

# **Studies on new pharmacological treatments for alcohol dependence**

**- and the importance of objective markers of  
alcohol consumption**

**Andrea de Bejczy**

**2016**

Addiction Biology Unit  
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Sweden



**UNIVERSITY OF GOTHENBURG**

Cover illustration: by A. de Bejczy

Inspired by Stan Lee's

"The Invincible Ironman. The empty shell", Marvel Comics

“...HIS VOICE IS HOARSE, HIS  
HAND TREMBLING AS HE  
REACHES FOR THE GLEAMING  
OBJECT THAT SEEMS BOTH  
WONDERFUL AND TERRIBLE  
TO HIM...”

Studies on new pharmacological treatments for alcohol dependence

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### ABSTRACT

This thesis will guide you through three randomized controlled trials (RCT) on three pharmacotherapies for alcohol dependence; the antidepressant drug mirtazapine, the smoking cessation drug varenicline and the glycine-uptake inhibitor Org 25935. The mirtazapine study was an investigator initiated single-center harm-reduction study with alcohol consumption measured by self-report in a diary as main outcome. The results indicated that mirtazapine reduced alcohol consumption in males with heredity for alcohol use disorder (AUD). The Org 25935 study was an international multi-center study with abstinence as treatment goal, main time to relapse and alcohol consumption was measured by self-report collected by the Time Line Follow Back method (TLFB). All subjects reduced their drinking compared to baseline, but Org 25935 failed to show superiority over placebo. The varenicline study was an investigator initiated multi-center harm-reduction study. In this study alcohol consumption was measured both by self-report in a diary and by alcohol biomarkers. In the analysis of self-reported data, varenicline failed to show efficacy, however, in the biomarker analysis varenicline reduced alcohol consumption compared to placebo. The direct alcohol marker phosphatidylethanol (PEth) was superior to the indirect biomarkers CDT and GGT in measuring alcohol consumption.

**Keywords:** Alcohol dependence, RCT, mirtazapine, glycine-transporter inhibitor, varenicline, alcohol marker, phosphatidylethanol, PEth

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# LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

## Papers

- I. The Effects of Mirtazapine Versus Placebo on Alcohol Consumption in Male High Consumers of Alcohol; A Randomized, Controlled Trial.**  
Andrea de Bejczy, MD and Bo Söderpalm, MD, PhD.  
*Journal of Clinical Psychopharmacology, Vol. 35, No.1, pp 43-50, February 2015*
- II. Efficacy and Safety of the Glycine Transporter-1 Inhibitor Org 25935 for the Prevention of Relapse in Alcohol-Dependent Patients: A Randomized, Double-Blind, Placebo-Controlled Trial.**  
Andrea de Bejczy, Kari R. Nations, Armin Szegedi, Joep Schoemaker, Frank Ruwe and Bo Söderpalm.  
*Alcoholism: Clinical and Experimental Research, Vol.38, No.9, pp. 2427-2435, September 2014*
- III. Varenicline for treatment of alcohol dependence: a randomized, placebo-controlled trial.**  
Andrea de Bejczy\*, MD, Elin Löf\*, PhD, Lisa Walther, MD, Joar Guterstam, MD, Anders Hammarberg, PhD, Gulber Asanovska, MD, Johan Franck, prof., Anders Isaksson, associate prof., and Bo Söderpalm, prof. \*shared first author  
*Alcoholism: Clinical and Experimental Research, Vol.39, No.11, pp. 2189-2199, November 2015*
- IV. Phosphatidylethanol is Superior to Carbohydrate-Deficient Transferrin and  $\gamma$ -Glutamyltransferase as an Alcohol Marker and is a Reliable Estimate of Alcohol Consumption Level.**  
Lisa Walther, Andrea de Bejczy, Elin Löf, Therese Hansson, Anders Andersson, Joar Guterstam, Anders Hammarberg, Gulber Asanovska, Johan Franck, Bo Söderpalm and Anders Isaksson.  
*Alcoholism: Clinical and Experimental Research, Vol.39, No.11, pp. 2200-2208, November 2015*

# POPULÄRVETENSKAPLIG SAMMANFATTNING

Alkoholberoende innebär ett stort lidande för de drabbade och deras närstående. Alkohol orsakar också stora kostnader för samhället och ca. 4 % av den totala sjukdomsburden i världen orsakas av alkohol. Idag finns i Europa fyra godkända läkemedel för alkoholberoende. Disulfiram, som verkar genom att påverka nedbrytningen av alkohol och orsaka symptom som kräkning och huvudvärk om man dricker alkohol när man tagit medicinen. Akamprosat, naltrexon och nalmefen verkar genom olika mekanismer för att minska suget efter alkohol och öka kontrollen över hur mycket man dricker. Tyvärr fungerar inte läkemedlen för alla och det skulle behövas fler behandlingar. Den här avhandlingen baseras på tre kliniska randomiserade placebokontrollerade prövningar som undersöker tre olika läkemedels effekter på alkoholkonsumtionen. Den första studien visade att mirtazapin, ett välkänt antidepressivt läkemedel (Remeron®) kan ha effekt på alkoholkonsumtionen hos högkonsumerande män med ärftlighet för alkoholberoende. Den andra studien undersökte hur en glycintransportör-blockare, Org 25935, påverkade återfallsfrekvensen hos avgiftade alkoholberoende individer. Båda grupperna drack mycket mindre under studien, men läkemedlet hade inte bättre effekt jämfört med placebo. På grund av övertygande resultat i djurstudier behövs det fler studier som utvärderar glycintransportör-blockerare innan man med säkerhet kan säga att de inte har en effekt på alkoholkonsumtionen. Studien var gjord i samarbete med ett läkemedelsföretag (MSD) men det underliggande konceptet kom från gruppens prekliniska forskning. Den tredje studien var också en fortsättning på gruppens prekliniska forskning. I den undersöktes vareniklin, den verksamma substansen i ett läkemedel mot rökning (Champix®), påverkar kolinerga system i hjärnan och som visat sig sänka alkoholkonsumtion hos råttor och hos rökare med hög alkoholkonsumtion. I vår studie mättes alkoholkonsumtionen på två sätt, dels genom att försökspersonerna fick anteckna vad de druckit i en dagbok, dels genom ett blodprov där man mätte phosphatidylethanol (PEth), som bildas då man druckit alkohol och är en markör för hur mycket man har druckit. När man analyserade alkoholkonsumtionen som rapporterats i dagböckerna så fanns det ingen skillnad mellan gruppen som fått vareniklin och gruppen som fått placebo, men när man analyserade uppmätta nivåer av PEth i blodet så hade de som fått vareniklin sänkt sin konsumtion. Eftersom flera markörer för alkoholkonsumtion mättes i studien, jämfördes dessa med varandra och med den mängd alkohol som uppgivits i dagböckerna. Den markör som verkade ge den mest korrekta uppskattningen av alkoholkonsumtionen var PEth. Det var större skillnad mellan den uppgivna konsumtionen och PEth-nivåer i blodet i placebogruppen än i gruppen som fått vareniklin. Förklaringen kan vara att om man starkt önskar att dricka mindre och man också förväntar sig en effekt av det läkemedel man får, så underrapporterar man omedvetet sin konsumtion. Hos de som fått vareniklin och

faktiskt har sänkt sin konsumtion under studien blev inte underrapporteringen lika tydlig. Det är möjligt att det finns en nivå av alkoholkonsumtion som känns accepterad av samhället och också upplevs som en acceptabel förbättring, och att det är på den nivån som placebogrupperna OCH vareniklinggruppen (som rapporterar lite mindre än den med PEth uppmätta konsumtionen) hamnar. Den sammanlagda slutsatsen från de tre studierna är att det är viktigt att mäta utfallet, dvs. alkoholkonsumtionen, på ett så objektiva sätt som möjligt, t.ex. med en alkoholmarkör. Av de markörer som utvärderades i studierna var PEth den som mätte alkoholkonsumtionen bäst.

