline}} < 0.05$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Leclercq et al. (2012) Belgium	52 individuals with AD admitted for alcohol detoxification (40 at 18–19 days of abstinence) (81% men; average age ± SD, 47 ± 11 years) 16 controls	Age and BMI Screened for liver disease; none had advanced fibrosis	Abstinence for 18–19 days	Plasma and urine IP (^{51}Cr-EDTA); CRP (multiplex immunoassay)	**IP (% ^{51}Cr-EDTA)** ***Small intestine*** Increased in AD vs controls at baseline $P_{AD\ vs\ controls} < 0.05$ Decreased in AD after abstinence vs baseline $P_{abstinence\ vs\ baseline} < 0.001$ Same levels in AD and controls after abstinence $P_{AD\ vs\ controls} = 0.32$ ***Colon*** Increased in AD vs controls at baseline $P_{AD\ vs\ controls}$: NS Decreased in AD after abstinence vs baseline $P_{abstinence\ vs\ baseline} < 0.05$ Same levels in AD and controls after abstinence $P_{AD\ vs\ controls}$: NR ***Total IP*** Increased in AD vs controls at baseline $P_{AD\ vs\ controls}$: NS Decreased in AD after abstinence vs baseline $P_{abstinence\ vs\ baseline} < 0.01$ Same levels in AD and controls after abstinence: $P_{AD\ vs\ controls}$: NR **LPS (EU/mL)** Increased in AD vs controls at baseline $P_{AD\ vs\ controls} < 0.05$ Decreased in AD after abstinence vs baseline $P_{abstinence\ vs\ baseline} < 0.05$ Same levels in AD and controls after abstinence $P_{AD\ vs\ controls}$: NS **TNF-α (pg/mL)** Increased in AD vs controls at baseline $P_{AD\ vs\ controls} < 0.001$ Decreased in AD after abstinence vs baseline $P_{abstinence\ vs\ baseline}$: NS Increased in AD vs controls after abstinence $P_{AD\ vs\ controls} < 0.01$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Leclercq et al. (2012) (cont.)					**IL-6 (pg/mL)** Increased in AD vs controls at baseline $P_{\text{AD vs controls}} < 0.05$ Decreased in AD after abstinence vs baseline $P_{\text{abstinence vs baseline}}$: NS Increased in AD after abstinence vs controls $P_{\text{AD vs controls}} < 0.05$ **hsCRP (mg/dL)** Increased in AD vs controls at baseline $P_{\text{AD vs controls}} < 0.05$ Decreased in AD after abstinence vs baseline $P_{\text{abstinence vs baseline}}$: NS But still increased in AD vs controls after abstinence $P_{\text{AD vs controls}} < 0.01$ **IL-10 (pg/mL)** Increased in AD vs controls at baseline $P_{\text{AD vs controls}}$: NR Decreased in AD after abstinence vs baseline $P_{\text{abstinence vs baseline}} < 0.001$ Same levels in AD and controls after abstinence $P_{\text{AD vs controls}}$: NR
Leclercq et al. (2014) Belgium	60 participants with AD entering a detoxification programme (47 men, 13 women) and 15 HC Exclusion criteria: obesity, diabetes, chronic inflammatory conditions, liver fibrosis or cirrhosis, and resumption of drinking during follow-up Participants with AD were divided into high-IP and low-IP groups	Age, sex, and BMI	Abstinence for 19 days	Plasma and urine IP (^{51}Cr-EDTA)	**IP (% ^{51}Cr-EDTA)**, mean ± SD ***Small intestine: 0–4 hours*** Controls: 2.36 ± 0.87 At baseline: $AD_{\text{high-IP}}$: 7.81 ± 5.46 $AD_{\text{low-IP}}$: 2.58 ± 0.79 $P_{\text{AD high-IP vs low-IP}} < 0.001$ $P_{\text{AD high-IP vs controls}} < 0.001$ Abstinence for 19 days: $AD_{\text{high-IP}}$: 3.26 ± 2.54 $AD_{\text{low-IP}}$: 1.93 ± 1.11 $P_{\text{AD high-IP vs low-IP}} < 0.05$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Leclercq et al. (2014) (cont.)					***Colon: 4–24 hours*** Controls: 1.08 ± 0.37 At baseline: $AD_{high\text{-}IP}$: 2.79 ± 1.73 $AD_{low\text{-}IP}$: 0.90 ± 0.26 $P_{AD\ high\text{-}IP\ vs\ low\text{-}IP} < 0.001$ $P_{AD\ high\text{-}IP\ vs\ controls} < 0.001$ Abstinence for 19 days: $AD_{high\text{-}IP}$: 1.46 ± 1.01 $AD_{low\text{-}IP}$: 1.00 ± 0.63 ***Total: 0–24 hours*** Controls: 1.34 ± 0.43 At baseline: $AD_{high\text{-}IP}$: 3.71 ± 2.15 $AD_{low\text{-}IP}$: 1.22 ± 0.28 $PAD_{high\text{-}IP\ vs\ low\text{-}IP} < 0.001$ $PAD_{high\text{-}IP\ vs\ controls} < 0.001$ Abstinence for 19 days: $AD_{high\text{-}IP}$: 1.86 ± 1.34 $AD_{low\text{-}IP}$: 1.23 ± 0.75 **TNF-α (pg/mL)** At baseline: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.01$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.05$ Abstinence for 19 days: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.01$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.05$ **IL-6 (pg/mL)** At baseline: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.10$ $AD_{low\text{-}IP}$ vs controls: no significant change

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Leclercq et al. (2014) (cont.)					Abstinence for 19 days: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.05$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.05$ **IL-10 (pg/mL)** At baseline: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.001$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.001$ Abstinence for 19 days: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.01$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.001$ **IL-8 (pg/mL)** At baseline: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.001$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.05$ Increased in $AD_{high\text{-}IP}$ vs $AD_{low\text{-}IP}$ $P < 0.05$ Abstinence for 19 days: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.001$ Increased in $AD_{low\text{-}IP}$ vs controls $P < 0.05$ **IL-1β (pg/mL)** At baseline: $AD_{high\text{-}IP}$ or $AD_{low\text{-}IP}$ vs controls: no significant change Abstinence for 19 days: $AD_{high\text{-}IP}$ or $AD_{low\text{-}IP}$ vs controls: no significant change

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Leclercq et al. (2014) (cont.)					**hsCRP (mg/mL)** At baseline: $AD_{high\text{-}IP}$ or $AD_{low\text{-}IP}$ vs controls: no significant change Abstinence for 19 days: Increased in $AD_{high\text{-}IP}$ vs controls $P < 0.05$ $AD_{low\text{-}IP}$ vs controls: no significant change
Voican et al. (2015) France	47 participants with ALD (38 men, 9 women) Participants divided into 2 groups: mild ALD (35 participants) and severe ALD (12 participants)	NR	Participants had drinking history of 50 g per day during the previous year and had not stopped drinking before admission Alcohol withdrawal was started at admission under strict medical surveillance	Blood and liver and subcutaneous adipose tissue Samples collected at 1 week of abstinence Cytokines and fibrosis markers (mRNAs); macrophage marker (real-time qPCR)	**Mild ALD (abstinence vs admission)** Decreased expression of macrophage markers in adipose tissue $P < 0.05$ Decreased mRNA expression of cytokines/chemokines (IL-18, CCL2, osteopontin, semaphorin 7A) and macrophage marker CD68 **Severe ALD (abstinence vs admission)** Increased expression of macrophage marker CCL18 $P < 0.01$
Donnadieu-Rigole et al. (2016) France	Longitudinal study of 40 participants with AUD who were admitted for detoxification and 20 matched HC (85% men)	Age and sex	Participants with AUD consumed mean ± SD of 202 ± 125 g of pure alcohol per day, and 87.5% were active tobacco smokers 2 weeks of abstinence	Blood (monocytes) Flow cytometry	Values are mean ± SD **$CD14^{+}CD16^{-}$ cells (%)** Controls: (median, 86.5) AUD at baseline: 75.3 ± 11.9 (median, 78.6) AUD after abstinence (2 weeks): 78.1 ± 13.4 $P_{\text{baseline vs controls}} < 0.0001$ $P_{\text{abstinence vs baseline}} = 0.09$ **$CD14^{dim}CD16^{+}$ cells (%)** Controls: (median, 2.5) AUD at baseline: 10.3 ± 7.7 (median, 8.6) AUD after abstinence (2 weeks): 6.7 ± 6.4 $P_{\text{baseline vs controls}} < 0.0001$ $P_{\text{abstinence vs baseline}} < 0.01$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
García-Marchena et al. (2017) Spain	85 participants with AUD who were abstinent and 55 HC (65% men; mean age, 46.6 years) from an outpatient psychiatry clinic	BMI, sex, and age Participants with AUD included individuals with pancreatic disease	Average duration of alcohol consumption, 13 years Mean duration of abstinence, 9 months; minimum, 4 weeks	Plasma Cytokines/ chemokines (multiplex immunoassay)	Values are mean (95% CI) **CXCL12 (pg/mL)** Controls: 349.95 (319.15–384.59) Abstinent with AUD: 285.76 (266.07–307.61) $P < 0.001$ **CX3CL1 (pg/mL)** Controls: 5.689 (4.966–6.516) Abstinent with AUD: 4.592 (4.140–5.105) $P < 0.05$ **IL-8, MCP1, and MIP1α**: no significant change [Levels of cytokines before abstinence not reported]
Li et al. (2017) USA	Longitudinal 12-month study 68 individuals with ARH (61% men; average age, 44 years) 65 HDC without liver disease (57% men; average age, 43 years) 20 HC (55% men; average age, 38 years)	Age, sex, and alcohol consumption for HDC Age and sex for HC	The HDC group had consumed significantly more drinks in the 30 days before enrolment than the ARH group	Plasma Multiplex immunoassay, ELISA	Values are median (IQR) **TNF-α (pg/mL)** HC: 6.5 (4.8–10) ARH at baseline: 17.9 (12.5–35.8) HDC at baseline: 10.2 (6.8–19.2) $P_{\text{ARH vs H}}C < 0.001$ $P_{\text{ARH vs HDC}} < 0.001$ ARH after abstinence (180 days): 27.7 (15.5–32.0) HDC after abstinence (180 days): 8.3 (5.6–13.4) $P_{\text{ARH vs HDC}} < 0.01$ ARH after abstinence (360 days): 27.3 (21.8–45.0) HDC after abstinence (360 days): 11.9 (5.1–17.9) $P_{\text{ARH vs HDC}} < 0.01$ **IL-6 (pg/mL)** HC: 1.4 (0.9–3.3) ARH at baseline: 13.5 (6.6–36.2) HDC at baseline: 2.6 (0.9–6) $P_{\text{ARH vs HC}} < 0.001$ $P_{\text{ARH vs HDC}} < 0.001$ ARH after abstinence (180 days): 7.3 (4.9–19.3) HDC after abstinence (180 days): 4.2 (0.9–7.8) $P_{\text{ARH vs HDC}} < 0.05$ ARH after abstinence (360 days): 6.2 (4.0–8.5) HDC after abstinence (360 days): 2.4 (1.4–5.7) $PA_{\text{RH vs HDC}}$: NS

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Li et al. (2017) (cont.)					**IL-8 (pg/mL)** HC: 6.9 (4.9–16.7) ARH at baseline: 314.2 (117.9–608.4) HDC at baseline: 8.0 (4.7–15.2) $P_{ARH\ vs\ HC} < 0.001$ $P_{ARH\ vs\ HDC} < 0.001$ ARH after abstinence (180 days): 40.4 (23.5–65.2) HDC after abstinence (180 days): 8.3 (5.8–47.6) $P_{ARH\ vs\ HDC} < 0.05$ ARH after abstinence (360 days): 44.2 (25.7–111.4) HDC after abstinence (360 days): 9.4 (5.4–22.8) $P_{ARH\ vs\ HDC} < 0.01$ **IP-10 (pg/mL)** HC: 507 (344–610) ARH at baseline: 1144 (767–1531) HDC at baseline: 629 (429–847) $P_{ARH\ vs\ HC} < 0.001$ $P_{ARH\ vs\ HDC} < 0.001$ ARH after abstinence (180 days): 1317 (882–1764) HDC after abstinence (180 days): 648 (324–1041) $P_{ARH\ vs\ HDC} < 0.01$ ARH after abstinence (360 days): 1169 (861–1274) HDC after abstinence (360 days): 689 (472–1214) $P_{ARH\ vs\ HDC}$: NS **IL-4 (pg/mL)** HC: 4.5 (4.5–14.7) ARH at baseline: 4.5 (4.5–17) HDC at baseline: 4.5 (4.5–7.1) $P_{ARH\ vs\ HC}$: NS $P_{ARH\ vs\ HDC} < 0.05$ ARH after abstinence (180 days): 8.5 (4.5–18.4) HDC after abstinence (180 days): 4.5 (4.5–8.1) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 8.7 (4.5–31.7) HDC after abstinence (360 days): 4.5 (4.5–8.7) $P_{ARH\ vs\ HDC}$: NS

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Li et al. (2017) (cont.)					**IL-9 (pg/mL)** HC: 1.4 (1.2–5.7) ARH at baseline: 1.4 (1.2–5.8) HDC at baseline: 1.2 (0.6–3.6) $P_{ARH\ vs\ HC}$: NS $P_{ARH\ vs\ HDC} < 0.05$ ARH after abstinence (180 days): 2.8 (1.2–5.6) HDC after abstinence (180 days): 1.9 (1.2–4.0) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 4.1 (1.2–15.3) HDC after abstinence (360 days): 1.2 (0.9–1.9) $P_{ARH\ vs\ HDC}$: NS **IL-10 (pg/mL)** HC: 2.7 (1.6–5.6) ARH at baseline: 14.3 (6.4–35.2) HDC at baseline: 5.6 (1.8–10.3) $P_{ARH\ vs\ HC}$: NS $P_{ARH\ vs\ HDC} < 0.001$ ARH after abstinence (180 days): 10.9 (2.8–27.4) HDC after abstinence (180 days): 8.3 (1.1–11.4) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 17.5 (7.6–34.2) HDC after abstinence (360 days): 9.3 (7.6–11.7) $P_{ARH\ vs\ HDC}$: NS **FGF-2 (pg/mL)** HC: 81.6 (54.1–133.1) ARH at baseline: 66.3 (49.3–136.7) HDC at baseline: 56 (26.4–95.9) $P_{ARH\ vs\ HC}$: NS $P_{ARH\ vs\ HDC} < 0.05$ ARH after abstinence (180 days): 102.8 (66.3–122) HDC after abstinence (180 days): 91.9 (62.4–104.3) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 109.1 (83.6–225.3) HDC after abstinence (360 days): 83.6 (58.2–91.9) $P_{ARH\ vs\ HDC} < 0.05$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Li et al. (2017) (cont.)					**IL-7 (pg/mL)** HC: 1.4 (1.4–3.3) ARH at baseline: 8.1 (1.4–15.2) HDC at baseline: 2.1 (1.4–9.7) $P_{ARH\ vs\ HC} < 0.05$ $P_{ARH\ vs\ HDC} < 0.05$ ARH after abstinence (180 days): 7.2 (4.7–10.9) HDC after abstinence (180 days): 5.5 (1.4–10.1) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 12 (8.4–18.9) HDC after abstinence (360 days): 7.4 (1.4–8.7) $P_{ARH\ vs\ HDC} < 0.05$ **IL-15 (pg/mL)** HC: 4.0 (2.1–8.1) ARH at baseline: 14.5 (8.5–23.7) HDC at baseline: 5.2 (2–9.3) $P_{ARH\ vs\ HC} < 0.001$ $P_{ARH\ vs\ HDC} < 0.001$ ARH after abstinence (180 days): 10.7 (5.3–23.1) HDC after abstinence (180 days): 6.4 (1.3–17.9) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 6.5 (3.5–27.6) HDC after abstinence (360 days): 4.5 (2.4–13.3) $P_{ARH\ vs\ HDC}$: NS **TGF-α (pg/mL)** HC: 1.7 (1.2–4.6) ARH at baseline: 5.8 (2.9–12.4) HDC at baseline: 2.4 (1–5.1) $P_{ARH\ vs\ HC} < 0.05$ $P_{ARH\ vs\ HDC} < 0.01$ ARH after abstinence (180 days): 4.7 (2.7–13) HDC after abstinence (180 days): 2.5 (0.8–16.1) $P_{ARH\ vs\ HDC}$: NS ARH after abstinence (360 days): 4.0 (2–40.3) HDC after abstinence (360 days): 5.1 (2.2–13.6) $P_{ARH\ vs\ HDC}$: NS