## I KORTHET

Alkohol orsakar lidande, för tidig död och stora kostnader.

Tre kliniska studier på tre möjliga nya läkemedel genomfördes.

Mirtazapin, ett antidepressivt läkemedel, minskade alkoholkonsumtion hos män med ärftlighet för alkoholkonsumtion.

Org 25935, som påverkar glycinivåer i hjärnan, förlängde inte tiden till återfall efter avgiftning, men fler studier behöver göras.

Vareniklin, ett läkemedel mot rökning, sänkte alkoholkonsumtionen när man mätte med en objektiv alkoholmarkör, men inte när man mätte med självrapporterad alkoholkonsumtion.

Alkoholkonsumtion i studier bör hellre mätas med ett objektiva mått, som t.ex. en alkoholmarkör, än med ett subjektivt mått.

I jämförelse med två andra vanliga alkoholmarkörer visade sig fosfatidylethanol (PEth) vara den som mätte alkoholkonsumtion bäst.



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- Appendix 1: Supplementary to Paper III
- Appendix 2: Alcohol-nicotine diary Paper III
- Appendix 3: ALKO-NACKA
- Appendix 4: AUDIT
- Appendix 5: OCDS

## **PAPERS**

- Paper I
- Paper II
- Paper III
- Paper IV

# LIST OF ABBREVIATIONS

AA	Alcoholics Anonymous
AAF	Alcohol Attributable Fraction
AC	Adenylyl Cyclase
ADHD	Attention Deficit Hyperactivity Disorder
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ARBD	Alcohol Related Brain Damage
AST	Aspartate Aminotransferase
AT	As-Treated
ATP	Adenosine 5'-triphosphate
AUD	Alcohol Use Disorders
AUDIT	Alcohol Use Disorder Identification Test
AUDIT-C	AUDIT- consumption items
BAC	Blood Alcohol Content
BMI	Body Mass Index
BP	Blood Pressure
CAN	Centralförbundet för alkohol- och narkotikaupplysning
CBI	Combined Behavioral Interaction
CD	Conduct Disorder
CDT	Carbohydrate Deficient Transferrin

CI	Confidence Interval
CNS	Central Nervous System
COA	Children of alcoholics
CRF	Case Report File
CSF	Cerebro Spinal Fluid
CVD	Cardio Vascular Disease
DA	Dopamine
DSM	Diagnostic and Statistical Manual of mental disorders
DT	Delirium Tremens
DUDIT	Drug Use Disorder Identification Test
DUI	Driving Under the Influence
EEG	Electroencephalogram
EMA	European Medicines Agency
EOA	Early Onset Alcoholics
EoT	End of Treatment
EtG	Ethyl Glucuronide
EtS	Ethyl Sulfate
FAS	Fetal Alcohol Syndrome
FAEE	Fatty Acid Ethyl Esters
FDA	Food and Drug Administration
FHN	Family History Negative for alcoholism
FHP	Family History Positive for alcoholism

$\gamma$ CDT	gamma Carbohydrate-Deficient Transferrin
GABA	Gamma Amino Butyric Acid
GAD	Generalized Anxiety Disorder
GDP	Gross Domestic Product
GGT	Gamma Glytanyltransferase
GHB	Gamma Hydroxy Butyrate
GI	Gastrointestinal
GlyT	Glycine Transporter
GWAS	Genom Wide Association Study
HDD	Heavy Drinking Days
IMP	Ivestigational Medicinal Product
ICD	International Classification of Diseases
ITT	Intention-To-Treat
LOA	Late Onset Alcoholics
LOCF	Last Observation Carried Forward
LR	Low Response to Alcohol
LSD	Lysergic Acid Diethylamide
LS means	Least Squares Means
MCH	Mean corpuscular haemoglobine
MCV	Mean corpuscular volume
N	Number
NA	Noradrenergic

nAc	nucleus Accumbens
nAChR	nicotinergetic Acetylcholine Receptor
NMDA	N-Metyl-D-Aspartate
NNT	Numbers Needed to Treat
NPV	Negative Predictive Value
NtBNP	N-terminal pro-BNP
OCDS	Obsessive Compulsive Drinking Scale
PEth	Phosphatidylethanol
PP	Per Protocol
PPSI	Patient-perceived Satisfactory Improvement
PPV	Positive Predictive Value
RCT	Randomized Controlled Trial
RR	Relative Risk
SAP	Statistical Analytical Plan
SD	Standard Deviation
SEM	Standard Error of the Mean
SNP	Single Nucleotide Polymorphism
SSRI	Selective Serotonergic Reuptake Inhibitor
SUD	Substance Use Disorder
TAS	Transdermal Alcohol Sensor
TD	Thiamine Deficiency
TLFB	Timeline Follow Back



VAS	Visual Analogue Scale
VTA	Ventral Tegmental Area
WHO	World Health Organisation
5-HT	5-hydroxytryptamine/ serotonin

# DEFINITIONS IN SHORT

**Agonist** – is a substance that acts like another substance and therefore stimulates an action.

**Antagonist** – is a substance that acts against and blocks an action.

**As-Treated** – subjects who took active treatment, regardless of assigned group

**Heavy Drinking Days** – days when 4 drinks of alcohol or more for women and 5 drinks or more for men are consumed.

**Global Burden of Disease** – the collective disease burden produced by all diseases around the world.

**Disability Adjusted Life Years** – the sum of years of potential life lost due to premature mortality and the years of productive life lost due to disability.

**Alcohol Attributable Fraction** – the extent to which alcohol contributes to a health outcome.

**Power calculation** – Statistical power is defined as the probability of rejecting the null hypothesis while the alternative hypothesis is true. Factors that affect statistical power include the sample size. A power calculation is made to estimate the sample size needed to detect a difference between groups, taken into consideration the expected effect size.

**Least squares means** – the group means used in analysis of covariance models after having controlled for a covariate (i.e. holding it constant at some typical value of the covariate, such as its mean value). Also referred to as marginal means or estimated marginal means or model based means.

**Type I error** – rejection of a null hypothesis (which in drug treatment studies means that the drug has no effect) that is actually true, i.e. false positive. With a confidence level of 95 %, there is a 5 % probability of type I error.

**Type II error** – accept that the drug has no effect (null hypothesis) when really it did have effect, i.e. false negative.

**Intention-to-treat (ITT)** – all subjects randomized into a study

**Modified ITT** – pre-defined criteria for inclusion according to ITT (modified ITT). Typical criteria are; ingestion of at least one dose of IMP, and one valid data point.

**Per protocol** – subjects that follow their assigned treatment according to pre-specified criteria.

**Cohen's  $d$**  – indicates the standardized difference between two means. This is a measure of effect size, which is a quantitative measure of the strength of a phenomenon.

**Hedge's  $g$**  – measures effect size with adaption for small sample size, with a larger number being a better effect size.

**Positive Predictive Value** – Positive predictive value is the probability that subjects with a positive screening test truly have the disease.

**Negative Predictive Value** – Negative predictive value is the probability that subjects with a negative screening test truly don't have the disease.

**Sensitivity** – true positive rate (if a person has the disease, how often will the test be positive).

**Specificity** – true negative rate (if a person does not have the disease, how often will the test be negative)

# CALCULATION FORMULAS

**To calculate alcohol content in beverages, use the following formula;**

volume of beverage in liters x volume percent alcohol  
x density of ethanol (7.89) = grams of pure alcohol

**To calculate blood alcohol content (BAC), use the simplified Widmark formula;**

$BAC = \left[ \frac{\text{Alcohol consumed in grams}}{\text{Body weight in grams} \times r} \right] \times 100$ .  
In this formula, "r" is the gender constant: r = 0.55 for females and 0.68 for males

**To calculate the metabolism of ethanol, use the following formula;**

BAC as a percentage - elapsed times in hours x 0.15

**To calculate  $\gamma$ -CDT, use the following formula;**

$(0.8 \times \ln(\text{GGT}) + 1.3 \times \ln(\text{CDT}))$  or  $(0.8 \times \ln(\text{GGT}) + 1.3 \times \ln(\% \text{CDT}))$ .

**To calculate sensitivity, use the following formula;**

true positives / (true positives + false negatives)

**To calculate specificity, use the following formula;**

true negatives / (true negatives + false positives)

**To calculate positive predictive value (PPV), use the following formula;**

true positives / (true positives + false positives)

**To calculate negative predictive value (NPV), use the following formula;**

true negatives / (true negatives + false negatives)

**To calculate pooled SD, use the following formula;**

$$SD_{\text{pooled}} = \sqrt{((SD_1^2 + SD_2^2) / 2)}$$

**To calculate Cohen's d, use the following formula;**

$$\text{Cohen's } d = (M_2 - M_1) / SD_{\text{pooled}}$$

# ALCOHOL AND ITS HISTORY

## Global history of alcohol

Mankind has been fermenting alcohol for at least 10 000 years and the earliest beer containers found are dated back to 8000 BC. The earliest alcoholic drinks are thought to have been made from honey and berries, however, by the introduction of grain farming in the Near East beer brewing was enabled and became a basic source of nourishment.

Winemaking occurred a bit later on and archeological findings of jars are dated back to 5400/5000 BC in Iran and to 7000/6600 BC in China. In Egypt the first picturing of wine appeared around 4000 BC and pyramid builders are described to have a daily ration of 5 liters of 5% beer. Beer was thought to be invented by Osiris and was brewed at home. Drinking was hence widespread but seems to have been fairly moderate and used for pleasure and nourishment as well as medicine and religious purposes. One of the first descriptions of alcohol intoxication is from the Old Testament, when Noah has been drinking wine and is lying drunk in his vineyard.

Oral traditions hold it that Hebrews began drinking beer during the Egyptian captivity at 1200 BC. When they were led to Canaan (Palestine) by Moses, they missed the wine left in Egypt and started growing wine in the new land. By the Exile of the Hebrews in 539 BCE, wine became a major source of nourishment also for children and an essential part of the Hebrew life.

Permanent settlement seems to be the working ground for alcohol making and drinking. By 2000 BC winemaking reached Greece and was soon commonplace. Indeed, at public festivities all were ensured an equal amount of wine and from this democracy is said to have been born. Greeks generally promoted drinking in moderation and frowned on drunkenness, except for the Dionysus cult that was all for debauchery.

Also in Rome moderate drinking was the norm until approx. 400 BC, but with the Roman Empire spreading throughout the Mediterranean region, this was replaced by heavy drinking, and alcohol misuse spread in the society. The destructive alcohol consumption seemed to peak around 50 BC with several abusive drinkers as emperors. However, with the introduction of Christianity drunkenness was again disapproved. According to Bible texts, moderate consumption and medical use of alcohol, in particular wine, was approved by Jesus, but intoxication was condemned and abstinence recommended for those incapable of controlling their drinking.

When the Roman Empire collapsed in 476 AD the monasteries took charge of the winemaking and the brewing and carefully guarded the knowledge down to the 12<sup>th</sup> century.

The exact start of fermentation with barley, with hops added for taste and conservation is not known, but from 850 to 1100 AD mead (but also the sweeter ale) had a central role in the Viking culture and their perception of heaven, defined as endless quantities of mead.

Beer, mead and wild fruit wines were preferred by Celts, Anglo-Saxons, Germans, and Scandinavians. Anglo-Saxons mostly drank in village halls, which became important centers of Anglo-Saxon culture. In England, during 1000 to 1500 AD, ale was an important food source for the commoners. Men women and children all had ale for breakfast, with dinner and before bed, and for an adult 4 liters a day was a normal consumption. Although popular among the gentry, few commoners had ever tasted wine as it was imported and expensive. German towns were allowed to brew their own local beer, and did so with great pride. In Italy, Spain and France wine was the beverage of choice and vineyards in Bordeaux blossomed during the 12<sup>th</sup> century.

A major advance in the alcohol history was the art of distillation. There are references of distilling in Chinese poems from 800 AD, and also documents indicating that the Egyptians had the theoretical knowledge of distilling around 300 AD. But most likely the Chinese learned from Arabians, and Russians then learned from the Chinese. In 1100 AD the skill of distilling came to Europe, and was first described by Albertus Magnus (1193-1280). Physicians and monks became interested of the aqua vitae (named by the professor in medicine Amaldus of Villanova (d. 1315)) as medication. During the 15<sup>th</sup> century, distilled spirits “the water of immortality” was believed to cure virtually everything.

By the end of the middle-ages, however, spirits were consumed as a drink rather than medication. At this time the Dutch introduced Brandy (originally named brandewijn which means burnt (distilled) wine) and they were also first with flavoring spirits, with juniper berries. The consumption of alcohol throughout Europe was high and alcohol was everywhere and consumed by all ages. Intoxication became natural and blameless.

In America the Mayan cultures had been fermented all kinds of fruits and seeds since 1000 BC. However, most tribes did not drink alcoholic beverages before the Europeans introduced them in the 1600s, and when Spanish alcohol was introduced social structures collapsed also in areas with long tradition of local alcohol use.

Some famous alcoholic beverages saw daylight in the 16th and 17th century. Champagne was actually stumbled upon in England as the wine had been left in the cellar over winter and was referred to as brisk champagne. At the time the French considered bubbles in wine an abomination. Dom Perignon later developed the bottles and corks necessary.

Whiskey, the first grain spirit, was probably invented in Ireland and was soon widespread.

However, the distilled spirits were still mostly for medicinal use, but when sugar production in the Caribbean increased, so did the production of Rum (first mentioned in 1651), and with importation of molasses the prices of rum dropped in North America and became popular. It also had a part in the triangle trade, traded for African slaves who were traded for more molasses to make more Rum.

In 1690, England experienced the gin epidemic when a nation of 6.5 million consumed 68 million liters of gin per year.