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Yen et al. (2017) Taiwan (China)	Longitudinal study 78 men with AD (mean age, 40.3 years) admitted into a 4-week detoxification programme Controls: 86 healthy men (mean age, 38.8 years)	Liver disease, age, and BMI	4 weeks of abstinence Only 48 men with AD completed the 4-week abstinence	Plasma Samples collected at baseline and after abstinence Multiplex immunoassay	Values are mean ± SD **IL-2 (pg/mL)** Controls: 1.26 ± 0.89 AD at baseline: 4.56 ± 2.84 AD after abstinence (4 weeks): 1.83 ± 1.47 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} = 0.022$ $P_{\text{abstinence vs baseline}} < 0.001$ **IFN-γ (pg/mL)** Controls: 1.36 ± 1.10 AD at baseline: 4.73 ± 3.87 AD after abstinence (4 weeks): 2.66 ± 1.89 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **TNF-α (pg/mL)** Controls: 2.12 ± 1.68 AD at baseline: 8.49 ± 5.41 AD after abstinence (4 weeks): 3.35 ± 2.19 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **IL-4 (pg/mL)** Controls: 4.76 ± 4.07 AD at baseline: 23.86 ± 17.08 AD after abstinence (4 weeks): 6.09 ± 4.08 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} = 0.031$ $P_{\text{abstinence vs baseline}} < 0.001$ **IL-5 (pg/mL)** Controls: 1.06 ± 0.95 AD at baseline: 3.45 ± 2.16 AD after abstinence (4 weeks): 2.56 ± 1.59 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} = 0.011$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Yen et al. (2017) (cont.)					**IL-6 (pg/mL)** Controls: 0.99 ± 0.82 AD at baseline: 3.43 ± 2.76 AD after abstinence (4 weeks): 1.98 ± 1.74 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} = 0.001$ **IL-10 (pg/mL)** Controls: 1.51 ± 1.22 AD at baseline: 4.42 ± 2.93 AD after abstinence (4 weeks): 2.78 ± 2.58 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **IL-1β (pg/mL)** Controls: 0.19 ± 0.20 AD at baseline: 0.89 ± 0.98 AD after abstinence (4 weeks): 0.63 ± 0.62 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} = 0.02$ **IL-8 (pg/mL)** Controls: 2.26 ± 2.17 AD at baseline: 12.28 ± 10.54 AD after abstinence (4 weeks): 5.04 ± 4.78 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **GM-CSF (pg/mL)** Controls: 1.01 ± 0.71 AD at baseline: 3.82 ± 3.06 AD after abstinence (4 weeks): 0.97 ± 0.49 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs controls}} = 0.731$ $P_{\text{abstinence vs baseline}} < 0.001$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Donnadieu-Rigole et al. (2018) France	41 participants with AUD (30 men, 11 women; average age, 48.2 years) entering a 6-week treatment programme Controls: 100 heathy volunteers from blood donation banks usually characterized by a population of 51% women and aged 44–55 years Exclusion criteria: history of chronic inflammation diseases, current infections, HIV, or undergoing immunomodulatory or immunosuppressive treatments	Controls not matched	NR	Blood IP assessed indirectly by measuring I-FABP or zonulin (ELISA) Microbial translocation monitored by measuring serum levels of LBP and sCD14 (ELISA) or plasma 16S or 23S rDNA (qPCR)	Values are mean ± SD **I-FABP (µg/mL)** Controls: 310.5 ± 196.7 AUD at baseline: 944.1 ± 442.5 AUD after abstinence (6 weeks): 1151.4 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} = 0.21$ AUD with heavy alcohol consumption at baseline: higher I-FABP vs AUD with less alcohol consumption $P = 0.03$ AUD with heavy alcohol consumption after abstinence (6 weeks): smaller decrease in I-FABP vs AUD with less alcohol consumption $P = 0.02$ **Zonulin (µg/mL)** Controls: 36.0 ± 17.02 AUD at baseline: 46.47 ± 16.82 AUD after abstinence (6 weeks): 34.2 $P_{\text{baseline vs controls}}$: NS $P_{\text{abstinence vs baseline}} = 0.639$ AUD with high BMI at baseline: higher zonulin vs AUD with low BMI $P = 0.0001$ AUD with high BMI after abstinence (6 weeks): larger decrease in zonulin vs AUD with low BMI $P = 0.005$ **LBP (µg/mL)** Controls: 5.3 ± 6.2 AUD at baseline: 41 ± 13.3 AUD after abstinence (6 weeks): 39 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} = 0.043$ Cannabis-smoking AUD after abstinence (6 weeks): smaller decrease in LBP vs non-cannabis-smoking AUD $P = 0.04$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Donnadieu-Rigole et al. (2018) (cont.)					**16S rDNA (copies/µL)** Controls: 9.2 ± 1.9 AUD at baseline: 13.9 ± 4.6 AUD after abstinence (6 weeks): 13.9 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}}$: NS **sCD14 (µg/mL)** Controls: 5.2 ± 1.6 AUD at baseline: 5.9 ± 1.6 AUD after abstinence (6 weeks): 5.1 $P_{\text{baseline vs controls}} = 0.024$ $P_{\text{abstinence vs baseline}} = 0.001$ Cannabis-smoking AUD after abstinence (6 weeks): smaller decrease in sCD14 vs non-cannabis-smoking AUD $P = 0.008$
Girard et al. (2019) France	115 participants with AD admitted for detoxification (88 men; average age, 47 years) 27 individuals relapsed (no abstinence at all), 44 relapsed at least once during the first 6 months after withdrawal (14 abstinent again at 6 months), and 30 remained abstinent at all follow-up visits (abstainers)	No control group No comment on presence of liver disease Variable periods of abstinence during 6 months All of the participants were smokers	Follow-up during 6-month abstinence Average duration of AD, 9.7 years	Serum Samples collected at 48 hours and 1, 2, 4, and 6 months after admission Multiplex immunoassay	Values are mean ± SD **TNF-α (pg/mL)** AD at baseline: 1.35 ± 4.11 AD after abstinence (1 month): 0.63 ± 1.36 $P = 0.002$ **IL-6 (pg/mL)** AD at baseline: 2.76 ± 3.49 AD after abstinence (1 month): 2.16 ± 3.31 $P = 0.01$ **IL-8 (pg/mL)** AD at baseline: 21.47 ± 32.42 AD after abstinence (1 month): 11.98 ± 17.23 $P < 0.001$ **IL-10 (pg/mL)** AD at baseline: 0.58 ± 0.91 AD after abstinence (1 month): 0.58 ± 1.07 $P = 0.408$ **MCP1 (pg/mL)** AD at baseline: 433.29 ± 266.24 AD after abstinence (1 month): 382.47 ± 161.77 $P = 0.054$

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Girard et al. (2019) France (cont.)					**IL-1β (pg/mL)** AD at baseline: 0.45 ± 0.83 AD after abstinence (1 month): 0.28 ± 0.58 $P < 0.001$ **IFN-γ (pg/mL)** AD at baseline: 0.07 ± 0.22 AD after abstinence (1 month): 0.09 ± 0.24 $P = 0.489$ **IL-12 (pg/mL)** AD at baseline: 17.85 ± 70.78 AD after abstinence (1 month): 15.16 ± 62.23 $P_{\text{abstinence vs baseline}} = 0.035$
Li et al. (2019) USA	56 participants with ARH, 45 HDC without overt liver disease, and 59 HC	Age, sex, race, and alcohol consumption	NR	Blood Samples collected at baseline and 6-month and 12-month follow-up MAIT cells (flow cytometry)	Values are median (Q1, Q3) **MAIT cells (% of T cells)** HC: 1.25 (0.63, 2.32) ARH at baseline: 0.16 (0.09, 0.34) HDC at baseline: 0.56 (0.23, 1.41) $P_{\text{ARH vs HC}} < 0.001$ $P_{\text{ARH vs HDC}} < 0.001$ $P_{\text{HC vs HDC}} < 0.01$ ARH after abstinence (180 days): increase HDC after abstinence (180 days): increase $P_{\text{ARH abstinence vs ARH baseline}}$: NS $P_{\text{HDC abstinence vs HDC baseline}}$: NS ARH after abstinence (360 days): 0.31 (0.14, 0.61) HDC after abstinence (360 days): 0.63 (0.17, 1.26) $P_{\text{ARH abstinence vs ARH baseline}} < 0.01$ $P_{\text{HDC abstinence vs HDC baseline}}$: NS

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Chaturvedi et al. (2020) India	17 men with AUD admitted for 7-day inpatient detoxification programme (average age, 32 years;) Controls: 12 men (average age, 34 years) with normal liver function tests and no known psychiatric or physical illnesses	Age and sex	Average duration of AUD, 6 years 7-day inpatient abstinence; patients followed up for 1 month after treatment	Serum CD200, a suppressor of inflammation (ELISA)	Values are mean ± SD **CD200 (pg/mL)** Controls: 23.30 ± 6.41 AUD at baseline: 16.25 ± 5.03 AUD after abstinence (1 week): 17.02 ± 4.16 AUD after abstinence (1 month): 17.83 ± 5.36 $P_{\text{baseline vs controls}} = 0.003$ $P_{\text{1-month abstinence vs baseline}} = 0.677$ $P_{\text{all time points vs controls}} < 0.05$
García-Marchena et al. (2020) Spain	85 abstinent participants with AUD and 55 HC (65% men; mean age, 46.6 years) Participants with AUD included individuals with pancreatic disease	BMI, sex, and age	Average duration of AUD, 13 years Mean duration of abstinence, 9 months; minimum, 4 weeks	Plasma Multiplex immunoassay	Abstinent AUD vs HC Increased IL-1β and IL-6 $P < 0.001$ Increased TNF-α $P < 0.01$ Decreased IL-4 and IL-17A $P < 0.001$ Decreased IFN-γ $P < 0.05$
Maccioni et al. (2020) Belgium	106 individuals with AUD (78 men; mean age ± SEM, 46 ± 9.2 years) entering a 3-week detoxification programme Controls: 24 healthy volunteers (14 men, 10 women; mean age ± SEM, 42 ± 11 years) who consumed < 20 g of alcohol per day, defined as social drinking	Age, sex, and BMI (1 to 4 ratio) Stratified based on degree of liver injury and IP	Individuals with AUD had consumed > 60 g of alcohol per day for ≥ 1 year Detoxification programme consisted of 1 week of inpatient detoxification, followed by 1 week of outpatient care and 1 week of inpatient treatment	Urine and stool IP (^{51}Cr-EDTA); faecal albumin content (ELISA)	**IP (% ^{51}Cr-EDTA) after 3 weeks of abstinence** Decreased in $AUD_{\text{high-IP}}$ $P_{\text{abstinence vs baseline}} = 0.0036$ $AUD_{\text{low-IP}}$: no change **Faecal albumin content (3 weeks abstinence) (µg/g of stool)** Decreased in $AUD_{\text{high-IP}}$ $P_{\text{abstinence vs baseline}} = 0.0025$ $AUD_{\text{low-IP}}$: no change

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Jung et al. (2021) Austria and Germany	37 participants with AUD (26 men, 11 women; average age, 49 years) entering an inpatient alcohol rehabilitation programme 17 controls (10 men, 7 women; average age, 45.1 years) who consumed < 10 g per day of alcohol	Sex and age	Participants had ALD (24 low fibrosis, 12 advanced fibrosis based on transient elastography) 1-week inpatient abstinence	Serum I-FABP (western blot); LPS (limulus amoebocyte lysate assay); LBP and zonulin (ELISA); TLR2 and TLR4 ligands (reporter gene assays)	**LPS (EU/mL)** Controls: 1 AUD at baseline: 1.6 AUD after abstinence: 1.4 $P_{\text{AUD vs abstinent}} < 0.05$ $P_{\text{AUD vs controls}}$: NS **LBP (µg/mL)** Controls: 25 AUD at baseline: 40 AUD after abstinence: 45 $P_{\text{AUD baseline or AUD abstinent vs controls}} < 0.05$ $P_{\text{AUD vs abstinent}}$: NS **Zonulin (ng/mL)** Controls: 2.25 AUD at baseline: 1.8 AUD after abstinence: 2.0 $P_{\text{AUD vs abstinent}} < 0.05$ $P_{\text{AUD baseline or AUD abstinent vs controls}}$: NS **I-FABP (intensity units)** Controls: 2.3 M AUD at baseline: 1.7 M AUD after abstinence: 2 M $P_{\text{AUD vs abstinent}} < 0.05$ $P_{\text{AUD baseline or AUD abstinent vs controls}}$: NS **TLR4 ligands (fold over controls)** AUD at baseline: 2.5 AUD after abstinence: 2.0 All comparisons: $P < 0.05$ **TLR2 ligands (fold over controls)** AUD at baseline: 4.5 AUD after abstinence: 3.0 All comparisons: $P < 0.05$ [Levels before and after 1-week abstinence were estimated from figures]

Table 3.5 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)[a]	Results
Shiba et al. (2021) Japan	Inpatient alcohol rehabilitation treatment 72 men with AD (average age, 52 years) 13 men without AD as controls	Sex	Participants consumed > 60 g per day; 8 with cirrhosis (none with Child–Pugh score of C) 4-week inpatient treatment ensuring abstinence	Circulating blood mononuclear cells Cytokine release (flow cytometry) after *ADH1B* and *ALDH2* genotyping (PCR)	**CD14+CD16− cells (% of PBMC)** Controls: 19 AD at baseline: 11 AD after abstinence: 15 $P_{\text{AD baseline vs controls}} < 0.05$ $P_{\text{AD baseline vs AD abstinence}} < 0.05$ $P_{\text{AD baseline vs controls}}$: NS **CD14intCD16+ or CD14−CD16+ cells (% of PBMC)** No difference for either subset between controls, AD at baseline, or AD after abstinence **TNF-α after LPS stimulation of CD14+CD16− cells (pg/nL)** Controls: 400 AD at baseline: 100 AD after abstinence: 200 All comparisons: $P < 0.05$ **IL-6 after LPS stimulation of CD14+CD16− cells (pg/nL)** Controls: 6000 AD at baseline: 1000 AD after abstinence: 2000 All comparisons: $P < 0.05$
Yang et al. (2021) USA	79 participants with ARH, 66 HDC without liver disease, and 46 HC	Sex and alcohol consumption for HDC; age and sex for HC	Follow-up of abstinence at 6 months and 12 months	Plasma REG3α and TFF3 (specific ELISA kits)	Baseline: increased TFF3 and REG3α in ARH vs HC and HDC $P < 0.001$ The elevated levels persisted at 6 months and 12 months after abstinence

[a] % ^{51}Cr-EDTA is the percentage of the ingested dose of ^{51}Cr-EDTA found in urine, normalized for creatinine.

16S rDNA, 16S ribosomal DNA subunit; AD, alcohol dependence; *ADH1B*, alcohol dehydrogenase 1B gene; ALD, alcohol-related liver disease; *ALDH2*, aldehyde dehydrogenase gene; ARH, alcohol-related hepatitis; AUD, alcohol use disorder; AWS, alcohol withdrawal syndrome; BMI, body mass index; CCL2, C–C motif chemokine ligand 2; CLA, common leukocyte antigen; CRP, C-reactive protein; CX3CL1, chemokine C–X3–C motif ligand 1; CXCL12, chemokine C–X–C motif ligand 12; EDTA, ethylenediaminetetraacetic acid; ELISA, enzyme-linked immunosorbent assay; EU, endotoxin unit; FGF-2, fibroblast growth factor 2; GM-CSF, granulocyte macrophage colony-stimulating factor; HC, healthy controls; HDC, heavy drinking controls; hsCRP, high-sensitivity C-reactive protein; I-FABP, intestinal fatty acid binding protein; IFN-γ, interferon γ; IL, interleukin; IP, intestinal permeability; IP-10, IFN-γ-induced protein 10; IQR, interquartile range; LBP, lipopolysaccharide binding protein; LPS, lipopolysaccharide; MAIT, mucosal-associated invariant T; MCP1, monocyte chemoattractant protein 1; MIP1α, macrophage inflammatory protein 1 alpha; mRNA, messenger RNA; NR, not reported; NS, not significant; PBMC, peripheral blood mononuclear cells; PCR, polymerase chain reaction; Q1, first quartile; Q3, third quartile; qPCR, quantitative polymerase chain reaction; rDNA, ribosomal DNA; REG3α, regenerating islet-derived protein 3α; sCD14, soluble CD14; SD, standard deviation; SEM, standard error of the mean; TFF3, trefoil factor 3; TGF-β, tumour growth factor β; TLR, toll-like receptor; TNF-α, tumour necrosis factor α.

known to cause oxidative stress, the correlation between improved oxidative stress measures (see Section 3.2.7) and improved intestinal permeability points towards a decrease in cancer risk upon cessation of alcohol consumption.]

In a study in France, Donnadieu-Rigole et al. (2018) assessed the beneficial effects of alcohol withdrawal on intestinal permeability and microbial translocation among 41 individuals (mostly men) with AUD. Intestinal permeability was assessed indirectly by measuring levels of I-FABP or zonulin, a modulator of intestinal tight junctions. In addition, plasma levels of three microbial translocation markers were measured: LBP, the LPS co-receptor sCD14, and the 16S or 23S ribosomal DNA subunits (16S or 23S rDNA). Levels of these markers were compared with those among controls (blood samples from 100 healthy women aged 40–55 years) and with samples collected again from the same individuals with AUD at 3 weeks and 6 weeks after alcohol withdrawal. At baseline, levels of the markers LBP, 16S rDNA, and sCD14 were significantly higher among the individuals with AUD than among the controls. The mean plasma levels of I-FABP were significantly higher among the individuals with AUD than among the controls; no significant difference was found in the zonulin levels. At 6 weeks after alcohol withdrawal, the plasma levels of sCD14 and LBP decreased significantly, but the mean levels of 16S rDNA remained unchanged. [Although these changes were statistically significant, they were numerically small and may not be clinically significant.] The levels of the intestinal permeability markers I-FABP and zonulin did not change significantly after 6 weeks of alcohol withdrawal. [These results could have been affected by cannabis use, high body mass index, and use of women as controls for men.]

Jung et al. (2021) examined the effect of 1 week of abstinence on markers of intestinal injury and microbial translocation. They compared 37 participants with heavy alcohol consumption and ALD (ranging from fatty liver without fibrosis to cirrhosis) with 17 age- and sex-matched individuals with very light alcohol consumption (< 10 g per day). Circulating levels of I-FABP and zonulin were not significantly lower among the participants with heavy alcohol consumption and increased after 1 week of abstinence. LPS levels among the participants with ALD were not significantly higher than those among the controls, because 24 of the 37 participants with ALD did not have elevated LPS levels at admission. LBP levels were higher at admission and remained higher after 1 week of abstinence. A novel method was used to assess bacterial translocation: cell lines expressing reporter genes responding to ligands of toll-like receptor 2 (TLR2) (lipoteichoic acid, from Gram-positive bacteria) and TLR4 (LPS, from Gram-negative bacteria). The levels of these ligands were significantly higher among the participants with heavy alcohol consumption and decreased significantly after 1 week of abstinence, although they remained significantly higher than those among the controls.

Yang et al. (2021) conducted a longitudinal study of 79 individuals with ARH, 66 individuals with heavy alcohol consumption and without overt liver disease at baseline, and 46 healthy controls. The levels of regenerating islet-derived protein 3α (REG3α) and trefoil factor 3 (TFF3) (identified as biomarkers for intestinal injury) at baseline were significantly higher only among the individuals with ARH compared with the controls, and the elevated levels persisted at 6 months and 12 months of follow-up upon abstinence.

(b) Mucosal immune cells in the gastrointestinal environment

A potential consequence of the changes in the microbiome, increased permeability, and microbial translocation is intestinal inflammation (Bishehsari et al., 2017), which is modulated by the effects of alcohol on mucosal immune cells.

In the study of Maccioni et al. (2020), there were no overt histological features of inflammation in duodenal biopsies from the participants with AUD; in fact, there were fewer haematopoietic cells ($CD45^+$), macrophages ($CD68^+$), and T cells ($CD3^+$) compared with controls. Assays of mRNA levels of interleukin-17 (IL-17), IL-1β, interferon γ (IFN-γ), and IL-22 showed increases only in the IL-1β transcripts.

A small study (Maier et al., 1999) compared duodenum samples from participants who consumed ≥ 80 g of alcohol per day for > 2 years and healthy controls. In the samples taken within 5 days of abstinence from the individuals with heavy alcohol consumption, increased numbers of B lymphocytes and decreased numbers of common leukocyte antigen (CLA)-positive intraepithelial lymphocytes and of macrophages were seen compared with controls. These changes were no longer seen after 5–10 days of abstinence among a small subset of the cohort. [The Working Group noted that this was a very small study, with a very short abstinence period and little control for other variables.]

(c) *Effects on circulating markers of immune activation*

Translocation of microbial products to the liver via the portal vein is thought to play a central role in the pathogenesis of ALD. The resident macrophages (Kupffer cells) are activated (especially via TLR4-mediated pathways), causing the release of numerous cytokines and increasing the generation of reactive chemical species. Microbial LPS levels also are increased in peripheral circulation, potentially stimulating immune responses in distant organs (Liangpunsakul et al., 2017). The understanding of the extent and time course for resolution of these effects is based largely on measuring changes in circulating cytokines and the properties of cells obtained from individuals who have heavy alcohol consumption and are entering a treatment programme for AUD or ALD, or from comparisons of individuals who have heavy alcohol consumption with individuals who are abstinent. These results are discussed below and summarized in Table 3.5.

(i) *Cytokines and chemokines and circulating markers of inflammation*

The immune system is sensitive to even short-term exposure to alcohol. Acute administration of 60 g of alcohol to 5 healthy volunteers caused an increase in IL-8 concentrations (González-Quintela et al., 2000). Among 221 individuals who never consumed alcohol, 140 who had light alcohol consumption, 53 who had moderate alcohol consumption, and 45 who had heavy alcohol consumption, the percentage of individuals with high IL-8 concentrations (defined as > 10 pg/mL) increased from 5.9% in those who never consumed alcohol to 10.7% in those with light alcohol consumption, 13.2% in those with moderate alcohol consumption, and 17.8% in those with heavy alcohol consumption (Gonzalez-Quintela et al., 2007). Increases in concentrations of IL-2, IL-4, IL-10, and IFN-γ were reported after 30 days of consumption of moderate amounts of beer by a group of 57 healthy adults who abstained for 30 days before this exposure period (Romeo et al., 2007). Walline et al. (2018) compared cytokine concentrations among 22 individuals who had heavy alcohol consumption with those among 20 individuals who had lower alcohol consumption and found increased LPS levels but no difference in IL-6 or IL-10 levels. Elevations in levels of the inflammatory cytokines IFN-γ-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP1) (Manzardo et al., 2016; Bjørkhaug et al., 2020), and IL-17 (Xu et al., 2020) have been observed among individuals with heavy alcohol consumption compared with individuals who are abstinent. [The effect of abstinence on these cytokines was not reported.]

One approach to understanding the effect of abstinence on alcohol-induced inflammation

is to compare cytokine concentrations among individuals who recently abstained with those in controls. In one study (García-Marchena et al., 2017), the concentrations of the chemokines CXCL12 and CX3CL1 were found to be reduced among 85 individuals who formerly had heavy alcohol consumption and had abstained for ≥ 4 weeks (average, 9 months) compared with 55 healthy controls. [The wide range of duration of abstinence is somewhat problematic, and it is not understood why the chemokine levels would remain persistently low or whether they would increase with even longer abstinence. The presence of other disease was not discussed.]

García-Marchena et al. (2020) also reported higher levels of inflammatory cytokines (IL-1β, IL-6, and tumour necrosis factor α [TNF-α]) and decreased levels of potentially anti-inflammatory cytokines (IL-4 and IL-17) among 85 individuals who formerly had heavy alcohol consumption and were now abstinent compared with 55 healthy controls [probably the same participants as in the study of García-Marchena et al. (2017)]. [Among the participants who formerly had heavy alcohol consumption and abstained, those with liver and pancreatic disease had higher IL-6 levels and lower IL-17 levels than the other participants who abstained, underscoring the importance of recognizing the presence of co-existing alcohol-related organ injury in any such studies.]

A more informative way of determining the effect of abstinence has been to measure cytokine concentrations at admission for rehabilitation treatment and at various durations of abstinence thereafter. [A common confounder in these studies is the stress of withdrawal on the markers under investigation, and the presence or absence of liver disease among the participants undergoing treatment.] González-Quintela et al. (2000) studied cytokine levels among 29 participants hospitalized for rehabilitation treatment and reported higher IL-6 and IL-10 concentrations at admission, which decreased after 6 days of abstinence but remained substantially higher than those among the controls. The levels of IL-8 and IL-12 did not differ from those among the controls and they did not change significantly with abstinence. [The Working Group noted that 6 days is a short duration of abstinence and that the control group was only 5 individuals.]

In the study of Leclercq et al. (2012) described previously, plasma levels of IL-6, TNF-α, and C-reactive protein (CRP) were significantly increased at the initial and final measurements among individuals with alcohol dependence compared with controls. Half of the participants had a decrease in IL-6 levels and two thirds had a decrease in TNF-α levels after 19 days of abstinence. IL-10 levels were higher at entry compared with controls and decreased significantly with abstinence. Compared with controls, the participants with alcohol dependence had significantly higher concentrations of high-sensitivity CRP (hsCRP) and LPS at entry; hsCRP levels remained significantly higher after abstinence.

In the subsequent study (Leclercq et al., 2014), plasma levels of TNF-α, IL-8, and IL-10 were increased (and there was a non-significant increase in IL-6 levels) among individuals with or without high intestinal permeability at baseline; after 19 days of abstinence, the levels remained elevated compared with controls. Also, IL-1β levels were slightly elevated at both time points. Plasma hsCRP concentrations were increased after abstinence only among the subset of participants with high intestinal permeability.

Girard et al. (2019) followed up a cohort of 115 individuals with AUD after admission for detoxification for up to 6 months. The degree of abstinence was assessed at each time point using the timeline follow-back method, and the participants served as their own controls. Of the participants, 26% abstained completely for 6 months, 38% had at least one relapse, and 23% were not abstinent at any time during follow-up. The concentrations of TNF-α, IL-6, IL-8, IL-12, and IL-1β decreased after 1 month; levels of MCP1 decreased and then plateaued at a lower

concentration at 2 months of abstinence. There were no changes in levels of IL-10, IL-12, or IFN-γ. Otherwise, there was no difference in cytokine concentration at the later time points between the individuals who abstained and those who did not abstain. [There was no control group, only the cytokine levels at the beginning of detoxification, and the data were combined for individuals who were abstinent, who were not abstinent, and who had a relapse. Not all cytokines were detectable in all participants at admission, except for MCP1 and IL-8, and they became detectable or undetectable at various time points, leading to large standard deviations.]

Yen et al. (2017) studied 78 men with AUD, at entry into detoxification treatment and 4 weeks later, for a panel of cytokines (IL-2, IFN-γ, TNF-α, IL-4, IL-5, IL-6, IL-10, IL-1β, IL-8, and granulocyte macrophage colony-stimulating factor [GM-CSF]). The control group was 86 healthy individuals. All cytokine concentrations were higher at admission among the individuals with AUD than among the controls. Within 4 weeks, all of the concentrations had decreased significantly in the 48 participants who abstained; the level of GM-CSF decreased to the control levels, whereas levels of the other cytokines were still significantly higher than the control levels [although, in most cases, quite close to the control levels].

Liangpunsakul et al. (2017) studied 97 individuals with excessive alcohol consumption and 51 individuals without excessive alcohol consumption. They found that the individuals with excessive alcohol consumption had higher concentrations of LPS, sCD14, and sCD163 (a marker of macrophage activation) than those without excessive alcohol consumption, and the concentrations correlated with the quantity of alcohol consumed in the previous 30 days. In a separate cohort, 31 individuals in rehabilitation treatment were studied for changes in these immune markers over 12 weeks. The concentrations decreased to normal after 4 weeks and remained low for 12 weeks.

A consortium evaluating the pathogenesis of ARH (Li et al., 2017) studied an array of 38 cytokines among 20 healthy controls, 65 individuals with heavy alcohol consumption but without liver disease (ascertained by serum liver tests), and 68 individuals with ARH. There was no difference in cytokine concentrations between the participants with heavy alcohol consumption and the controls. There was an increase in 11 inflammatory cytokines, anti-inflammatory cytokines, and growth factors, including TNF-α, IL-6, IL-8, IP-10, IL-10, IL-7, IL-15, and tumour growth factor α (TGF-α), among the individuals with ARH compared with the individuals with heavy alcohol consumption but without liver disease, and some of the changes persisted for 12 months (Li et al., 2017). [This study reinforces the caveat that studies of the effect of alcohol among individuals with heavy alcohol consumption must consider the possibility that there is occult liver disease confounding the conclusions, because histological alcohol-related steatohepatitis can be present among individuals who have only mild abnormalities in aminotransferases (Seitz et al., 2018).]

Voican et al. (2015) studied 47 individuals with ALD at baseline and at 1 week after withdrawal of alcohol. They measured mRNA expression levels in blood samples and subcutaneous fat biopsies for a host of adipokines and cytokines, including IL-1β, IL-18, caspase-1, C–C motif chemokine ligand 2 (CCL2), osteopontin, and semaphorin 7A. The levels of these chemokines and inflammasome components were correlated with liver damage at baseline. After 1 week of abstinence, macrophage infiltration of subcutaneous fat decreased and the macrophages were reoriented towards an anti-inflammatory M2 phenotype.

Chaturvedi et al. (2020) examined the effect of alcohol consumption on the concentration of neuroimmune regulators (molecules in the

nervous system that can silence innate immune responses and suppress inflammation). The concentration of the neuroimmune regulator CD200 was lower among the individuals with AUD at baseline compared with the controls, and no significant improvement was reported at 1 month of abstinence.

(ii) Circulating immune cells

Donnadieu-Rigole et al. (2016) studied flow cytometric characteristics of peripheral blood mononuclear cells among individuals with AUD at admission for detoxification and after 2 weeks of abstinence. At admission, an altered distribution of circulating monocytes was found among the participants with AUD, with a decrease in the classical $CD14^+/CD16^-$ monocyte subset and an increase in the non-classical $CD14^{dim}/CD16^+$ subset compared with healthy controls. These changes improved partially during abstinence.

In another study (Li et al., 2019), individuals with heavy alcohol consumption had reduced numbers of MAIT cells (expressed as a percentage of T cells) of 0.56% compared with individuals who were abstinent (1.25%), and individuals with ARH had even lower levels (0.16%). Among the individuals with ARH, the cells were hyperactivated, which was associated with increased plasma levels of IL-7, IL-15, IL-17, IL-18, IL-23, IFN-γ, and TNF-α. No changes were observed in the levels of other circulating innate T cells, including natural killer (NK) T cells, invariant NK T cells, and γδ T cells. The numbers of MAIT cells increased among individuals with heavy alcohol consumption and among individuals with ARH after 6 months and 12 months of abstinence but remained significantly lower than those among healthy controls. [The activation of the MAIT cells may have resulted from the changes in the intestinal mucosa discussed previously, ultimately leading to activation-induced cell death.]

Shiba et al. (2021) studied 72 men with alcohol dependence who were admitted to the hospital for inpatient treatment for 4 weeks. They examined changes in levels of LPS and responsiveness to LPS stimulation of circulating blood monocytes, and the interaction between these changes and the genotypes of the individuals at the *ADH1B* and *ALDH2* loci. The control group consisted of 13 healthy men. There were lower percentages of $CD14^+CD16^-$ cells at entry among the participants with alcohol dependence compared with the controls; these increased at 4 weeks but not to control levels. There was no difference in the levels of $CD14^{int}CD16^+$ or $CD14^-CD16^+$ subtypes, nor were there changes in the levels of other circulating cells (plasmacytoid dendritic cells, NK cells, or NK T cells). When the $CD14^+CD16^-$ cells were incubated with LPS overnight, they produced less TNF-α and IL-6 than control cells, and this difference partially reverted at 4 weeks. $CD14^+CD16^-$ cells from individuals with alcohol dependence who were heterozygous for *ALDH2*2* had an even more suppressed response to LPS, which was potentiated by the presence of an *ADH1B*2* allele, compared with cells from participants who were homozygous for *ADH1B*1*. [This suggests that the concentrations of acetaldehyde reached during heavy alcohol consumption contributed to this effect.] Incubation of the $CD14^+CD16^-$ cells with malondialdehyde–acetaldehyde–albumin adducts attenuated the response of the cells to a second stimulation with LPS after an initial 24-hour exposure, again supporting a role for acetaldehyde in this effect on the circulating monocytes. The mechanism for this effect was suggested to involve induction of IL-1 receptor-associated kinase M, which regulates the response to LPS, in the cells from the individuals with alcohol dependence.

Shiba et al. (2021) extended their study to mice that were fed ethanol by gavage for 42 days, mice that were fed ethanol and then had ethanol withdrawn for 21 days, and control mice. Hepatic macrophages ($CD11b^+$ cells) were isolated and stimulated with LPS. Intracellular

TNF-α expression was reduced in the cells from the ethanol-fed animals and returned to normal 21 days after withdrawal of ethanol. Finally, the authors compared the responses of hepatic $CD11b^+$ cells from wild-type and *ALDH2*2* transgenic mice that were fed ethanol and found that the *ALDH2*2* mice had lower levels of LPS-stimulated TNF-α.

3.2.7 Oxidative stress

Ethanol oxidation, especially via CYP2E1, is associated with the generation of reactive oxygen species. CYP2E1 is considered a "leaky" enzyme, which can readily transfer electrons from NAD phosphate to oxygen rather than to ethanol, generating hydroxyl radicals, superoxide anions, and hydrogen peroxide (IARC, 2010); in addition, the activity of CYP2E1 is induced by alcohol consumption. Reactive oxygen species are detoxified by superoxide dismutase, the selenoprotein glutathione peroxidase (GPx), glutathione reductase, and catalase, and by reaction with retinol, carotene, and vitamin E. Increased production of reactive oxygen species can lead to increased breakdown products of lipid peroxides. Evidence of oxidative stress and of impaired ability to detoxify reactive oxygen species has been found among individuals with chronic heavy alcohol consumption; five studies have assessed the time course for resolution of these effects (Table 3.6).