In the early 1700s the agricultural revolution produced so much grain and fruit that the supply of alcohol met the high demand, leading to the democratization of alcohol as workers and peasants could drink at the same levels as the gentry. As also transportation improved, alcohol became a market commodity. However, with increased availability, drunkenness started to be viewed as a liability. In the industrialized society where you needed to be punctual and alert alcohol was increasingly recognized as causing crime, poverty and high infant mortality. The protestant church began to turn against the former belief that alcohol was a gift from God and in the second part of the 1800s launched the "two wine" theory, stating that it was grape juice that was the gift and wine was causing the problems. Over time, personal, economic, criminal, family, social, moral, and religious problems were attributed to alcohol. This led to the rise of the temperance movement during the first and second decade of the 1800, which in turn resulted in the prohibition era, mainly during the 1920s.

(Hanson, 2013, Courtwright, 2002, Rydving, 1996, Pontén, 1967, Ericsson, 2003, WHO, 2014, Boyle, 2013)



## TO SUMMARIZE

Mankind has been using alcohol for at least 10 000 years.

Beer is thought to first appear in the Near East in 8000 BC and with permanent settlement and grain farming, beer became an important source of nourishment, even for children. For Celts, Anglo-Saxons, Germans and Scandinavians beer became an important part of life.

First records of winemaking is dated back to 5000-7000 BC, in Iran and China, in 2000 BC it reached Greece and later spread with the Roman Empire. For the Mediterranean countries, wine remained the beverage of choice.

The art of distillation is thought to have originated in Arabia and travelled via China and Russia to Europe by the 1100 century. At first, spirits were used mainly for medicine and gun-powder production, but by the middle-ages consumption started spreading and with cheaper production costs, spirits were made more available.

By the 1800s increased consumption resulted in more problems in society which resulted in the rise of the temperance movement.

## The history of alcohol in Sweden

In the beginning of Swedish relationship with alcohol the Gods were thought to immerse with the intoxication. Preferred beverages were beer and mead. In the 1500s Swedish soldiers fighting in Russia brought home the knowledge of distilling spirits, although at first it was mainly used in the making of gun powder. In the 16th century it was tried as a medication for depression and for the Plague and used as a stimulatory tonic as a part of the pharmacy assortment. At first distilling spirits was made of "mäsk" (a mix of dried, sprouted grains and water). In the 1500ies as many as 60 % of the farmers owned a distiller, a quite expensive apparatus (valued at 1 ½ cows) made out of copper. When grains were scarce distilling was prohibited and for a ten year period the crown tried to monopolize distilling. However, when allowed, farmers probably produced around 12-13 liters per person and year. A bi-product of the distilling process was "drank", a product containing soluble proteins and highly suited as animal feed, and hence valuable.

In 1746 the very first description of distilling from potatoes is documented. This new process was invented by a scientist called Eva de la Gardie (first woman in The Swedish Academy of Science at 24 years of age). Using potatoes for distilling saved the grains for bread and by the 1800s potatoes were widely used for distillation. Spirits were by now also a commodity and used in the colonial trade.

The debauchery increased and by 1829 the average Swede drank 45 liters of spirits a year. The temperance movement grew stronger and 1850 the first "Systembolaget" opened in Falun. Laws regulating sales and serving of alcohol were taken in 1855 and in 1860 household distilling were prohibited. No individual profit from sales was allowed and physician Ivan Bratt implemented the Bratt system with the ration book in 1917. This included 1.) All production and sale should be controlled within the governmental monopoly, 2.) Healthcare should be available for sobriety, and 3.) There should be an individual sale control, meaning that every person's alcohol consumption was controlled by the ration book. Rations were determined according to gender, age, law abiding-ness and civil status. Several occasions of drunkenness resulted in withdrawal of rations. A man over 30 years of age could obtain at the most 4 liters of spirit per month (averaging on 1.82 liters/month) while an unmarried woman was allowed only 1-2 bottles per 3 months. Married women did not get a ration book of their own at all. Wine was also registered but not limited and extra rations were given at festivities. 1922 a vote on prohibition was lost with 51 to 49 %, and the Bratt-system became permanent as a compromise on both sides and continued until 1955. When the ration book was abandoned the consumption increased drastically.

Up until 1995, the alcohol policies were strict, but with the entry into EU, the rules were becoming more allowing and e.g. individual import of alcohol was increased from a few liters to, practically, limitless.

(Drugsmart, Systembolaget, Solhem, Prestjan, 2006, Presjtjan, 2004, Courtwright, 2002, Spiritus, 2000-2003, Pontén, 1967, Ericsson, 2003, CAN, 2011, Lantbruksakademin, Systembolaget, 1999)

## TO SUMMARIZE

Beer and mead was the first alcohol beverages used in Sweden and intoxication was thought to be related to the Gods.

The art of distillation was imported by soldiers from Russia in the 1500s.

Home-distilling was common, but prohibited when there was a lack of grains.

Distilling from potatoes was first described by Eva de la Gardie and was widely used by the 1800s, saving the grains for bread.

Debauchery increased and in response also the temperance movement. In 1850 the first "Systembolaget" opened.

In 1917, the physician Ivan Bratt implemented the ration book, which remained until 1955.

Alcohol policies remained strict until the entry in the EU in 1995.

# ALCOHOL AND SOCIETY

## Alcohol consumption in the Swedish population

The estimated yearly consumption of pure alcohol per person 15 years of age and older was 9.9 liters in 2013. The registered purchase accounted for 75 %, import in traveling 16 %, smuggling 6 %, moon shining 2 %, and purchase via internet 2 %. In the ninth grade (15 years of age) boys' alcohol consumption amounted to 1.6 liters pure alcohol and girls' to 1.4 liters. At age 17 the numbers were 4.3 and 2.7 liters, respectively. Consumption has gone down slightly since a peak in the 00s. In the latest figures, it was estimated that 5.9 % of the Swedish population suffered from alcohol abuse (285 000 males and 161 000 women). Fifteen percent stated that they had been experiencing negative consequences from a relative's or friend's excessive drinking, while 10 % had suffered consequences from a stranger's excessive drinking (more women than men). Approx. 16 % are considered to be in the risk zone by their consumption. Alcohol use disorders (AUD) are estimated to yearly kill in-between 3000-7000 persons. In 2010 alcohol was considered to cause 3.4 of the total burden of disease in Sweden with 84 000 Disability adjusted life years (DALYs), considerably more men than women affected (66000 vs 18000). Also, 18 % of death casualties in drivers displayed above 0.2 per mille blood alcohol (the legal limit for DUI in Sweden). Deaths have been decreasing but in-ward occasions increasing from the 80-ies. In-ward events due to alcohol reached 3.5 % in 2011-2013, more for men than women (427/100 000 for men and 216/100 000 for women) (CAN, 2014).

## The monetary costs of alcohol

An attempt to estimate the global costs of alcohol was made in 2006 in a crude calculation based upon the best studies available. The costs were estimated at 210 to 650 billion US dollars in the year 2002, including 40 to 105 billion US dollars for health, 55 to 210 billion US dollars for premature mortality, 30 to 65 billion US dollars for absenteeism, 0 to 80 for unemployment, 30 to 85 billion US dollars for criminal justice system, and 15 to 50 billion US dollars for criminal damage. This represents a total of 0.6 to 2.0 % of the global GDP, or somewhere between the GDP of Austria and India (Baumberg, 2006). The costs for EU were estimated to 125 billion US dollars (Andersson, 2006). The cost of alcohol to the Swedish society has been estimated at approx. 100 billion Swedish crowns (SEK) (Johnson, 2000a).