D'Antonio et al. (1986) measured carotene, retinol, and α-tocopherol on day 0 and day 5 among 192 men with AUD admitted for detoxification. Plasma carotene levels were somewhat low at admission (94 μg/dL among 149 participants) and increased by 30% by day 5. Dietary history suggested an intake of carotene ~1 unit lower than that considered normal. Retinol and α-tocopherol levels were normal at admission and on day 5. Among a subset of 19 participants, carotene levels increased after 33 days of abstinence to 190 μg/dL. [The increase in carotene levels could have resulted from abstinence or improved dietary intake.]

Girre et al. (1990) assessed selenium, vitamin E (α-tocopherol measurement), and GPx levels among 25 individuals with AUD and 25 age- and sex-matched controls. The plasma and erythrocyte levels of GPx and α-tocopherol and the plasma and whole-blood levels of selenium were reduced among the participants with AUD compared with the controls. After 14 days of abstinence in hospital (and no dietary supplementation), plasma levels of selenium remained low but whole-blood levels increased. Plasma levels of GPx were unchanged after abstinence, whereas erythrocyte enzyme activity increased significantly (not reaching the normal level). Plasma and erythrocyte levels of α-tocopherol remained low with abstinence.

Lettéron et al. (1993) measured exhaled ethane as a measure of oxidative stress among 42 healthy individuals who were controls, 52 participants with liver disease other than ALD who were controls, and 89 participants who had heavy alcohol consumption and were admitted to the hospital for alcohol withdrawal and liver disease assessment. Among the participants with heavy alcohol consumption, 73 had advanced liver disease (ARH, cirrhosis, or both). Samples of breath were obtained from the participants at various times after admission to the hospital. The level of exhaled ethane was 5 times as high among the individuals with heavy alcohol consumption and ALD as among the controls or the participants without ALD. The participants without ALD served as a control for the effect of liver disease. In a small subset of that group (9 participants followed up over time), there was a slow resolution towards normal with abstinence, over a period of weeks. The level of exhaled ethane was positively but weakly correlated with levels of daily alcohol consumption before admission.

In Taiwan (China), Peng et al. (2005) investigated the activity of antioxidant enzymes and malondialdehyde in plasma from 29 participants

Table 3.6 Effects of cessation of alcohol consumption on oxidative stress

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
D'Antonio et al. (1986) USA	192 participants (men) with AUD (126 White, 66 Black; average age, 40 years) entering a 4-day inpatient treatment followed by a 28-day outpatient detoxification programme	No control group (population data) Dietary survey done at admission	Total pure alcohol per day (mL), mean ± SD: 341 ± 353 149 participants (96 White, 53 Black) completed the 4-day inpatient programme. Among them, 19 patients (16 White, 3 Black) completed the 28-day outpatient programme	Blood Plasma concentration of carotene (colorimetry); serum retinol and α-tocopherol (HPLC)	**Carotene (µg/dL)** At baseline: 94 After 4-day abstinence: 145 (comparable to "non-alcoholic population") After 33-day abstinence: 190 (within normal range) **Retinol (µg/dL) and α-tocopherol (µg/10 mL)** At baseline: within normal range After 4-day abstinence: no change After 33-day abstinence: no change [Levels were estimated from figures]
Girre et al. (1990) France	25 participants (20 men, 5 women; mean age, 40 years) entering a 14-day inpatient detoxification programme Controls: 25 healthy hospital staff members (mean age, 39 years) who consumed alcohol occasionally (weekly consumption < 100 g)	Sex and age No patients with cirrhosis; no dietary supplements	Alcohol consumption: mean, 253 g per day for a duration (mean ± SD) of 11.8 ± 8.1 years 14 days of inpatient abstinence	Blood Plasma and whole-blood selenium (atomic absorption spectrometry), GPx (enzyme assay), α-tocopherol (HPLC)	Values are mean ± SD **Plasma selenium (µmol/L)** Baseline: 0.92 ± 0.20 Abstinence: 0.88 ± 0.21 Controls: 1.15 ± 0.20 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **Whole-body selenium (µmol/L)** Baseline: 0.90 ± 0.26 Abstinence: 1.09 ± 0.31 Controls: 1.33 ± 0.29 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.025$ $P_{\text{abstinence vs controls}} < 0.001$ **Plasma GPx (U/L)** Baseline: 292.1 ± 102.2 Abstinence: 281.2 ± 63.9 Controls: 328.3 ± 51.4 $P_{\text{baseline vs controls}} < 0.05$ $P_{\text{abstinence vs baseline}}$: NS **Erythrocyte GPx (U/g Hb)** Baseline: 28.2 ± 4.7 Abstinence: 31.09 ± 5.62 Controls: 34.1 ± 3.1

Table 3.6 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Girre et al. (1990) (cont.)					$P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}} < 0.001$ **Plasma α-tocopherol (mg/L)** Baseline: 10.36 ± 3.06 Abstinence: 10.86 ± 3.29 Controls: 14.63 ± 4.21 $P_{\text{baseline vs controls}} < 0.005$ $P_{\text{abstinence vs baseline}}$: NS **Erythrocyte α-tocopherol (mg/L)** Baseline: 2.29 ± 0.69 Abstinence: 2.38 ± 0.69 Controls: 3.25 ± 0.95 $P_{\text{baseline vs controls}} < 0.001$ $P_{\text{abstinence vs baseline}}$: NS
Lettéron et al. (1993) France	89 HD participants (45 men, 44 women; average age, 49 years) admitted to a hospital for alcohol withdrawal and assessment of liver disease Control group 1: 42 healthy laboratory or clinical staff members (22 men, 20 women; average age, 40 years) Control group 2: 52 patients with liver disease other than ALD (29 men, 23 women; average age, 49 years)	Liver conditions in HD participants and controls: HD participants: 10 with hepatic steatosis, 6 with ARH, 29 with cirrhosis without ARH, 34 with hepatic cirrhosis and ARH, and 4 with alcohol-related cirrhosis and hepatocellular carcinoma Control group 2: 2 with liver transplants, 6 with acute hepatitis, 11 with chronic hepatitis, 17 with viral cirrhosis, 1 with liver polyadenomatosis, 5 with non-alcohol-related hepatocellular carcinoma, 2 with liver metastases, 5 with sclerosing cholangitis, 1 with biliary cirrhosis, and 2 with extrahepatic bile duct obstruction	Varying times after admission; 9 HD participants studied serially	Exhaled breath Ethane (gas-liquid chromatography)	**Exhaled ethane (pmol/L)** HD: ~800 Control group 1: ~200 Control group 2: ~200 $P < 0.0001$ [Levels were estimated from figures] Positively correlated with previous daily alcohol consumption ($r = 0.275$; $P = 0.009$) Inversely correlated with duration of abstinence ($r = 0.235$; $P = 0.026$) Serially studied HD participants had variable rates of normalization

Table 3.6 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Peng et al. (2005) Taiwan (China)	29 participants with AUD (28 men, 1 woman; average age, 43.81 years) admitted for detoxification Controls: 19 healthy participants (11 men, 8 women; average age, 30.33 years)	Participants with AUD: HBsAg-negative and no liver cirrhosis	Alcohol consumption (mean ± SD): 271.2 ± 123.6 g per day for a duration (mean ± SD) of 22.2 ± 10.5 years 14 days of abstinence	Blood (serum) MDA (thiobarbituric acid assay); CAT, GPx, and GR (enzyme assay); SOD (ELISA)	Values are mean ± SD **MDA (μM)** 24 hours after admission AUD: 6.50 ± 2.14 Controls: 3.96 ± 0.86 $P < 0.05$ Normalized after 7 days and 14 days of abstinence **SOD (ng/mL)** 24 hours after admission AUD: 0.10 ± 0.05 Controls: 0.80 ± 0.53 $P < 0.05$ Remained low during up to 14 days of abstinence $P < 0.05$ **GPx (nmol/min/mL)** 24 hours after admission AUD: 156.46 ± 73.79 Controls: 205.08 ± 44.71 $P < 0.05$ Remained low after 7 days and 14 days of abstinence $P < 0.05$ **CAT (U/mL)** 24 hours after admission: no significant difference between AUD and controls Lower after 14 days of abstinence vs controls and baseline $P < 0.05$ **GR (nmol/min/mL)** 24 hours after admission: no significant difference between AUD and controls Higher after 7 days of abstinence vs controls and baseline $P < 0.05$ Lower after 14 days of abstinence (similar to controls)

Table 3.6 (continued)

Reference Study location	Setting, study design, number, age, sex, ethnicity, and health status of exposed and control groups	Matching factors Covariates controlled for	Alcohol exposure and duration of abstinence	Sampling matrix End-point biomarker (test used)	Results
Varella Morandi Junqueira-Franco et al. (2006) Brazil	10 participants (men) with alcohol-related pellagra (average age, 35 years) entering a 27-day inpatient detoxification programme Exclusion criteria: not previously treated with vitamins	Not reported	Alcohol consumption: > 90 g of ethanol per day Patients entered inpatient treatment (abstinence) for 27 days and were given 100 mg of niacin per day	Urine and blood NMN (HPLC); vitamin E (HPLC); GPx (enzyme assay); MDA (thiobarbituric acid and quantified by HPLC); protein carbonyl levels (colorimetric assay)	Values are mean ± SD **NMN (mg/24 hours)** Baseline: 0.82 ± 1.21 Treatment: 9.97 ± 9.89 $P < 0.05$ **MDA (µmol/L)** Baseline: 1.19 ± 0.40 Treatment: 0.89 ± 0.27 $P < 0.05$ **Protein carbonyls (nmol/mg protein)** Baseline: 2.22 ± 0.36 Treatment: 1.84 ± 0.40 $P < 0.05$ **Plasma vitamin E (µmol/L)** Baseline: 12.66 ± 4.23 Treatment: 20.49 ± 7.74 $P < 0.05$ **GPx (U/g Hb)** Baseline: 21.70 ± 7.46 Treatment: 29.54 ± 8.72 $P < 0.05$

ALD, alcohol-related liver disease; ARH, alcohol-related hepatitis; AUD, alcohol use disorder; CAT, catalase; ELISA, enzyme-linked immunosorbent assay; GPx, glutathione peroxidase; GR, glutathione reductase; Hb, haemoglobin; HBsAg, hepatitis B surface antigen; HD, heavy drinking; HPLC, high-performance liquid chromatography; MDA, malondialdehyde; NMN, N^1-methylnicotinamide; NS, not significant; SD, standard deviation; SOD, superoxide dismutase.

with AUD (28 men and 1 woman) and 19 healthy controls. None of them were reported to carry the *ALDH2*2* allele. Concentrations of malondialdehyde were 2-fold higher and levels of GPx and superoxide dismutase were lower among the participants with AUD at entry compared with the controls. Levels of catalase and glutathione reductase were not affected. With 14 days of abstinence, malondialdehyde levels decreased to control levels, whereas superoxide dismutase and GPx levels remained low.

In a study in Brazil (Varella Morandi Junqueira-Franco et al., 2006), participants with AUD who had pellagra were treated with niacin for 27 days (niacin is a non-traditional antioxidant that affects macrophage function; Montserrat-de la Paz et al., 2017). There was an expected large (10-fold) increase in the urinary concentration of the niacin metabolite N^1-methylnicotinamide, an increase of ~40% in erythrocyte GPx activity, an increase of ~60% in vitamin E levels (both GPx activity and vitamin E level were below reference values before treatment), a decrease of ~20% in plasma carbonyl groups (a marker of protein oxidation), and a decrease of ~25% in plasma malondialdehyde levels. [This study did not have any comparison with samples from a control group, and any positive effects resulting from abstinence remain unclear because the participants experienced it together with administration of daily doses of niacin and, no doubt, an improved diet.]

Kang et al. (2022) examined the effect of 3 weeks of abstinence on hepatic markers of oxidative stress in a mouse model of ARH. The model entailed a ramp-up in alcohol administration over 5 days, 10 days on the Lieber–DeCarli liquid diet, and bolus gavage of ethanol on day 11, followed by a return to an alcohol-free diet. [The number of animals studied was small, but this was a unique assessment of the time course of alcohol withdrawal.] The ethanol treatment protocol caused an increase in serum and liver triglycerides, serum malondialdehyde levels, and CYP2E1 mRNA levels, and a 2–3-fold increase in the level of alanine transaminase (ALT), which resolved by 1–2 weeks after the return to an alcohol-free diet. Expression of several genes that encode enzymes involved in antioxidant responses was decreased with alcohol feeding and increased again during abstinence: Nfe2/2, which encodes the NRF2 protein; Sod1, which encodes superoxide dismutase; and GSHPx, which encodes GPx. The mRNA level of Hmox1, which encodes haem oxygenase, increased with alcohol feeding and decreased during abstinence. mRNA levels reflective of macrophage numbers (Adgre1 and Cd68 mRNA) and inflammatory cytokine levels (Tnf for TNF and Ccl2 for CCL2) increased with alcohol feeding and then decreased to normal over 3 weeks. [The relevance of these data to humans without overt liver disease is unknown.]

References

Allen DC, Gonzales SW, Grant KA (2018). Effect of repeated abstinence on chronic ethanol self-administration in the rhesus monkey. *Psychopharmacology (Berl).* 235(1):109–20. doi:10.1007/s00213-017-4748-9 PMID:29051997

Alvisa-Negrín J, González-Reimers E, Santolaria-Fernández F, García-Valdecasas-Campelo E, Valls MR, Pelazas-González R, et al. (2009). Osteopenia in alcoholics: effect of alcohol abstinence. *Alcohol Alcohol.* 44(5):468–75. doi:10.1093/alcalc/agp038 PMID:19535494

American Psychiatric Association (1987). Diagnostic and statistical manual of mental disorders. 3rd revised ed. Washington (DC), USA: American Psychiatric Association.

American Psychiatric Association (1994). Diagnostic and statistical manual of mental disorders. 4th ed. Washington (DC), USA: American Psychiatric Association.

American Psychiatric Association (2013). Diagnostic and statistical manual of mental disorders. 5th ed. Washington (DC), USA: American Psychiatric Association.

Ames NJ, Barb JJ, Schuebel K, Mudra S, Meeks BK, Tuason RTS, et al. (2020). Longitudinal gut microbiome changes in alcohol use disorder are influenced by abstinence and drinking quantity. *Gut Microbes.*

11(6):1608–31. doi:10.1080/19490976.2020.1758010 PMID:32615913

Anantharaman D, Marron M, Lagiou P, Samoli E, Ahrens W, Pohlabeln H, et al. (2011). Population attributable risk of tobacco and alcohol for upper aerodigestive tract cancer. *Oral Oncol*. 47(8):725–31. doi:10.1016/j.oraloncology.2011.05.004 PMID:21684805

Anderson NM, Simon MC (2020). The tumor microenvironment. *Curr Biol*. 30(16):R921–5. doi:10.1016/j.cub.2020.06.081 PMID:32810447

Assi N, Rinaldi S, Viallon V, Dashti SG, Dossus L, Fournier A, et al. (2020). Mediation analysis of the alcohol–postmenopausal breast cancer relationship by sex hormones in the EPIC cohort. *Int J Cancer*. 146(3):759–68. doi:10.1002/ijc.32324 PMID:30968961

Bajaj JS, Heuman DM, Hylemon PB, Sanyal AJ, White MB, Monteith P, et al. (2014). Altered profile of human gut microbiome is associated with cirrhosis and its complications. *J Hepatol*. 60(5):940–7. doi:10.1016/j.jhep.2013.12.019 PMID:24374295

Balbo S, Juanes RC, Khariwala S, Baker EJ, Daunais JB, Grant KA (2016). Increased levels of the acetaldehyde-derived DNA adduct N^2-ethyldeoxyguanosine in oral mucosa DNA from rhesus monkeys exposed to alcohol. *Mutagenesis*. 31(5):553–8. doi:10.1093/mutage/gew016 PMID:27056945

Balbo S, Meng L, Bliss RL, Jensen JA, Hatsukami DK, Hecht SS (2012). Kinetics of DNA adduct formation in the oral cavity after drinking alcohol. *Cancer Epidemiol Biomarkers Prev*. 21(4):601–8. doi:10.1158/1055-9965.EPI-11-1175 PMID:22301829

Berkel TD, Pandey SC (2017). Emerging role of epigenetic mechanisms in alcohol addiction. *Alcohol Clin Exp Res*. 41(4):666–80. doi:10.1111/acer.13338 PMID:28111764

Bishehsari F, Magno E, Swanson G, Desai V, Voigt RM, Forsyth CB, et al. (2017). Alcohol and gut-derived inflammation. *Alcohol Res*. 38(2):163–71.

Bjørkhaug ST, Neupane SP, Bramness JG, Aanes H, Skar V, Medhus AW, et al. (2020). Plasma cytokine levels in patients with chronic alcohol overconsumption: relations to gut microbiota markers and clinical correlates. *Alcohol*. 85:35–40. doi:10.1016/j.alcohol.2019.10.002 PMID:31610228

Blekhman R, Goodrich JK, Huang K, Sun Q, Bukowski R, Bell JT, et al. (2015). Host genetic variation impacts microbiome composition across human body sites. *Genome Biol*. 16(1):191. doi:10.1186/s13059-015-0759-1 PMID:26374288

Bosron WF, Li TK (1986). Genetic polymorphism of human liver alcohol and aldehyde dehydrogenases, and their relationship to alcohol metabolism and alcoholism. *Hepatology*. 6(3):502–10. doi:10.1002/hep.1840060330 PMID:3519419

Burim RV, Canalle R, Takahashi CS, Tavares DC, Martinelli AL, Sakamoto-Hojo ET (2004). Clastogenic effect of ethanol in chronic and abstinent alcoholics. *Mutat Res*. 560(2):187–98. doi:10.1016/j.mrgentox.2004.03.004 PMID:15157656

Castelli E, Hrelia P, Maffei F, Fimognari C, Foschi FG, Caputo F, et al. (1999). Indicators of genetic damage in alcoholics: reversibility after alcohol abstinence. *Hepatogastroenterology*. 46(27):1664–8. PMID:10430317

Castro GD, Castro JA (2014). Alcohol drinking and mammary cancer: pathogenesis and potential dietary preventive alternatives. *World J Clin Oncol*. 5(4):713–29. doi:10.5306/wjco.v5.i4.713 PMID:25300769

Cederbaum AI (2012). Alcohol metabolism. *Clin Liver Dis*. 16(4):667–85. doi:10.1016/j.cld.2012.08.002 PMID:23101976

Chang JS, Straif K, Guha N (2012). The role of alcohol dehydrogenase genes in head and neck cancers: a systematic review and meta-analysis of *ADH1B* and *ADH1C*. *Mutagenesis*. 27(3):275–86. doi:10.1093/mutage/ger073 PMID:22042713

Chaturvedi A, Rao G, Praharaj SK, Guruprasad KP, Pais V (2020). Reduced serum levels of cluster of differentiation 200 in alcohol-dependent patients. *Alcohol Alcohol*. 55(4):391–4. doi:10.1093/alcalc/agaa033 PMID:32363396

Chen J, Pan W, Chen Y, Wen L, Tu J, Liu K (2020). Relationship of *ALDH2* rs671 and *CYP2E1* rs2031920 with hepatocellular carcinoma susceptibility in East Asians: a meta-analysis. *World J Surg Oncol*. 18(1):21. doi:10.1186/s12957-020-1796-0 PMID:31987047

Chen YC, Yang L-F, Lai C-L, Yin S-J (2021). Acetaldehyde enhances alcohol sensitivity and protects against alcoholism: evidence from alcohol metabolism in subjects with variant *ALDH2*2* gene allele. *Biomolecules*. 11(8):1183. doi:10.3390/biom11081183 PMID:34439848

Chiang C-P, Jao S-W, Lee S-P, Chen P-C, Chung C-C, Lee S-L, et al. (2012). Expression pattern, ethanol-metabolizing activities, and cellular localization of alcohol and aldehyde dehydrogenases in human large bowel: association of the functional polymorphisms of *ADH* and *ALDH* genes with hemorrhoids and colorectal cancer. *Alcohol*. 46(1):37–49. doi:10.1016/j.alcohol.2011.08.004 PMID:21940137

Crabb DW, Edenberg HJ, Bosron WF, Li T-K (1989). Genotypes for aldehyde dehydrogenase deficiency and alcohol sensitivity. The inactive $ALDH2^2$ allele is dominant. *J Clin Invest*. 83(1):314–6. doi:10.1172/JCI113875 PMID:2562960

D'Antonio JA, LaPorte RE, Dai WS, Hom DL, Wozniczak M, Kuller LH (1986). Lipoprotein cholesterol, vitamin A, and vitamin E in an alcoholic population. *Cancer*. 57(9):1798–802. doi:10.1002/1097-0142(19860501)57:9<1798::AID-CNCR2820570915>3.0.CO;2-X PMID:3485469

Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR (2002). Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized

controlled trial. *JAMA*. 287(19):2559–62. doi:10.1001/jama.287.19.2559 PMID:12020337

DeMaster EG, Redfern B, Weir K, Pierpont GL, Crouse LJ (1983). Elimination of artifactual acetaldehyde in the measurement of human blood acetaldehyde by the use of polyethylene glycol and sodium azide: normal blood acetaldehyde levels in the dog and human after ethanol. *Alcohol Clin Exp Res*. 7(4):436–42. doi:10.1111/j.1530-0277.1983.tb05502.x PMID:6362469

Ding J, Li S, Wu J, Gao C, Zhou J, Cao H, et al. (2008). Alcohol dehydrogenase-2 and aldehyde dehydrogenase-2 genotypes, alcohol drinking and the risk of primary hepatocellular carcinoma in a Chinese population. *Asian Pac J Cancer Prev*. 9(1):31–5. PMID:18439068

Dong YJ, Peng TK, Yin SJ (1996). Expression and activities of class IV alcohol dehydrogenase and class III aldehyde dehydrogenase in human mouth. *Alcohol*. 13(3):257–62. doi:10.1016/0741-8329(95)02052-7 PMID:8734840

Donnadieu-Rigole H, Mura T, Portales P, Duroux-Richard I, Bouthier M, Eliaou JF, et al. (2016). Effects of alcohol withdrawal on monocyte subset defects in chronic alcohol users. *J Leukoc Biol*. 100(5):1191–9. doi:10.1189/jlb.5A0216-060RR PMID:27256567

Donnadieu-Rigole H, Pansu N, Mura T, Pelletier S, Alarcon R, Gamon L, et al. (2018). Beneficial effect of alcohol withdrawal on gut permeability and microbial translocation in patients with alcohol use disorder. *Alcohol Clin Exp Res*. 42(1):32–40. doi:10.1111/acer.13527 PMID:29030980

Dorgan JF, Baer DJ, Albert PS, Judd JT, Brown ED, Corle DK, et al. (2001). Serum hormones and the alcohol–breast cancer association in postmenopausal women. *J Natl Cancer Inst*. 93(9):710–5. doi:10.1093/jnci/93.9.710 PMID:11333294

Du X, Squier CA, Kremer MJ, Wertz PW (2000). Penetration of *N*-nitrosonornicotine (NNN) across oral mucosa in the presence of ethanol and nicotine. *J Oral Pathol Med*. 29(2):80–5. doi:10.1034/j.1600-0714.2000.290205.x PMID:10718403

Dugué PA, Wilson R, Lehne B, Jayasekara H, Wang X, Jung CH, et al. (2021). Alcohol consumption is associated with widespread changes in blood DNA methylation: analysis of cross-sectional and longitudinal data. *Addict Biol*. 26(1):e12855. doi:10.1111/adb.12855 PMID:31789449

Dumitrescu RG, Shields PG (2005). The etiology of alcohol-induced breast cancer. *Alcohol*. 35(3):213–25. doi:10.1016/j.alcohol.2005.04.005 PMID:16054983

Edenberg HJ, McClintick JN (2018). Alcohol dehydrogenases, aldehyde dehydrogenases and alcohol use disorders: a critical review. *Alcohol Clin Exp Res*. 42(12):2281–97. doi:10.1111/acer.13904 PMID:30320893

Eng MY, Luczak SE, Wall TL (2007). *ALDH2*, *ADH1B*, and *ADH1C* genotypes in Asians: a literature review. *Alcohol Res Health*. 30(1):22–7. PMID:17718397

Fang JL, Vaca CE (1995). Development of a ^{32}P-postlabelling method for the analysis of adducts arising through the reaction of acetaldehyde with 2′-deoxyguanosine-3′-monophosphate and DNA. *Carcinogenesis*. 16(9):2177–85. doi:10.1093/carcin/16.9.2177 PMID:7554072

Feller L, Chandran R, Khammissa RA, Meyerov R, Lemmer J (2013). Alcohol and oral squamous cell carcinoma. *SADJ*. 68(4):176–80. PMID:23971298

Forquer MR, Hashimoto JG, Roberts ML, Wiren KM (2011). Elevated testosterone in females reveals a robust sex difference in altered androgen levels during chronic alcohol withdrawal. *Alcohol*. 45(2):161–71. doi:10.1016/j.alcohol.2010.08.013 PMID:20843636

Freudenheim JL (2020). Alcohol's effects on breast cancer in women. *Alcohol Res*. 40(2):11. doi:10.35946/arcr.v40.2.11 PMID:32582503

Gao B, Emami A, Zhou R, Lang S, Duan Y, Wang Y, et al. (2020). Functional microbial responses to alcohol abstinence in patients with alcohol use disorder. *Front Physiol*. 11:370. doi:10.3389/fphys.2020.00370 PMID:32390870

Gao J, Wang Z, Wang GJ, Gao N, Li J, Zhang YF, et al. (2018). From hepatofibrosis to hepatocarcinogenesis: higher cytochrome P450 2E1 activity is a potential risk factor. *Mol Carcinog*. 57(10):1371–82. doi:10.1002/mc.22851 PMID:29917271

García-Marchena N, Araos PF, Barrios V, Sánchez-Marín L, Chowen JA, Pedraz M, et al. (2017). Plasma chemokines in patients with alcohol use disorders: association of CCL11 (eotaxin-1) with psychiatric comorbidity. *Front Psychiatry*. 7:214. doi:10.3389/fpsyt.2016.00214 PMID:28149283

García-Marchena N, Maza-Quiroga R, Serrano A, Barrios V, Requena-Ocaña N, Suárez J, et al. (2020). Abstinent patients with alcohol use disorders show an altered plasma cytokine profile: identification of both interleukin 6 and interleukin 17A as potential biomarkers of consumption and comorbid liver and pancreatic diseases. *J Psychopharmacol*. 34(11):1250–60. doi:10.1177/0269881120928176 PMID:32536325

Gattás GJ, Saldanha PH (1997). Chromosomal aberrations in peripheral lymphocytes of abstinent alcoholics. *Alcohol Clin Exp Res*. 21(2):238–43. doi:10.1111/j.1530-0277.1997.tb03755.x PMID:9113258

Gianoulakis C, Dai X, Brown T (2003). Effect of chronic alcohol consumption on the activity of the hypothalamic pituitary-adrenal axis and pituitary beta-endorphin as a function of alcohol intake, age, and gender. *Alcohol Clin Exp Res*. 27(3):410–23. doi:10.1097/01.ALC.0000056614.96137.B8 PMID:12658106

Ginsburg ES, Mello NK, Mendelson JH, Barbieri RL, Teoh SK, Rothman M, et al. (1996). Effects

of alcohol ingestion on estrogens in postmenopausal women. *JAMA*. 276(21):1747–51. doi:10.1001/jama.1996.03540210055034 PMID:8940324

Girard M, Malauzat D, Nubukpo P (2019). Serum inflammatory molecules and markers of neuronal damage in alcohol-dependent subjects after withdrawal. *World J Biol Psychiatry*. 20(1):76–90. doi:10.1080/15622975.2017.1349338 PMID:28669319

Giraud J, Saleh M (2021). Host-microbiota interactions in liver inflammation and cancer. *Cancers (Basel)*. 13(17):4342. doi:10.3390/cancers13174342 PMID:34503151

Girre C, Hispard E, Therond P, Guedj S, Bourdon R, Dally S (1990). Effect of abstinence from alcohol on the depression of glutathione peroxidase activity and selenium and vitamin E levels in chronic alcoholic patients. *Alcohol Clin Exp Res*. 14(6):909–12. doi:10.1111/j.1530-0277.1990.tb01836.x PMID:2088128

Gonzalez-Quintela A, Campos J, Gude F, Perez L-F, Tomé S (2007). Serum concentrations of interleukin-8 in relation to different levels of alcohol consumption. *Cytokine*. 38(1):54–60. doi:10.1016/j.cyto.2007.05.006 PMID:17576072

González-Quintela A, Dominguez-Santalla MJ, Pérez LF, Vidal C, Lojo S, Barrio E (2000). Influence of acute alcohol intake and alcohol withdrawal on circulating levels of IL-6, IL-8, IL-10 and IL-12. *Cytokine*. 12(9):1437–40. doi:10.1006/cyto.2000.0715 PMID:10976010

Greten FR, Grivennikov SI (2019). Inflammation and cancer: triggers, mechanisms, and consequences. *Immunity*. 51(1):27–41. doi:10.1016/j.immuni.2019.06.025 PMID:31315034

Guidolin V, Carlson ES, Carrà A, Villalta PW, Maertens LA, Hecht SS, et al. (2021). Identification of new markers of alcohol-derived DNA damage in humans. *Biomolecules*. 11(3):366. doi:10.3390/biom11030366 PMID:33673538

Guo H, Zhang G, Mai R (2012). Alcohol dehydrogenase-1B Arg47His polymorphism and upper aerodigestive tract cancer risk: a meta-analysis including 24,252 subjects. *Alcohol Clin Exp Res*. 36(2):272–8. doi:10.1111/j.1530-0277.2011.01621.x PMID:21895720

Harada S, Agarwal DP, Goedde HW, Takagi S (1983). Blood ethanol and acetaldehyde levels in Japanese alcoholics and controls. *Pharmacol Biochem Behav*. 18(Suppl 1):139–40. doi:10.1016/0091-3057(83)90161-2 PMID:6634829

Hartman TJ, Baer DJ, Graham LB, Stone WL, Gunter EW, Parker CE, et al. (2005). Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. *Eur J Clin Nutr*. 59(2):161–8. doi:10.1038/sj.ejcn.1602051 PMID:15367922

Hartmann P, Lang S, Zeng S, Duan Y, Zhang X, Wang Y, et al. (2021). Dynamic changes of the fungal microbiome in alcohol use disorder. *Front Physiol*. 12:699253. doi:10.3389/fphys.2021.699253 PMID:34349667

Hashibe M, Brennan P, Chuang SC, Boccia S, Castellsague X, Chen C, et al. (2009). Interaction between tobacco and alcohol use and the risk of head and neck cancer: pooled analysis in the International Head and Neck Cancer Epidemiology Consortium. *Cancer Epidemiol Biomarkers Prev*. 18(2):541–50. doi:10.1158/1055-9965.EPI-08-0347 PMID:19190158

Heberlein A, Büscher P, Schuster R, Kleimann A, Lichtinghagen R, Rhein M, et al. (2015). Do changes in the BDNF promoter methylation indicate the risk of alcohol relapse? *Eur Neuropsychopharmacol*. 25(11):1892–7. doi:10.1016/j.euroneuro.2015.08.018 PMID:26404404

Helminen A, Väkeväinen S, Salaspuro M (2013). *ALDH2* genotype has no effect on salivary acetaldehyde without the presence of ethanol in the systemic circulation. *PLoS One*. 8(9):e74418. doi:10.1371/journal.pone.0074418 PMID:24058561

Higuchi S, Matsushita S, Masaki T, Yokoyama A, Kimura M, Suzuki G, et al. (2004). Influence of genetic variations of ethanol-metabolizing enzymes on phenotypes of alcohol-related disorders. *Ann N Y Acad Sci*. 1025(1):472–80. doi:10.1196/annals.1316.058 PMID:15542751

Higuchi S, Matsushita S, Muramatsu T, Murayama M, Hayashida M (1996). Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. *Alcohol Clin Exp Res*. 20(3):493–7. doi:10.1111/j.1530-0277.1996.tb01080.x PMID:8727242

Hoes L, Dok R, Verstrepen KJ, Nuyts S (2021). Ethanol-induced cell damage can result in the development of oral tumors. *Cancers (Basel)*. 13(15):3846. doi:10.3390/cancers13153846 PMID:34359747

Homann N, Jousimies-Somer H, Jokelainen K, Heine R, Salaspuro M (1997). High acetaldehyde levels in saliva after ethanol consumption: methodological aspects and pathogenetic implications. *Carcinogenesis*. 18(9):1739–43. doi:10.1093/carcin/18.9.1739 PMID:9328169

Homann N, König IR, Marks M, Benesova M, Stickel F, Millonig G, et al. (2009). Alcohol and colorectal cancer: the role of alcohol dehydrogenase 1C polymorphism. *Alcohol Clin Exp Res*. 33(3):551–6. doi:10.1111/j.1530-0277.2008.00868.x PMID:19120062

Homann N, Stickel F, König IR, Jacobs A, Junghanns K, Benesova M, et al. (2006). Alcohol dehydrogenase 1C*1 allele is a genetic marker for alcohol-associated cancer in heavy drinkers. *Int J Cancer*. 118(8):1998–2002. doi:10.1002/ijc.21583 PMID:16287084

Homann N, Tillonen J, Meurman JH, Rintamäki H, Lindqvist C, Rautio M, et al. (2000a). Increased salivary acetaldehyde levels in heavy drinkers and smokers: a microbiological approach to oral cavity cancer. *Carcinogenesis*. 21(4):663–8. doi:10.1093/carcin/21.4.663 PMID:10753201