## Global Burden of Disease (GBD) and mortality

Alcohol is top five for risk factor for disease and disability in Europe and top one in Eastern Europe (IMHE, Lim et al., 2012, Nutt and Rehm, 2014). Europeans above 15 years of age drink 9800 g pure alcohol per year (approx. 12.5 liters), which is twice the amount in comparison of the rest of the world (P Andersson, 2012). The global number per year for persons 15 years and older is 6.2 liters pure alcohol, however, 60 % had not drunk at all during the past 12 months. In all regions females were more inclined to be lifetime abstainers (WHO, 2014).

In 2012, 3.3 million deaths (5.9 % of all deaths, 7.6 % among males and 4.0 % among women) were attributable to alcohol. The GBD for alcohol was estimated at 5.1 % (as measured in Disability Adjusted Life Years (DALYs), 7.4 % for males and 2.3 % for women). Numbers vary from 0.9 % in the poorest countries to 13.8 % in Europe, with former socialist countries and European high-income countries being particularly affected (WHO, 2014).

As a risk factor, alcohol use is ranked no. 5 in middle-income countries, surpassed only by high blood pressure, tobacco use, overweight and physical inactivity. Globally, unsafe sex, high cholesterol and high blood glucose are greater risk factors. Low-income countries have additional problems, as childhood underweight, suboptimal breast-feeding and unsafe water (Room et al., 2005).

Another way to describe the consequences of alcohol is the attributable proportion of alcohol as a cause of a specific diagnose. AUD obviously has a 100 % attributable proportion. Violence, such as homicide has a 24 % attributional proportion. Unintentional injury has a high proportion due to alcohol; 20 % of motor vehicle accidents are attributable to alcohol, 18 % of poisoning and 10 % of drowning. Esophageal cancer (29 %), cancers in the liver (25 %) as well as in the mouth and oropharynx (19 %) and breast (7 %) have been shown to have causality to alcohol. Hemorrhagic epilepsy (18 %) and stroke (10 %) are besides liver cirrhosis (32 %) also major groups (Room et al., 2005). The risk for cardiovascular disease is also causally impacted by alcohol, as are infectious diseases, especially pulmonary disease (WHO, 2013, Lonnroth et al., 2008). Except for the case of breast cancer, the attributable factor is larger for men (Room et al., 2005). In Europe approx. 80 % of the alcohol-related deaths were attributable to cirrhosis, injuries and neoplasms (Agardh et al., 2016).

When looking at relative mortality risk with pooled standardized mortality ratios (SMRs), after 10 years follow up on AUD patients, the top 4 conditions were liver cirrhosis (14.8 %), mental disorder (10.8 %), death by injury (6.6 %), and cancer (2 %). Cause specific mortality for AUD was high in all categories, compared to the general population. Also, for many risk variables, the risk increased over time. No beneficial effects were found for any diagnosis, but instead an elevated risk both for

cardiovascular disease (CVD) and diabetes (diagnoses sometimes considered to benefit from alcohol), especially in the highest consumption group. A clear dose-response relationship was shown for all cancers described. The risk for gastrointestinal diseases also grew exponentially with higher consumption. There was evidence of high co-morbidity with mental disorder, however, the causality was not established, as seldom is possible. Heavy drinking clearly increased risk for respiratory disease, via immune-system impairment. The risks of different patterns of drinking, binge vs chronic heavy drinking, with the same exposure could not be determined (Roerecke and Rehm, 2014).

## TO SUMMARIZE

5.9 % of the Swedish population suffer from AUD and 16 % are considered to be in the risk consumption zone.

Sweden and Europe have a high annual consumption of approx. 10-12 liters pure alcohol in adults of 15 years or older.

The monetary costs of alcohol consumption are calculated as high as 650 billion dollars a year, the corresponding figure for Sweden is estimated at 100 billion SEK.

Alcohol consumption causes 3.3 million deaths yearly worldwide and accounts for 5.1 % of the total Global Burden of Disease.

Alcohol is one of the leading risk factors for death and alcohol intake has been shown to be causally related to many diseases, e.g. cirrhosis, injuries and neoplasms.

# THE CONSEQUENCES OF ALCOHOL CONSUMPTION

## Neurological complications

Alcohol can lead to harm by intoxication, affecting behavior, risk taking, cognition, coordination and consciousness, and by leading to dependence (as discussed in sections above). However, alcohol also has a toxic effect on organs and tissues causing a wide range of damage to the body and mind (WHO, 2014).

The most severe damage from alcohol is caused by acute and chronic exposure prenatally, which i.a. is associated with neuronal loss. Exposure in the womb may lead to Fetal Alcohol Syndrome (FAS), characterized by craniofacial abnormalities, growth deficiency, and cognitive and behavioral impairment (Hernandez et al., 2016).

Chronic alcohol use in adulthood has been associated with permanent neuronal loss in e.g. the hippocampus and the cerebellum brain regions, and with neurological impairments in memory, cognition and motor function. Mechanisms involved in ethanol's neurotoxic and neurodegenerative effects could be oxidative stress associated with the metabolism of alcohol (Hernandez et al., 2016).

With long and heavy drinking malnutrition often occurs and especially harmful is thiamine deficiency (TD), which is thought to be an important factor in the alcohol-related brain damage (ARBD) (Vedder et al., 2015). TD can lead to Wernicke encephalopathy, and worsen into Wernicke-Korsakoff syndrome, including encephalopathy, ophtalmoplegia, ataxia, confusion, and neuropathic damages and a psychotic chronic component with memory disturbances, polyneuropathy, and confabulation (Vedder et al., 2015, Sternebring, 2001).

Not only is the CNS vulnerable to the toxic effects of alcohol, but also peripheral nerves. Polyneuropathy, the symmetric sensibility impairment of distal extremities, could be followed by neuro-arthropathy and result in e.g. destroyed joints in the foot (Charcot's foot) (Sternebring, 2001, Arapostathi et al., 2013).

Muscles are also sensitive to alcohol exposure, alcohol myopathy, a slowly progressive process resulting in muscular loss of pelvis and upper leg muscles (Sternebring, 2001, Preedy et al., 2007, Preedy et al., 2001, Preedy et al., 2003).

A potentially deadly acute withdrawal syndrome, delirium tremens (DT), can occur within the first days of abstinence after a longer time (weeks) of heavy drinking. DT includes seizures, hyperthermia, hallucination and arrhythmia. Withdrawal seizures may occur without DT and its etiology is not entirely known, but is probably related to changes of subtype configurations of GABA<sub>A</sub> receptors (Rogawski, 2005), and a kindling effect, when repeated withdrawal lowers the threshold for seizures (Mehta, 2016, Sternebring, 2001).

## **Somatic complications**

Alcohol is liver toxic, but probably also causes damage by acting on the immune system and by biochemical cell changes. In between 8-15 % of AUD patients develop liver disease, more women than men and high BMI is a risk factor. Fatty liver, hepatitis, and liver cirrhosis can all be caused by alcohol. The risk limit of intake is probably around 20 g per day for women and 40 g/day for men. Apart from GI symptoms, bleedings, vomiting, flapping tremor, and rigidity can appear, Liver diseases can also cause liver encephalopathy with lowered consciousness, stupor and coma (Sternebring, 2001, Mehta, 2016).

The gastro-intestinal tract also suffers damages from alcohol consumption, the most common side effects being diarrhea, dyspepsia, and vomiting. Other common problems are reflux, gastritis, helicobacter infection and ulcers. Neuropathy could cause impaired mobility of the esophagus, which in combination with voluminous vomiting can cause rifts and ruptures. Most bleedings, however, stop spontaneously (called Mallory-Weiss bleedings), but in combination with elevated abdominal pressure the bleedings are life-threatening (Boerhaaves syndrome), especially with co-morbidity of liver disease. Long time excessive alcohol consumption is also the major cause of acute and chronic pancreatitis (Mehta, 2016, Sternebring, 2001).

B-vitamin deficiency, involved in the CNS damage due to alcohol, is one of the great problems in marginalized alcohol dependent individuals. Accelerated duodenal transport, delayed ventricle evacuation and epithelial damage cause malabsorption. This leads to B-vitamin deficiencies, with thiamine deficiency (TD) being correlated with neuropathic problems. Also folate acid deficiency, B<sub>2</sub>, B<sub>6</sub>, B<sub>12</sub> deficiency and zink deficiency (causing less production of alcohol dehydrogenase and impaired healing and deficient immune system) is often present. Malnutrition is also worsened by bad health status in the mouth with glossitis, stomatitis and caries (Sternebring, 2001).

Heavy drinking is an indisputable risk factor for cardio vascular disease (CVD) including cardiomyopathy, hypertension, arrhythmia, cerebrovascular disease, stroke, and bleeding and coagulation disturbances. An early sign of alcohol cardiac



damage is the “holiday heart syndrome”, an arrhythmia after a shorter period of heavy drinking. This could be the first sign of cardiomyopathy, a deadly diagnose most often occurring in males over 40 years. A consumption of over 80 g a day for more than 10 years is probably required. “Bierherz” (“beer heart”) is a non-specific dilatation of the heart, probably due to thiamin deficiency and more common in malnutrition (Sternebring, 2001).

Cutaneous manifestations, as worsening of psoriasis, acne rosacea, ecthyma (wine lesions), palmar plantar erythema, spider naevi, rhinophyma, erysipelas, and eczema are common in excessive drinkers. Also hematological changes, as megaloblastic anemia, and immunological impairment are common. The elevated risk for infections has impact on the cutaneous manifestations and lung infections are common and may become severe (Sternebring, 2001). Indeed, the connection between alcohol use and respiratory infections is discussed since far back. The connection between AUD and pneumonia could be a changed oropharyngeal flora and decreased airway reflexes as well as impaired defense and immunity functions (Mehta, 2016).

Alcohol consumption, in relatively low doses, has been associated with esophageal, stomach, colorectal, pancreas, lung, larynx, oral cavity, pharynx, breast, endometrial, prostate, CNS, and thyroid cancer (de Menezes et al., 2013, de Menezes et al., 2015).

Finally, high alcohol consumption may also cause impotency, insomnia, depression, dementia and confusion. And acute intoxication is, needless to say, a great risk factor for violence, both as victim and offender, for drowning and for vehicle accidents, and also for hypothermia and bad decisions.

## TO SUMMARIZE

The detrimental effects of alcohol to the brain are profound, both acute and chronic alcohol can lead to brain damage and impaired functions in cognition, memory and motor functions.

Delirium tremens (DT) is a potentially deadly withdrawal syndrome, causing seizures, arrhythmia, hyperthermia, circulatory collapse, and hallucinations.

Wernicke-Korsakoff is a serious syndrome due to thiamine-deficiency, in turn due to long-term alcohol consumption, manifesting with i.a. ataxia, memory disturbances and confabulation.

Alcohol is toxic to almost all organs in the human body causing liver cirrhosis, esophageal bleedings, arrhythmia, cardiovascular disease, impaired immune system response, pulmonary infections, malnutrition, and cancer.

# RISK FACTORS FOR ALCOHOL DEPENDENCE

Often the alcohol consumption peaks in the mid-twenties but often stabilizes after the start of family life and steady job. However, some do not have the ability to reduce but instead keep up their high consumption and continue into a dependence career. However, of all individuals using alcohol (in the western world often approx. 90 % of male population and 80 % of female population) only a minor group develops alcohol dependence. In a large American survey including over 40.000 respondents the risks of transition from substance use to dependence was examined. After the first year of onset of alcohol use, the risk of transition into dependence was almost 2 %. After a decade the number was 11 % and as many as 22.7 % of alcohol users had a lifetime cumulative risk to develop dependence. Half of the cases had transcended within 13 years. Early debut is one risk-factor commonly associated with adult alcohol dependence, as are being of male gender, and never being married or employed. In this study however, neither education nor income predicted transition to dependence (Lopez-Quintero et al., 2011). Using other substances of dependence (e.g. nicotine and cannabis) was associated with later dependence of alcohol (Lopez-Quintero et al., 2011). Neuroadaptation from chronic drug exposure may increase the sensitivity to the reinforcing drug effects and may be a mechanism in the tendency to poly-drug dependence (Winstanley et al., 2007). Any kind of mental disorder and family history of AUD or substance use disorder (SUD) also constitute risks, mainly due to genetic factors (Kendler et al., 2003). Conduct disorder (CD), ADHD and maternal and paternal AUD were the most potent predictors for early initiation of alcohol use. Childhood risk factors for risk for progression to alcohol dependence are nicotine dependence, generalized anxiety disorder (GAD), CD, and cannabis abuse (Sartor et al., 2007). Also, in adolescence a drinking pattern characterized by loss of control was associated with increased risk of AUD in young adulthood (Olsson et al., 2016).

Knowledge of risk factors for developing alcohol dependence can be used for identifying groups or individuals at risk. This could facilitate early interventions, but also enable identification of target mechanisms for different treatments and also identify subpopulations for individualized treatment.

## TO SUMMARIZE

In the Western World approx. 90 % of males and 80 % of females use alcohol but only a minor group develops alcohol dependence.

However, as many as 22.7 % of alcohol users have been shown to have a lifetime cumulative risk to develop dependence.

Early debut, other substance use, unemployment, male gender and to never have been married, as well as mental disorder and family history of alcoholism are risk factors for developing AUD.

# THE ALCOHOL USE DISORDER (AUD)

The WHO ICD-10 and the DSM-IV-R (the version available at the time of the study) alcohol dependence definition include a cluster of physiological, behavioral and cognitive variables where the substance of abuse takes priority over other values and where the return to drinking after a period of abstinence (relapse) reinstates the symptoms. The WHO Lexicon of Alcohol and Drug Terms also defines a lapse, or slip, which is an isolated event of excessive drinking. Most patients who have suffered from alcohol dependence will have a life-long vulnerability for relapse (most likely due to neuroadaptive changes). The majority of relapses occur within a year.