Homann N, Tillonen J, Rintamäki H, Salaspuro M, Lindqvist C, Meurman JH (2001). Poor dental status increases acetaldehyde production from ethanol in saliva: a possible link to increased oral cancer risk among heavy drinkers. *Oral Oncol*. 37(2):153–8. doi:10.1016/S1368-8375(00)00076-2 PMID:11167142

Homann N, Tillonen J, Salaspuro M (2000b). Microbially produced acetaldehyde from ethanol may increase the risk of colon cancer via folate deficiency. *Int J Cancer*. 86(2):169–73. doi:10.1002/(SICI)1097-0215(20000415)86:2<169::AID-IJC4>3.0.CO;2-3 PMID:10738242

Howie NM, Trigkas TK, Cruchley AT, Wertz PW, Squier CA, Williams DM (2001). Short-term exposure to alcohol increases the permeability of human oral mucosa. *Oral Dis*. 7(6):349–54. doi:10.1034/j.1601-0825.2001.00731.x PMID:11834098

Hsiao J-R, Chang C-C, Lee W-T, Huang C-C, Ou C-Y, Tsai S-T, et al. (2018). The interplay between oral microbiome, lifestyle factors and genetic polymorphisms in the risk of oral squamous cell carcinoma. *Carcinogenesis*. 39(6):778–87. doi:10.1093/carcin/bgy053 PMID:29668903

Hsu CL, Zhang X, Jiang L, Lang S, Hartmann P, Pride D, et al. (2022). Intestinal virome in patients with alcohol use disorder and after abstinence. *Hepatol Commun*. 6(8):2058–69. doi:10.1002/hep4.1947 PMID:35368152

Huang CC, Hsiao JR, Lee WT, Lee YC, Ou CY, Chang CC, et al. (2017). Investigating the association between alcohol and risk of head and neck cancer in Taiwan. *Sci Rep*. 7(1):9701. doi:10.1038/s41598-017-08802-4 PMID:28851901

Hüttner E, Matthies U, Nikolova T, Ehrenreich H (1999). A follow-up study on chromosomal aberrations in lymphocytes of alcoholics during early, medium, and long-term abstinence. *Alcohol Clin Exp Res*. 23(2):344–8. doi:10.1111/j.1530-0277.1999.tb04120.x PMID:10069566

IARC (1988). Alcohol drinking. *IARC Monogr Eval Carcinog Risks Hum*. 44:1–416. Available from: https://publications.iarc.who.int/62 PMID:3236394

IARC (2010). Alcohol consumption and ethyl carbamate. *IARC Monogr Eval Carcinog Risks Hum*. 96:1–1424. Available from: https://publications.iarc.who.int/114 PMID:21735939

IARC (2012). Personal habits and indoor combustions. *IARC Monogr Eval Carcinog Risks Hum*. 100E:1–538. Available from https://publications.iarc.who.int/122 PMID:23193840

Im PK, Yang L, Kartsonaki C, Chen Y, Guo Y, Du H, et al.; China Kadoorie Biobank (CKB) Collaborative Group (2022). Alcohol metabolism genes and risks of site-specific cancers in Chinese adults: an 11-year prospective study. *Int J Cancer*. 150(10):1627–39. doi:10.1002/ijc.33917 PMID:35048370

Inamura K, Hamada T, Bullman S, Ugai T, Yachida S, Ogino S (2022). Cancer as microenvironmental, systemic and environmental diseases: opportunity for transdisciplinary microbiomics science. *Gut*. 71(10):gutjnl-2022-327209. doi:10.1136/gutjnl-2022-327209 PMID:35820782

Inenaga K, Ono K, Hitomi S, Kuroki A, Ujihara I (2017). Thirst sensation and oral dryness following alcohol intake. *Jpn Dent Sci Rev*. 53(3):78–85. doi:10.1016/j.jdsr.2016.12.001 PMID:28725298

Iturriaga H, Valladares L, Hirsch S, Devoto E, P'erez C, Bunout D, et al. (1995). Effects of abstinence on sex hormone profile in alcoholic patients without liver failure. *J Endocrinol Invest*. 18(8):638–44. doi:10.1007/BF03349782 PMID:8655923

Ji YB, Tae K, Ahn TH, Lee SH, Kim KR, Park CW, et al. (2011). *ADH1B* and *ALDH2* polymorphisms and their associations with increased risk of squamous cell carcinoma of the head and neck in the Korean population. *Oral Oncol*. 47(7):583–7. doi:10.1016/j.oraloncology.2011.04.007 PMID:21576033

Jokelainen K, Matysiak-Budnik T, Mäkisalo H, Höckerstedt K, Salaspuro M (1996b). High intracolonic acetaldehyde values produced by a bacteriocolonic pathway for ethanol oxidation in piglets. *Gut*. 39(1):100–4. doi:10.1136/gut.39.1.100 PMID:8881818

Jokelainen K, Nosova T, Koivisto T, Väkeväinen S, Jousimies-Somer H, Heine R, et al. (1997). Inhibition of bacteriocolonic pathway for ethanol oxidation by ciprofloxacin in rats. *Life Sci*. 61(18):1755–62. doi:10.1016/S0024-3205(97)00799-6 PMID:9365222

Jokelainen K, Siitonen A, Jousimies-Somer H, Nosova T, Heine R, Salaspuro M (1996a). In vitro alcohol dehydrogenase-mediated acetaldehyde production by aerobic bacteria representing the normal colonic flora in man. *Alcohol Clin Exp Res*. 20(6):967–72. doi:10.1111/j.1530-0277.1996.tb01932.x PMID:8892513

Jung F, Burger K, Staltner R, Brandt A, Mueller S, Bergheim I (2021). Markers of intestinal permeability are rapidly improved by alcohol withdrawal in patients with alcohol-related liver disease. *Nutrients*. 13(5):1659. doi:10.3390/nu13051659 PMID:34068838

Kang H, Kim MB, Park YK, Lee JY (2022). A mouse model of the regression of alcoholic hepatitis: monitoring the regression of hepatic steatosis, inflammation, oxidative stress, and NAD^+ metabolism upon alcohol withdrawal. *J Nutr Biochem*. 99:108852. doi:10.1016/j.jnutbio.2021.108852 PMID:34525389

Kapucuoglu N, Coban T, Raunio H, Pelkonen O, Edwards RJ, Boobis AR, et al. (2003). Immunohistochemical demonstration of the expression of CYP2E1 in human breast tumour and non-tumour tissues. *Cancer Lett*. 196(2):153–9. doi:10.1016/S0304-3835(03)00277-5 PMID:12860273

Kesäniemi YA (1974). Ethanol and acetaldehyde in the milk and peripheral blood of lactating women

after ethanol administration. *J Obstet Gynaecol Br Commonw.* 81(1):84–6. doi:10.1111/j.1471-0528.1974.tb00369.x PMID:4818321

Key TJ, Appleby PN, Reeves GK, Roddam AW, Helzlsouer KJ, Alberg AJ, et al.; Endogenous Hormones and Breast Cancer Collaborative Group (2011). Circulating sex hormones and breast cancer risk factors in postmenopausal women: reanalysis of 13 studies. *Br J Cancer.* 105(5):709–22. doi:10.1038/bjc.2011.254 PMID:21772329

Koivisto T, Salaspuro M (1996). Aldehyde dehydrogenases of the rat colon: comparison with other tissues of the alimentary tract and the liver. *Alcohol Clin Exp Res.* 20(3):551–5. doi:10.1111/j.1530-0277.1996.tb01091.x PMID:8727253

Koyanagi YN, Suzuki E, Imoto I, Kasugai Y, Oze I, Ugai T, et al. (2020). Across-site differences in the mechanism of alcohol-induced digestive tract carcinogenesis: an evaluation by mediation analysis. *Cancer Res.* 80(7):1601–10. doi:10.1158/0008-5472.CAN-19-2685 PMID:32005715

Kurkivuori J, Salaspuro V, Kaihovaara P, Kari K, Rautemaa R, Grönroos L, et al. (2007). Acetaldehyde production from ethanol by oral streptococci. *Oral Oncol.* 43(2):181–6. doi:10.1016/j.oraloncology.2006.02.005 PMID:16859955

Kwa M, Plottel CS, Blaser MJ, Adams S (2016). The intestinal microbiome and estrogen receptor-positive female breast cancer. *J Natl Cancer Inst.* 108(8):djw029. doi:10.1093/jnci/djw029 PMID:27107051

Lachenmeier DW, Monakhova YB (2011). Short-term salivary acetaldehyde increase due to direct exposure to alcoholic beverages as an additional cancer risk factor beyond ethanol metabolism. *J Exp Clin Cancer Res.* 30(1):3. doi:10.1186/1756-9966-30-3 PMID:21211027

Lachenmeier DW, Salaspuro M (2017). *ALDH2*-deficiency as genetic epidemiologic and biochemical model for the carcinogenicity of acetaldehyde. *Regul Toxicol Pharmacol.* 86:128–36. doi:10.1016/j.yrtph.2017.02.024 PMID:28257851

Lai C-L, Yao C-T, Chau G-Y, Yang L-F, Kuo T-Y, Chiang C-P, et al. (2014). Dominance of the inactive Asian variant over activity and protein contents of mitochondrial aldehyde dehydrogenase 2 in human liver. *Alcohol Clin Exp Res.* 38(1):44–50. doi:10.1111/acer.12215 PMID:23909789

Laufer EM, Hartman TJ, Baer DJ, Gunter EW, Dorgan JF, Campbell WS, et al. (2004). Effects of moderate alcohol consumption on folate and vitamin B_{12} status in postmenopausal women. *Eur J Clin Nutr.* 58(11):1518–24. doi:10.1038/sj.ejcn.1602002 PMID:15138463

Leclercq S, Cani PD, Neyrinck AM, Stärkel P, Jamar F, Mikolajczak M, et al. (2012). Role of intestinal permeability and inflammation in the biological and behavioral control of alcohol-dependent subjects. *Brain Behav Immun.* 26(6):911–8. doi:10.1016/j.bbi.2012.04.001 PMID:22521198

Leclercq S, Matamoros S, Cani PD, Neyrinck AM, Jamar F, Stärkel P, et al. (2014). Intestinal permeability, gut-bacterial dysbiosis, and behavioral markers of alcohol-dependence severity. *Proc Natl Acad Sci USA.* 111(42):E4485–93. doi:10.1073/pnas.1415174111 PMID:25288760

Lettéron P, Duchatelle V, Berson A, Fromenty B, Fisch C, Degott C, et al. (1993). Increased ethane exhalation, an in vivo index of lipid peroxidation, in alcohol-abusers. *Gut.* 34(3):409–14. doi:10.1136/gut.34.3.409 PMID:8472992

Li H, Mukherjee N, Soundararajan U, Tarnok Z, Barta C, Khaliq S, et al. (2007). Geographically separate increases in the frequency of the derived *ADH1B**47His allele in eastern and western Asia. *Am J Hum Genet.* 81(4):842–6. doi:10.1086/521201 PMID:17847010

Li J, Zhou C, Wang R, Liu R, Huang Z, Tang C (2010). Irreversible exocrine pancreatic insufficiency in alcoholic rats without chronic pancreatitis after alcohol withdrawal. *Alcohol Clin Exp Res.* 34(11):1843–8. doi:10.1111/j.1530-0277.2010.01272.x PMID:20662806

Li W, Amet T, Xing Y, Yang D, Liangpunsakul S, Puri P, et al. (2017). Alcohol abstinence ameliorates the dysregulated immune profiles in patients with alcoholic hepatitis: a prospective observational study. *Hepatology.* 66(2):575–90. doi:10.1002/hep.29242 PMID:28466561

Li W, Lin EL, Liangpunsakul S, Lan J, Chalasani S, Rane S, et al. (2019). Alcohol abstinence does not fully reverse abnormalities of mucosal-associated invariant T cells in the blood of patients with alcoholic hepatitis. *Clin Transl Gastroenterol.* 10(6):e00052. doi:10.14309/ctg.0000000000000052 PMID:31211759

Liangpunsakul S, Toh E, Ross RA, Heathers LE, Chandler K, Oshodi A, et al. (2017). Quantity of alcohol drinking positively correlates with serum levels of endotoxin and markers of monocyte activation. *Sci Rep.* 7(1):4462. doi:10.1038/s41598-017-04669-7 PMID:28667254

Lieber CS (1999). Microsomal ethanol-oxidizing system (MEOS): the first 30 years (1968–1998) – a review. *Alcohol Clin Exp Res.* 23(6):991–1007. doi:10.1097/00000374-199906000-00006 PMID:10397283

Linderborg K, Salaspuro M, Väkeväinen S (2011). A single sip of a strong alcoholic beverage causes exposure to carcinogenic concentrations of acetaldehyde in the oral cavity. *Food Chem Toxicol.* 49(9):2103–6. doi:10.1016/j.fct.2011.05.024 PMID:21641957

Lindros KO (1983). Human blood acetaldehyde levels: with improved methods, a clearer picture emerges. *Alcohol Clin Exp Res.* 7(1):70–5. doi:10.1111/j.1530-0277.1983.tb05414.x PMID:6342452

Linhart K, Bartsch H, Seitz HK (2014). The role of reactive oxygen species (ROS) and cytochrome P-450 2E1 in the generation of carcinogenic etheno-DNA adducts.

Redox Biol. 3:56–62. doi:10.1016/j.redox.2014.08.009 PMID:25462066

Liu C, Marioni RE, Hedman ÅK, Pfeiffer L, Tsai PC, Reynolds LM, et al. (2018). A DNA methylation biomarker of alcohol consumption. *Mol Psychiatry*. 23(2):422–33. doi:10.1038/mp.2016.192 PMID:27843151

Liu Y, Nguyen N, Colditz GA (2015). Links between alcohol consumption and breast cancer: a look at the evidence. *Womens Health (Lond)*. 11(1):65–77. doi:10.2217/WHE.14.62 PMID:25581056

Loosen PT, Wilson IC, Dew BW, Tipermas A (1983). Thyrotropin-releasing hormone (TRH) in abstinent alcoholic men. *Am J Psychiatry*. 140(9):1145–9. doi:10.1176/ajp.140.9.1145 PMID:6412572

Lu Y, Zhu X, Zhang C, Jiang K, Huang C, Qin X (2017). Role of CYP2E1 polymorphisms in breast cancer: a systematic review and meta-analysis. *Cancer Cell Int*. 17(1):11. doi:10.1186/s12935-016-0371-9 PMID:28074086

Luckey TD (1977). Bicentennial overview of intestinal microecology. *Am J Clin Nutr*. 30(11):1753–61. doi:10.1093/ajcn/30.11.1753 PMID:920634

Maccioni L, Gao B, Leclercq S, Pirlot B, Horsmans Y, De Timary P, et al. (2020). Intestinal permeability, microbial translocation, changes in duodenal and fecal microbiota, and their associations with alcoholic liver disease progression in humans. *Gut Microbes*. 12(1):1782157. doi:10.1080/19490976.2020.1782157 PMID:32588725

Maffei F, Forti GC, Castelli E, Stefanini GF, Mattioli S, Hrelia P (2002). Biomarkers to assess the genetic damage induced by alcohol abuse in human lymphocytes. *Mutat Res*. 514(1–2):49–58. doi:10.1016/S1383-5718(01)00318-7 PMID:11815244

Mahabir S, Baer DJ, Johnson LL, Dorgan JF, Campbell W, Brown E, et al. (2004). The effects of moderate alcohol supplementation on estrone sulfate and DHEAS in postmenopausal women in a controlled feeding study. *Nutr J*. 3(1):11. doi:10.1186/1475-2891-3-11 PMID:15353002

Mahabir S, Pfeiffer R, Xu X, Baer DJ, Taylor PR (2017). Effects of low-to-moderate alcohol supplementation on urinary estrogen metabolites in postmenopausal women in a controlled feeding study. *Cancer Med*. 6(10):2419–23. doi:10.1002/cam4.1153 PMID:28879665

Maier A, Bode C, Fritz P, Bode JC (1999). Effects of chronic alcohol abuse on duodenal mononuclear cells in man. *Dig Dis Sci*. 44(4):691–6. doi:10.1023/A:1026697305769 PMID:10219824

Maier E, Anderson RC, Roy NC (2014). Understanding how commensal obligate anaerobic bacteria regulate immune functions in the large intestine. *Nutrients*. 7(1):45–73. doi:10.3390/nu7010045 PMID:25545102

Manzardo AM, Poje AB, Penick EC, Butler MG (2016). Multiplex immunoassay of plasma cytokine levels in men with alcoholism and the relationship to psychiatric assessments. *Int J Mol Sci*. 17(4):472. doi:10.3390/ijms17040472 PMID:27043532

Marchesi C, De Risio C, Campanini G, Maggini C, Piazza P, Grassi M, et al. (1992). TRH test in alcoholics: relationship of the endocrine results with neuroradiological and neuropsychological findings. *Alcohol Alcohol*. 27(5):531–7. PMID:1476556

Matsuda T, Yabushita H, Kanaly RA, Shibutani S, Yokoyama A (2006). Increased DNA damage in *ALDH2*-deficient alcoholics. *Chem Res Toxicol*. 19(10):1374–8. doi:10.1021/tx060113h PMID:17040107

Matsuo K, Hamajima N, Hirai T, Kato T, Koike K, Inoue M, et al. (2002). Aldehyde dehydrogenase 2 (*ALDH2*) genotype affects rectal cancer susceptibility due to alcohol consumption. *J Epidemiol*. 12(2):70–6. doi:10.2188/jea.12.70 PMID:12033531

Matsushima Y (1987). Chromosomal aberrations in the lymphocytes of alcoholics and former alcoholics. *Neuro-psychobiology*. 17(1–2):24–9. doi:10.1159/000118336 PMID:3627389

McCarthy DM, Pedersen SL, Lobos EA, Todd RD, Wall TL (2010). ADH1B*3 and response to alcohol in African-Americans. *Alcohol Clin Exp Res*. 34(7):1274–81. doi:10.1111/j.1530-0277.2010.01205.x PMID:20477764

McCullough ML, Zoltick ES, Weinstein SJ, Fedirko V, Wang M, Cook NR, et al. (2019). Circulating vitamin D and colorectal cancer risk: an international pooling project of 17 cohorts. *J Natl Cancer Inst*. 111(2):158–69. doi:10.1093/jnci/djy087 PMID:29912394

Mehta G, Macdonald S, Cronberg A, Rosselli M, Khera-Butler T, Sumpter C, et al. (2018). Short-term abstinence from alcohol and changes in cardiovascular risk factors, liver function tests and cancer-related growth factors: a prospective observational study. *BMJ Open*. 8(5):e020673. doi:10.1136/bmjopen-2017-020673 PMID:29730627

Melander A, Thorell J, Wadstein J (1982). Alteration of thyroid hormone economy during alcohol withdrawal in alcoholics. *Drug Nutr Interact*. 1(2):113–8. PMID:6926819

Millonig G, Wang Y, Homann N, Bernhardt F, Qin H, Mueller S, et al. (2011). Ethanol-mediated carcinogenesis in the human esophagus implicates CYP2E1 induction and the generation of carcinogenic DNA-lesions. *Int J Cancer*. 128(3):533–40. doi:10.1002/ijc.25604 PMID:20715111

Mizoi Y, Yamamoto K, Ueno Y, Fukunaga T, Harada S (1994). Involvement of genetic polymorphism of alcohol and aldehyde dehydrogenases in individual variation of alcohol metabolism. *Alcohol Alcohol*. 29(6):707–10. PMID:7695788

Montserrat-de la Paz S, Naranjo MC, Lopez S, Abia R, Muriana FJG, Bermudez B (2017). Niacin and its metabolites as master regulators of macrophage activation. *J Nutr Biochem*. 39:40–7. doi:10.1016/j.jnutbio.2016.09.008 PMID:27771381

Mori T, Okamoto Y, Mu A, Ide Y, Yoshimura A, Senda N, et al. (2023). Lack of impact of the *ALDH2* rs671 variant on breast cancer development in Japanese *BRCA1/2*-mutation carriers. *Cancer Med*. 12(6):6594–602. doi:10.1002/cam4.5430 PMID:36345163

Moritani K, Takeshita T, Shibata Y, Ninomiya T, Kiyohara Y, Yamashita Y (2015). Acetaldehyde production by major oral microbes. *Oral Dis*. 21(6):748–54. doi:10.1111/odi.12341 PMID:25809116

Murata M, Tagawa M, Watanabe S, Kimura H, Takeshita T, Morimoto K (1999). Genotype difference of aldehyde dehydrogenase 2 gene in alcohol drinkers influences the incidence of Japanese colorectal cancer patients. *Jpn J Cancer Res*. 90(7):711–9. doi:10.1111/j.1349-7006.1999.tb00805.x PMID:10470282

Muto M, Hitomi Y, Ohtsu A, Shimada H, Kashiwase Y, Sasaki H, et al. (2000). Acetaldehyde production by non-pathogenic *Neisseria* in human oral microflora: implications for carcinogenesis in upper aerodigestive tract. *Int J Cancer*. 88(3):342–50. doi:10.1002/1097-0215(20001101)88:3<342::AID-IJC4>3.0.CO;2-I PMID:11054661

NIAAA (2021). Alcohol use disorder: a comparison between DSM-IV and DSM-5. Bethesda (MD), USA: National Institute on Alcohol Abuse and Alcoholism. Available from: https://www.niaaa.nih.gov/publications/brochures-and-fact-sheets/alcohol-use-disorder-comparison-between-dsm.

Nieminen MT, Salaspuro M (2018). Local acetaldehyde – an essential role in alcohol-related upper gastrointestinal tract carcinogenesis. *Cancers (Basel)*. 10(1):1–23. doi:10.3390/cancers10010011 PMID:29303995

Nieminen MT, Uittamo J, Salaspuro M, Rautemaa R (2009). Acetaldehyde production from ethanol and glucose by non-*Candida albicans* yeasts *in vitro*. *Oral Oncol*. 45(12):e245–8. doi:10.1016/j.oraloncology.2009.08.002 PMID:19793674

Nosova T, Jokelainen K, Kaihovaara P, Heine R, Jousimies-Somer H, Salaspuro M (1998). Characteristics of aldehyde dehydrogenases of certain aerobic bacteria representing human colonic flora. *Alcohol Alcohol*. 33(3):273–80. doi:10.1093/oxfordjournals.alcalc.a008391 PMID:9632053

Nosova T, Jokelainen K, Kaihovaara P, Jousimies-Somer H, Siitonen A, Heine R, et al. (1996). Aldehyde dehydrogenase activity and acetate production by aerobic bacteria representing the normal flora of human large intestine. *Alcohol Alcohol*. 31(6):555–64. doi:10.1093/oxfordjournals.alcalc.a008191 PMID:9010546

Nosova T, Jokelainen K, Kaihovaara P, Väkeväinen S, Rautio M, Jousimies-Somer H, et al. (1999). Ciprofloxacin administration decreases enhanced ethanol elimination in ethanol-fed rats. *Alcohol Alcohol*. 34(1):48–54. doi:10.1093/alcalc/34.1.48 PMID:10075401

Nosova T, Jousimies-Somer H, Kaihovaara P, Jokelainen K, Heine R, Salaspuro M (1997). Characteristics of alcohol dehydrogenases of certain aerobic bacteria representing human colonic flora. *Alcohol Clin Exp Res*. 21(3):489–94. doi:10.1111/j.1530-0277.1997.tb03795.x PMID:9161610

Nuutinen HU, Salaspuro MP, Valle M, Lindros KO (1984). Blood acetaldehyde concentration gradient between hepatic and antecubital venous blood in ethanol-intoxicated alcoholics and controls. *Eur J Clin Invest*. 14(4):306–11. doi:10.1111/j.1365-2362.1984.tb01186.x PMID:6434326

O'Grady I, Anderson A, O'Sullivan J (2020). The interplay of the oral microbiome and alcohol consumption in oral squamous cell carcinomas. *Oral Oncol*. 110:105011. doi:10.1016/j.oraloncology.2020.105011 PMID:32980528

Ohtani N, Hara E (2021). Gut-liver axis-mediated mechanism of liver cancer: a special focus on the role of gut microbiota. *Cancer Sci*. 112(11):4433–43. doi:10.1111/cas.15142 PMID:34533882

Oneta CM, Lieber CS, Li J, Rüttimann S, Schmid B, Lattmann J, et al. (2002). Dynamics of cytochrome P4502E1 activity in man: induction by ethanol and disappearance during withdrawal phase. *J Hepatol*. 36(1):47–52. doi:10.1016/S0168-8278(01)00223-9 PMID:11804663

Ozsoy S, Esel E, Izgi HB, Sofuoglu S (2006). Thyroid function in early and late alcohol withdrawal: relationship with aggression, family history, and onset age of alcoholism. *Alcohol Alcohol*. 41(5):515–21. doi:10.1093/alcalc/agl056 PMID:16855000

Ozsoy S, Esel E, Turan T, Kula M (2007). Growth hormone response to the GABA-B agonist baclofen in 3-week abstinent alcoholics. *Alcohol*. 41(8):551–6. doi:10.1016/j.alcohol.2007.09.003 PMID:18047908

Parida S, Sharma D (2019). The microbiome-estrogen connection and breast cancer risk. *Cells*. 8(12):8. doi:10.3390/cells8121642 PMID:31847455

Park B, Kim J-H, Lee ES, Jung S-Y, Lee SY, Kang HS, et al. (2020). Role of aldehyde dehydrogenases, alcohol dehydrogenase 1B genotype, alcohol consumption, and their combination in breast cancer in East-Asian women. *Sci Rep*. 10(1):6564. doi:10.1038/s41598-020-62361-9 PMID:32300124

Pavlova SI, Jin L, Gasparovich SR, Tao L (2013). Multiple alcohol dehydrogenases but no functional acetaldehyde dehydrogenase causing excessive acetaldehyde production from ethanol by oral streptococci. *Microbiology (Reading)*. 159(Pt 7):1437–46. doi:10.1099/mic.0.066258-0 PMID:23637459

Pearson-Stuttard J, Papadimitriou N, Markozannes G, Cividini S, Kakourou A, Gill D, et al. (2021). Type 2 diabetes and cancer: an umbrella review of observational and Mendelian randomization studies. *Cancer Epidemiol Biomarkers Prev*. 30(6):1218–28. doi:10.1158/1055-9965.EPI-20-1245 PMID:33737302

Peng FC, Tang SH, Huang MC, Chen CC, Kuo TL, Yin SJ (2005). Oxidative status in patients with alcohol dependence: a clinical study in Taiwan. *J Toxicol Environ Health A*. 68(17–18):1497–509. doi:10.1080/15287390590967432 PMID:16076762

Peng GS, Wang MF, Chen CY, Luu SU, Chou HC, Li TK, et al. (1999). Involvement of acetaldehyde for full protection against alcoholism by homozygosity of the variant allele of mitochondrial aldehyde dehydrogenase gene in Asians. *Pharmacogenetics*. 9(4):463–76. PMID:10780266

Peng G-S, Yin S-J (2009). Effect of the allelic variants of aldehyde dehydrogenase *ALDH2*2* and alcohol dehydrogenase *ADH1B*2* on blood acetaldehyde concentrations. *Hum Genomics*. 3(2):121–7. doi:10.1186/1479-7364-3-2-121 PMID:19164089

Petagine L, Zariwala MG, Patel VB (2021). Alcoholic liver disease: current insights into cellular mechanisms. *World J Biol Chem*. 12(5):87–103. doi:10.4331/wjbc.v12.i5.87 PMID:34630912

Piccardo MG, Pacini G, Nardi E, Rosa MS, De Vito R (1994). Beta-cell response and insulin hepatic extraction in noncirrhotic alcoholic patients soon after withdrawal. *Metabolism*. 43(3):367–71. doi:10.1016/0026-0495(94)90106-6 PMID:8139486

Price JL, Nixon SJ (2021). Retrospective hair cortisol concentrations from pretreatment to early recovery in alcohol use disorder. *Alcohol Alcohol*. 56(2):181–4. doi:10.1093/alcalc/agaa127 PMID:33279964

Proskynitopoulos PJ, Rhein M, Petersson LP, Sperling CH, Wöhler J, Deest M, et al. (2021). Differences in the promoter methylation of atrial natriuretic peptide and vasopressin in alcohol use disorder. A longitudinal case-control-study during withdrawal therapy. *Psychoneuroendocrinology*. 133:105387. doi:10.1016/j.psyneuen.2021.105387 PMID:34399149

Qin N, Yang F, Li A, Prifti E, Chen Y, Shao L, et al. (2014). Alterations of the human gut microbiome in liver cirrhosis. *Nature*. 513(7516):59–64. doi:10.1038/nature13568 PMID:25079328

Radoï L, Paget-Bailly S, Cyr D, Papadopoulos A, Guida F, Schmaus A, et al. (2013). Tobacco smoking, alcohol drinking and risk of oral cavity cancer by subsite: results of a French population-based case-control study, the ICARE study. *Eur J Cancer Prev*. 22(3):268–76. doi:10.1097/CEJ.0b013e3283592cce PMID:22976386

Rajzer M, Kawecka-Jaszcz K, Czarnecka D, Dragan J, Betkowska B (1997). Blood pressure, insulin resistance and left ventricular function in alcoholics. *J Hypertens*. 15(11):1219–26. doi:10.1097/00004872-199715110-00005 PMID:9383170

Rao R (2009). Endotoxemia and gut barrier dysfunction in alcoholic liver disease. *Hepatology*. 50(2):638–44. doi:10.1002/hep.23009 PMID:19575462

Rasmussen DD, Boldt BM, Bryant CA, Mitton DR, Larsen SA, Wilkinson CW (2000). Chronic daily ethanol and withdrawal: 1. Long-term changes in the hypothalamo-pituitary-adrenal axis. *Alcohol Clin Exp Res*. 24(12):1836–49. doi:10.1111/j.1530-0277.2000.tb01988.x PMID:11141043

Rodriguez FD, Coveñas R (2021). Biochemical mechanisms associating alcohol use disorders with cancers. *Cancers (Basel)*. 13(14):3548. doi:10.3390/cancers13143548 PMID:34298760

Rolla R, Vay D, Mottaran E, Parodi M, Traverso N, Aricó S, et al. (2000). Detection of circulating antibodies against malondialdehyde-acetaldehyde adducts in patients with alcohol-induced liver disease. *Hepatology*. 31(4):878–84. doi:10.1053/he.2000.5373 PMID:10733543

Romeo J, Wärnberg J, Nova E, Díaz LE, González-Gross M, Marcos A (2007). Changes in the immune system after moderate beer consumption. *Ann Nutr Metab*. 51(4):359–66. doi:10.1159/000107679 PMID:17726314

Rumgay H, Murphy N, Ferrari P, Soerjomataram I (2021). Alcohol and cancer: epidemiology and biological mechanisms. *Nutrients*. 13(9):3173. doi:10.3390/nu13093173 PMID:34579050

Salaspuro M (2017). Key role of local acetaldehyde in upper GI tract carcinogenesis. *Best Pract Res Clin Gastroenterol*. 31(5):491–9. doi:10.1016/j.bpg.2017.09.016 PMID:29195668

Salaspuro M (2020). Local acetaldehyde: its key role in alcohol-related oropharyngeal cancer. *Visc Med*. 36(3):167–73. [Erratum in: Visc Med. 2021;37(2):158.] doi:10.1159/000507234 PMID:32775346

Salaspuro M, Lachenmeier DW (2020). Unique human cancer model for acetaldehyde based on Mendelian randomization. *Arch Toxicol*. 94(8):2887–8. doi:10.1007/s00204-020-02851-x PMID:32681186

Salaspuro MP (2003). Acetaldehyde, microbes, and cancer of the digestive tract. *Crit Rev Clin Lab Sci*. 40(2):183–208. doi:10.1080/713609333 PMID:12755455

Salaspuro MP, Kesäniemi YA (1973). Intravenous galactose elimination tests with and without ethanol loading in various clinical conditions. *Scand J Gastroenterol*. 8(7):681–6. doi:10.1080/00365521.1973.12096768 PMID:4768313

Salaspuro MP, Shaw S, Jayatilleke E, Ross WA, Lieber CS (1981). Attenuation of the ethanol-induced hepatic redox change after chronic alcohol consumption in baboons: metabolic consequences in vivo and in vitro. *Hepatology*. 1(1):33–8. doi:10.1002/hep.1840010106 PMID:6793493

Salaspuro V, Nyfors S, Heine R, Siitonen A, Salaspuro M, Jousimies-Somer H (1999). Ethanol oxidation and acetaldehyde production in vitro by human intestinal strains of *Escherichia coli* under aerobic, microaerobic, and anaerobic conditions. *Scand J Gastroenterol*. 34(10):967–73. doi:10.1080/003655299750025057 PMID:10563665

Salaspuro V, Salaspuro M (2004). Synergistic effect of alcohol drinking and smoking on *in vivo* acetaldehyde

concentration in saliva. *Int J Cancer*. 111(4):480–3. doi:10.1002/ijc.20293 PMID:15239123

Seitz HK, Bataller R, Cortez-Pinto H, Gao B, Gual A, Lackner C, et al. (2018). Alcoholic liver disease. *Nat Rev Dis Primers*. 4(1):16. [Erratum in: Nat Rev Dis Primers. 2018;4(1):18.] doi:10.1038/s41572-018-0014-7 PMID:30115921

Seitz HK, Egerer G, Oneta C, Krämer S, Sieg A, Klee F, et al. (1996). Alcohol dehydrogenase in the human colon and rectum. *Digestion*. 57(2):105–8. doi:10.1159/000201322 PMID:8785998

Seitz HK, Pelucchi C, Bagnardi V, La Vecchia C (2012). Epidemiology and pathophysiology of alcohol and breast cancer: Update 2012. *Alcohol Alcohol*. 47(3):204–12. doi:10.1093/alcalc/ags011 PMID:22459019