## Diagnose criteria

The DSM-5 states that in order for a person to be diagnosed with a disorder due to a substance, they must display 2 of the following 11 symptoms within 12-months:

- Consuming more alcohol or other substance than originally planned.
- Worrying about stopping or consistently failed efforts to control one's use.
- Spending a large amount of time using drugs/alcohol, or doing whatever is needed to obtain them.
- Use of the substance results in failure to "fulfill major role obligations" such as at home, work, or school.
- "Craving" the substance (alcohol or drug).
- Continuing the use of a substance despite health problems caused or worsened by it. This can be in the domain of mental health (psychological problems may include depressed mood, sleep-disturbance, anxiety, or "blackouts") or physical health.
- Continuing the use of a substance despite its having negative effects in relationships with others (for example, using even though it leads to fights or despite people's objecting to it).
- Repeated use of the substance in a dangerous situation (for example, when having to operate heavy machinery, when driving a car).
- Giving up or reducing activities in a person's life because of the drug/alcohol use.
- Building up a tolerance to the alcohol or drug. Tolerance is defined by the DSM-5 as "either needing to use noticeably larger amounts over time to get the desired effect or noticing less of an effect over time after repeated use of the same amount."
- Experiencing withdrawal symptoms after stopping use. Withdrawal symptoms typically include, according to the DSM-5: "anxiety, irritability, fatigue, nausea/vomiting, hand tremor or seizure in the case of alcohol."

## The AUD diagnose and heavy drinking

In an estimation of the 1-year prevalence of alcohol dependence in EU (including Iceland Norway and Switzerland) by Rehm et.al.in 2010, the numbers reached 3.4 % among persons 18-64 years of age (1.7 % women and 5.2 % men). This roughly results in 11 million afflicted. When also including all ages, abuse and harmful drinking the figure is close to 23 million affected. Prevalence varies among countries, but the prevalence of AUD seems to be stable between 2000 and 2010s, or shows just a slight increase in both genders. Heavy drinking greatly exceeds alcohol dependence diagnosis in all countries with available statistics, for men in the EU-region the ratio were 2.7 times and for women 4.9 times. Nordic countries showed the lowest difference of 1.9 times and southern countries the highest of 8.1 times, maybe illustrating cultural differences in the perception of heavy drinking and the pattern and beverages drunk in different countries. Also loss of control is viewed differently in different cultures. Numbers may be underestimated as they are based on general population and not including marginalized groups and populations with higher risks. For example, incarcerated and homeless people have a higher prevalence of AUD. Heavy drinking is not included in the AUD diagnose, and since heavy drinking has so much higher numbers, this might be a better indicator of public health (Rehm et al., 2011).

In the “International guide for monitoring alcohol consumption and related harm” different levels of risk consumption is defined for a single day for acute problems and for levels for chronic use (Table 1) (WHO, 2000).

**Table 1 *Risk intake of alcohol in grams***

***Single occasion risk,  
daily intake***

	<b>males</b>	<b>females</b>
<i>Low risk</i>	1-40 g	1-20 g
<i>Medium risk</i>	41-60 g	21-40 g
<i>High risk</i>	61-100 g	41-60 g
<i>Very high risk</i>	101+ g	61+ g
<b><i>Chronic consumption risk, daily intake</i></b>		
	<b>males</b>	<b>females</b>
<i>Low risk</i>	1-40 g	1-20 g
<i>Medium risk</i>	41-60 g	21-40 g
<i>High risk</i>	61+ g	41+ g

The approximate alcohol content of common beverages is 13 grams of alcohol in a bottle of beer (33 cl, 4.5 %), in a glass of wine (15 cl, 12 %) and in a shot of liquor of 40 % (please see section for calculation formulas).

Daily consumption limit should actually be as low as 7 g pure alcohol, as risk for e.g. cancer appears at very low doses. For some groups, e.g. pregnant women and patients with liver diseases no level of alcohol intake is free of risk (EMA, 2010).

There are several evidence-based society measures available to lower alcohol consumption on a population level. Prices (e.g. taxes) on alcohol are among the most effective, but also restrictions in availability (e.g. sales restrictions, age limits and prohibited to be intoxicated in public places) and drinking-driving countermeasures are effective (Room et al., 2005).

## TO SUMMARIZE

Alcohol use disorder (AUD) is diagnosed with DSM or ICD international classification systems.

Approximately 11 million people suffer from AUD in the EU and individuals with risk consumption greatly exceed this number.

The approximate alcohol content of common beverages is 13 grams of alcohol in a bottle of beer (33 cl, 4.5 %), in a glass of wine (15 cl, 12 %) and in a shot of liquor (4 cl, 40 %).

The risk-limit to avoid cancer is 7 grams of pure alcohol a day.

High risk drinking levels are considered to be daily intake of over 40 grams for women and over 60 grams for men.

Increased taxes, restriction in availability, and drink-driving legislations, lower alcohol consumption in a population.



# SUBTYPING OF A HETEROGENEOUS DISEASE

Alcohol dependence is a heterogeneous disease and examining the endophenotypes could help in developing effective strategies for treatments. There have been many endeavors to identify different phenotypes of alcoholism (McBride et al., 2004).

In 1960 Jellinek et al described a “delta” and a “gamma” type of alcoholism, based on drinking severity and psychopathology (Jellinek, 1960). In the 80s Cloninger and co-workers launched the type I and type II classification, where type I has a greater environmental component and type II is considered to be more inheritable and associated with heavier drinking and more antisocial behavior as well as debut at an earlier age (Cloninger, 1987, Gilligan et al., 1987). A somewhat similar classification is the type A and type B definition, where type B is characterized mainly by severity, age of onset and prognosis (Babor et al., 1992, Schuckit et al., 1995). The early onset alcoholism (EOA) is suggested to be a proxy for inherited biological vulnerability (Johnson, 2000b, Johnson et al., 2000a). Such a proxy can be used to phenotype patients a priori, instead of doing a post hoc sorting (McBride et al., 2004). The EOA and the late onset alcoholism (LOA) seem to be equivalent to Cloninger’s type I and type II as well as Babor’s Type A and type B (Johnson, 2000b, Johnson et al., 2000a, Schuckit, 2000, Schuckit et al., 1995). Another attempt for classification is the Lesch Typology, consisting of four subtypes based on drinking pattern and origin of craving (craving caused by 1, alcohol 2, stress 3, mood and 4, compulsion) The Lesch typology is thought to be of guidance in the psychosocial therapeutic approaches to maintain abstinence (Lesch et al., 1988, Lesch et al., 1990, Schlaff et al., 2011).

Factors associated as markers for individuals at increased risk to become dependent on alcohol are called trait markers (in contrast to state markers that are related to recent drinking, please see section Biomarkers). Trait markers can help identify individuals with vulnerability to develop AUD. A trait marker should be present before AUD development and found to be associated with AUD. Ideally, trait markers should also be high in sensitivity and specificity and the assessments should be non-invasive and cost-effective (Schuckit, 1986). Identifying trait markers could help in early diagnosis, in predicting disease progress, in preventing escalating into illegal substances, in sub-typing different phenotypes, as well as helping in choice of treatment and treatment goal (low alcohol vs no alcohol) (Mann et al., 2009).