Seitz HK, Simanowski UA, Garzon FT, Rideout JM, Peters TJ, Koch A, et al. (1990). Possible role of acetaldehyde in ethanol-related rectal cocarcinogenesis in the rat. *Gastroenterology*. 98(2):406–13. doi:10.1016/0016-5085(90)90832-L PMID:2295396

Sender R, Fuchs S, Milo R (2016). Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol*. 14(8):e1002533. doi:10.1371/journal.pbio.1002533 PMID:27541692

Sharma J, Krupenko SA (2020). Folate pathways mediating the effects of ethanol in tumorigenesis. *Chem Biol Interact*. 324:109091. doi:10.1016/j.cbi.2020.109091 PMID:32283069

Shiba S, Nakamoto N, Chu PS, Ojiro K, Taniki N, Yamaguchi A, et al. (2021). Acetaldehyde exposure underlies functional defects in monocytes induced by excessive alcohol consumption. *Sci Rep*. 11(1):13690. doi:10.1038/s41598-021-93086-y PMID:34211048

Song BJ, Abdelmegeed MA, Cho YE, Akbar M, Rhim JS, Song MK, et al. (2019). Contributing roles of CYP2E1 and other cytochrome P450 isoforms in alcohol-related tissue injury and carcinogenesis. *Adv Exp Med Biol*. 1164:73–87. doi:10.1007/978-3-030-22254-3_6 PMID:31576541

Soundararajan S, Agrawal A, Purushottam M, Anand SD, Shankarappa B, Sharma P, et al. (2021). Changes in DNA methylation persist over time in males with severe alcohol use disorder – a longitudinal follow-up study. *Am J Med Genet B Neuropsychiatr Genet*. 186(3):183–92. doi:10.1002/ajmg.b.32833 PMID:33491855

Stote KS, Tracy RP, Taylor PR, Baer DJ (2016). The effect of moderate alcohol consumption on biomarkers of inflammation and hemostatic factors in postmenopausal women. *Eur J Clin Nutr*. 70(4):470–4. doi:10.1038/ejcn.2015.182 PMID:26554758

Sun Q, Xie W, Wang Y, Chong F, Song M, Li T, et al. (2020). Alcohol consumption by beverage type and risk of breast cancer: a dose-response meta-analysis of prospective cohort studies. *Alcohol Alcohol*. 55(3):246–53. doi:10.1093/alcalc/agaa012 PMID:32090238

Tagaino R, Washio J, Otani H, Sasaki K, Takahashi N (2021). Bifacial biological effects of ethanol: acetaldehyde production by oral *Streptococcus* species and the antibacterial effects of ethanol against these bacteria. *J Oral Microbiol*. 13(1):1937884. doi:10.1080/20002297.2021.1937884 PMID:34178291

Thomasson HR, Edenberg HJ, Crabb DW, Mai X-L, Jerome RE, Li T-K, et al. (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet*. 48(4):677–81. PMID:2014795

Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M (1999a). Role of yeasts in the salivary acetaldehyde production from ethanol among risk groups for ethanol-associated oral cavity cancer. *Alcohol Clin Exp Res*. 23(8):1409–15. doi:10.1111/j.1530-0277.1999.tb04364.x PMID:10470985

Tillonen J, Homann N, Rautio M, Jousimies-Somer H, Salaspuro M (1999b). Ciprofloxacin decreases the rate of ethanol elimination in humans. *Gut*. 44(3):347–52. doi:10.1136/gut.44.3.347 PMID:10026319

Tillonen J, Kaihovaara P, Jousimies-Somer H, Heine R, Salaspuro M (1998). Role of catalase in in vitro acetaldehyde formation by human colonic contents. *Alcohol Clin Exp Res*. 22(5):1113–9. doi:10.1111/j.1530-0277.1998.tb03709.x PMID:9726283

Tillonen J, Väkeväinen S, Salaspuro V, Zhang Y, Rautio M, Jousimies-Somer H, et al. (2000). Metronidazole increases intracolonic but not peripheral blood acetaldehyde in chronic ethanol-treated rats. *Alcohol Clin Exp Res*. 24(4):570–5. doi:10.1111/j.1530-0277.2000.tb02026.x PMID:10798595

Triano EA, Slusher LB, Atkins TA, Beneski JT, Gestl SA, Zolfaghari R, et al. (2003). Class I alcohol dehydrogenase is highly expressed in normal human mammary epithelium but not in invasive breast cancer: implications for breast carcinogenesis. *Cancer Res*. 63(12):3092–100. PMID:12810634

Tsai M-C, Yang S-S, Lin C-C, Wang W-L, Hsu Y-C, Chen Y-S, et al. (2022). Association of heavy alcohol intake and *ALDH2* rs671 polymorphism with hepatocellular carcinoma and mortality in patients with hepatitis B virus-related cirrhosis. *JAMA Netw Open*. 5(7):e2223511. doi:10.1001/jamanetworkopen.2022.23511 PMID:35877121

Tsuchishima M, Tsutsumi M, Shiroeda H, Yano H, Ueshima Y, Shimanaka K, et al. (2000). Study of mitochondrial DNA deletion in alcoholics. *Alcohol Clin Exp Res*. 24(4 Suppl):12S–5S. doi:10.1111/j.1530-0277.2000.tb00004.x PMID:10803772

Tuma DJ (2002). Role of malondialdehyde-acetaldehyde adducts in liver injury. *Free Radic Biol Med*. 32(4):303–8. doi:10.1016/S0891-5849(01)00742-0 PMID:11841919

Ugai T, Milne RL, Ito H, Aronson KJ, Bolla MK, Chan T, et al. (2019). The functional *ALDH2* polymorphism is associated with breast cancer risk: a pooled analysis from the Breast Cancer Association Consortium. *Mol*

Genet Genomic Med. 7(6):e707. doi:10.1002/mgg3.707 PMID:31066241

Uittamo J, Siikala E, Kaihovaara P, Salaspuro M, Rautemaa R (2009). Chronic candidosis and oral cancer in APECED-patients: production of carcinogenic acetaldehyde from glucose and ethanol by *Candida albicans*. *Int J Cancer*. 124(3):754–6. doi:10.1002/ijc.23976 PMID:18975379

Uribe LA, Bacchetti P, Gelman N, Burchard E, Fitch M, Hellerstein M, et al. (2018). Impact of moderate alcohol discontinuation on insulin action and secretion in Latinos with and without hepatitis C. *Alcohol Clin Exp Res*. 42(3):492–9. doi:10.1111/acer.13576 PMID:29220547

Väkeväinen S, Tillonen J, Agarwal DP, Srivastava N, Salaspuro M (2000). High salivary acetaldehyde after a moderate dose of alcohol in *ALDH2*-deficient subjects: strong evidence for the local carcinogenic action of acetaldehyde. *Alcohol Clin Exp Res*. 24(6):873–7. doi:10.1111/j.1530-0277.2000.tb02068.x PMID:10888077

Väkeväinen S, Tillonen J, Salaspuro M (2001). 4-Methylpyrazole decreases salivary acetaldehyde levels in ALDH2-deficient subjects but not in subjects with normal ALDH2. *Alcohol Clin Exp Res*. 25(6):829–34. doi:10.1111/j.1530-0277.2001.tb02286.x PMID:11410717

Välimäki M, Pelkonen R, Härkönen M, Ylikahri R (1984). Hormonal changes in noncirrhotic male alcoholics during ethanol withdrawal. *Alcohol Alcohol*. 19(3):235–42. PMID:6508878

Välimäki M, Salaspuro M, Härkönen M, Ylikahri R (1982). Liver damage and sex hormones in chronic male alcoholics. *Clin Endocrinol (Oxf)*. 17(5):469–77. doi:10.1111/j.1365-2265.1982.tb01614.x PMID:6816489

van Zyl PM, Joubert G (2015). Acetaldehyde production capacity of salivary microflora in alcoholics during early recovery. *Alcohol*. 49(3):283–90. doi:10.1016/j.alcohol.2015.01.011 PMID:25819530

Varela-Rey M, Woodhoo A, Martinez-Chantar M-L, Mato JM, Lu SC (2013). Alcohol, DNA methylation, and cancer. *Alcohol Res*. 35(1):25–35. PMID:24313162

Varella Morandi Junqueira-Franco M, Ernesto Troncon L, Garcia Chiarello P, do Rosário Del Lama Unamuno M, Afonso Jordao A, Vannucchi H (2006). Intestinal permeability and oxidative stress in patients with alcoholic pellagra. *Clin Nutr*. 25(6):977–83. doi:10.1016/j.clnu.2006.03.010 PMID:16713031

Visapää J-P, Götte K, Benesova M, Li J, Homann N, Conradt C, et al. (2004). Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. *Gut*. 53(6):871–6. doi:10.1136/gut.2003.018994 PMID:15138216

Visapää J-P, Jokelainen K, Nosova T, Salaspuro M (1998). Inhibition of intracolonic acetaldehyde production and alcoholic fermentation in rats by ciprofloxacin. *Alcohol Clin Exp Res*. 22(5):1161–4. doi:10.1111/j.1530-0277.1998.tb03716.x PMID:9726290

Visapää J-P, Tillonen J, Salaspuro M (2002). Microbes and mucosa in the regulation of intracolonic acetaldehyde concentration during ethanol challenge. *Alcohol Alcohol*. 37(4):322–6. doi:10.1093/alcalc/37.4.322 PMID:12107032

Visvanathan K, Mondul AM, Zeleniuch-Jacquotte A, Wang M, Gail MH, Yaun SS, et al. (2023). Circulating vitamin D and breast cancer risk: an international pooling project of 17 cohorts. *Eur J Epidemiol*. 38(1):11–29. doi:10.1007/s10654-022-00921-1 PMID:36593337

Voican CS, Njiké-Nakseu M, Boujedidi H, Barri-Ova N, Bouchet-Delbos L, Agostini H, et al. (2015). Alcohol withdrawal alleviates adipose tissue inflammation in patients with alcoholic liver disease. *Liver Int*. 35(3):967–78. doi:10.1111/liv.12575 PMID:24766056

Walline CC, Blum JS, Linton T, Mangiacarne D, Liangpunsakul S (2018). Early activation of peripheral monocytes with hallmarks of M1 and M2 monocytic cells in excessive alcohol drinkers: a pilot study. *J Investig Med*. 66(5):1–4. doi:10.1136/jim-2017-000706 PMID:29519798

Wang F, Li G, Ning J, Chen L, Xu H, Kong X, et al. (2018). Alcohol accumulation promotes esophagitis via pyroptosis activation. *Int J Biol Sci*. 14(10):1245–55. doi:10.7150/ijbs.24347 PMID:30123073

Wang W, Wang C, Xu H, Gao Y (2020). Aldehyde dehydrogenase, liver disease and cancer. *Int J Biol Sci*. 16(6):921–34. doi:10.7150/ijbs.42300 PMID:32140062

Wilson R, Wahl S, Pfeiffer L, Ward-Caviness CK, Kunze S, Kretschmer A, et al. (2017). The dynamics of smoking-related disturbed methylation: a two time-point study of methylation change in smokers, non-smokers and former smokers. *BMC Genomics*. 18(1):805. doi:10.1186/s12864-017-4198-0 PMID:29047347

Witt SH, Frank J, Frischknecht U, Treutlein J, Streit F, Foo JC, et al. (2020). Acute alcohol withdrawal and recovery in men lead to profound changes in DNA methylation profiles: a longitudinal clinical study. *Addiction*. 115(11):2034–44. doi:10.1111/add.15020 PMID:32080920

Wolstenholme JT, Warner JA, Capparuccini MI, Archer KJ, Shelton KL, Miles MF (2011). Genomic analysis of individual differences in ethanol drinking: evidence for non-genetic factors in C57BL/6 mice. *PLoS One*. 6(6):e21100. doi:10.1371/journal.pone.0021100 PMID:21698166

Wu C, Wang Z, Song X, Feng XS, Abnet CC, He J, et al. (2014). Joint analysis of three genome-wide association studies of esophageal squamous cell carcinoma in Chinese populations. *Nat Genet*. 46(9):1001–6. doi:10.1038/ng.3064 PMID:25129146

Xu J, Ma HY, Liu X, Rosenthal S, Baglieri J, McCubbin R, et al. (2020). Blockade of IL-17 signaling reverses alcohol-induced liver injury and excessive alcohol

drinking in mice. *JCI Insight*. 5(3):e131277. doi:10.1172/jci.insight.131277 PMID:32051339

Yang J, Syed F, Xia Y, Sanyal AJ, Shah VH, Chalasani N, et al. (2021). Blood biomarkers of intestinal epithelium damage regenerating islet-derived protein 3α and trefoil factor 3 are persistently elevated in patients with alcoholic hepatitis. *Alcohol Clin Exp Res*. 45(4):720–31. doi:10.1111/acer.14579 PMID:33587293

Yang SJ, Yokoyama A, Yokoyama T, Huang YC, Wu SY, Shao Y, et al. (2010). Relationship between genetic polymorphisms of *ALDH2* and *ADH1B* and esophageal cancer risk: a meta-analysis. *World J Gastroenterol*. 16(33):4210–20. doi:10.3748/wjg.v16.i33.4210 PMID:20806441

Yao CT, Liao CS, Yin SJ (1997). Human hepatic alcohol and aldehyde dehydrogenases: genetic polymorphism and activities. *Proc Natl Sci Counc Repub China B*. 21(3):106–11. PMID:9309874

Yen CH, Ho PS, Yeh YW, Liang CS, Kuo SC, Huang CC, et al. (2017). Differential cytokine levels between early withdrawal and remission states in patients with alcohol dependence. *Psychoneuroendocrinology*. 76:183–91. doi:10.1016/j.psyneuen.2016.10.015 PMID:27951519

Yin SJ, Bosron WF, Magnes LJ, Li TK (1984). Human liver alcohol dehydrogenase: purification and kinetic characterization of the beta 2 beta 2, beta 2 beta 1, alpha beta 2, and beta 2 gamma 1 "Oriental" isoenzymes. *Biochemistry*. 23(24):5847–53. doi:10.1021/bi00319a026 PMID:6395883

Yin SJ, Chou FJ, Chao SF, Tsai SF, Liao CS, Wang SL, et al. (1993). Alcohol and aldehyde dehydrogenases in human esophagus: comparison with the stomach enzyme activities. *Alcohol Clin Exp Res*. 17(2):376–81. doi:10.1111/j.1530-0277.1993.tb00779.x PMID:8488982

Yin SJ, Liao CS, Lee YC, Wu CW, Jao SW (1994). Genetic polymorphism and activities of human colon alcohol and aldehyde dehydrogenases: no gender and age differences. *Alcohol Clin Exp Res*. 18(5):1256–60. doi:10.1111/j.1530-0277.1994.tb00115.x PMID:7847616

Yokoyama A, Kamada Y, Imazeki H, Hayashi E, Murata S, Kinoshita K, et al. (2016). Effects of *ADH1B* and *ALDH2* genetic polymorphisms on alcohol elimination rates and salivary acetaldehyde levels in intoxicated Japanese alcoholic men. *Alcohol Clin Exp Res*. 40(6):1241–50. doi:10.1111/acer.13073 PMID:27087413

Yokoyama A, Muramatsu T, Ohmori T, Yokoyama T, Okuyama K, Takahashi H, et al. (1998). Alcohol-related cancers and aldehyde dehydrogenase-2 in Japanese alcoholics. *Carcinogenesis*. 19(8):1383–7. doi:10.1093/carcin/19.8.1383 PMID:9744533

Yokoyama A, Tsutsumi E, Imazeki H, Suwa Y, Nakamura C, Mizukami T, et al. (2008). Salivary acetaldehyde concentration according to alcoholic beverage consumed and aldehyde dehydrogenase-2 genotype. *Alcohol Clin Exp Res*. 32(9):1607–14. doi:10.1111/j.1530-0277.2008.00739.x PMID:18616675

Yokoyama A, Tsutsumi E, Imazeki H, Suwa Y, Nakamura C, Yokoyama T (2007). Contribution of the alcohol dehydrogenase-1B genotype and oral microorganisms to high salivary acetaldehyde concentrations in Japanese alcoholic men. *Int J Cancer*. 121(5):1047–54. doi:10.1002/ijc.22792 PMID:17471563

Yokoyama S, Takeuchi K, Shibata Y, Kageyama S, Matsumi R, Takeshita T, et al. (2018). Characterization of oral microbiota and acetaldehyde production. *J Oral Microbiol*. 10(1):1492316. doi:10.1080/20002297.2018.1492316 PMID:30013723

Yoshida A, Huang I-Y, Ikawa M (1984). Molecular abnormality of an inactive aldehyde dehydrogenase variant commonly found in Orientals. *Proc Natl Acad Sci USA*. 81(1):258–61. doi:10.1073/pnas.81.1.258 PMID:6582480

Zhou D, Xiao L, Zhang Y, Xian J, Jiang J, Zong W, et al. (2012). Genetic polymorphisms of *ALDH2* and *ADH2* are not associated with risk of hepatocellular carcinoma among East Asians. *Tumour Biol*. 33(3):841–6. doi:10.1007/s13277-011-0309-8 PMID:22231434