The heritability of alcohol dependence, i.e. the combined genetic risk of developing AUD, has been studied e.g. in twin studies (Pickens et al., 1991, Kendler et al., 1992, Kendler et al., 2007, Heath et al., 1997) and is estimated to explain 50-60 % of disease risk (Schuckit, 2009, Heath et al., 1999, Viken et al., 2003) and as high as 73 % in early onset men (McGue et al., 1992). In terms of Family History of Alcoholism (FHP/FHN), a proxy for the hereditary risk of AUD, as much as 80 % of AUD afflicted individuals also have family members who are afflicted (Schuckit, 2009, Heath et al., 1999, Viken et al., 2003).

Personality traits associated with excessive alcohol consumption are impulsivity and disinhibition, novelty seeking, harm avoidance and reward dependence, which are all predictors of a later AUD (Cloninger et al., 1988).

The response to alcohol is shown to be heritable and may predict later alcohol problems. Low response on certain variables as feeling intoxicated, body swaying and sedation has been shown to be more common in children of alcoholics (COAs). Studies show 40 % of COAs having low response (LR) (Schuckit et al., 1996). Those who need more alcohol to gain the pleasures, are at risk for heavy drinking (Enoch, 2003), e.g. Native Americans and Koreans have a high rate of LR and also a high incidence of AUD (Ehlers et al., 1999). In children of alcoholics the LR was correlated with a 5-year alcohol-related outcome ( $r = 0.48$ ), mediated both directly and indirectly by heavier drinking peers, expectations and stress-coping drinking (Schuckit et al., 2012).

Endeavors have been made to identify risk gene loci for AUD in genom-wide association studies (GWAS), but as of yet the individual loci identified only explain a very small part of the difference in risk. Very large samples are needed to detect the small effects of individual gene loci. Cluster analyses, phenotype based analysis and e.g. consumption level based analysis could improve statistical power in studies relating specific genes to risk of disease (Hart and Kranzler, 2015). When combining very many single nucleotide polymorphisms (SNPs) as much as 23% of the phenotype variance could be explained (Yang et al., 2014). Otherwise the strongest association demonstrated so far is between AUD and the alcohol dehydrogenase (ADH) gene cluster (Park et al., 2013).

One approach to the genetic component of AUD is to develop personalized treatment strategies. Genetic variations seem to influence the effect of several pharmacotherapies for alcohol dependence (Jones et al., 2015, Enoch, 2014). One candidate is the Asn 40Asp SNP of the my-opioid receptor gene OPRM1, which has been associated with heavy drinking and may predict treatment outcome from naltrexone (Oslin et al., 2003). However, the findings are contradictory and need to be replicated (Jones et al., 2015). Other possible candidates are the GATA4 polymorphism, that may moderate acamprosate treatment response (Kiefer et al., 2011), the GRIK1 polymorphism, that may moderate topiramate treatment

(Kranzler et al., 2014) and the 5-HTTLPR and DRD4 polymorphisms that may moderate ondansetron and sertraline treatment response (Kenna et al., 2014).

Other trait markers for endophenotyping could be increased basal activity of the serotonin transporter in platelets and increased responsiveness of the pituitary beta-endorphin system to alcohol challenge (Ratsma et al., 2002). Apart from pharmacologically based examinations, also neuroimaging and EEG could be used as objective trait markers.

Chronic alcohol intake is related to volume reduction in white and grey matter and disruption of white matter tracts (Buhler and Mann, 2011). Behavioral problems such as externalizing disorders, anxiety disorders, mood-disorders and substance abuse can all be related to structural changes seen in amygdala, hippocampus, nucleus accumbens, putamen and thalamus (Wrase et al., 2008, Benegal et al., 2007, Makris et al., 2008).

Electroencephalogram (EEG) changes in alcoholics and COA, associated with GABA<sub>A</sub> receptor gene on chromosome 4 (GABRA2) seem to be highly hereditary (Dick et al., 2006, Edenberg et al., 2004) and could be associated with the disinhibition seen in some alcohol dependent individuals (Begleiter and Porjesz, 1999, Rodriguez Holguin et al., 1999). The P300 waveform (P3) of the event related potential is associated with cognitive function and could be related to disinhibition. Alcohol individuals have been shown to have reduced P3 amplitude and children of alcoholics with low P3 are at higher risk for AUD (Porjesz et al., 1998).

Alcohol metabolism also has genetic influences in peak blood alcohol concentrations and the rate of elimination of alcohol (Babor et al., 1992). There is a 2-3 fold increased risk for AUD for the highest quartile compared to lowest quartile of BAC, suggesting that alcohol metabolism actually influences vulnerability to develop AUD (Whitfield et al., 2001).

Strong desire to consume alcohol in association with loss of control, an important component of chronic high alcohol consumption, also seems to be, at least partly, genetically predisposed. The long variant of the D4 dopamine receptor gene (DRD4 VNTR) (7 or longer than 7 repeated alleles), was associated with higher craving after alcohol consumption (Hutchison et al., 2002). A clinical trial on olanzapine showed greater reduction in craving and consumption during treatment in individuals with a long allele of DRD4 VNTR (Hutchison et al., 2003).

The D4 dopamine receptor is linked to ADHD, schizophrenia and AUD and polymorphisms of serotonin transporter genes are associated with AUD, anxiety and depression. Co-morbidity is a risk factor for AUD and there might be common genes as well as shared risk factors (Maxwell, 2013).

Increased activity of endogenous opioids, peptides binding to opioid receptors, may be influential in initiating and maintaining excessive alcohol drinking (Gianoulakis et al., 2003). Early morning levels of plasma beta-endorphin were shown to be lower in FHP individuals, which also displayed a larger increase after ingestion of alcohol. This could be a part of the hereditary response to alcohol, and part of the explanation to the difference in individual response to pharmacotherapies, e.g. naltrexone (Froehlich et al., 2000).

Another pathway of interest is that of Adenylyl cyclase (AC), which turns adenosine 5'-triphosphate (ATP) in to the second messenger cAMP. AC activity is regulated by among others dopamine, opiate, adenosine, adrenergic, serotonergic and muscarinic cholinergic receptors. Many drugs produce their effects by stimulation or inhibition of the AC activity, which is also altered by acute and chronic alcohol exposure (Tabakoff and Hoffman, 1998).

## TO SUMMARIZE

Alcohol use disorder (AUD) is a heterogeneous disease.

AUD can be subtyped according to several classifications; delta and gamma type, type I and Type II, type A and type B, Early Onset Alcoholism (EOA) and Late Onset Alcoholism (LOA) and the Lesch typology.

Trait markers, a heritable marker associated with AUD, can help identify individuals at high risk to develop the disorder.

Heredity is thought to explain 50-60 % of the risk of developing AUD.

Impulsivity, disinhibition, harm avoidance and reward dependency are personality traits associated with AUD, as is low response to alcohol, e.g. to need more alcohol to feel intoxicated.

Several genes with correlations to AUD have been identified, but explain only a small portion of the difference in risk between individuals.

Genes have also been proposed to influence treatment responses to pharmacotherapies.

Neuroimaging and brain waves measured with e.g. EEG could also be possible trait markers.

# ALCOHOL AND THE REWARD PATHWAY

Dopamine is a neurotransmitter in the brain involved in the feeling of euphoria in response to natural rewards as e.g. sex and food intake. Dopamine is also released in response to drugs of abuse, creating a strong feeling of euphoria, and well-being.

The dopamine system spans different brain regions including the mesolimbic and mesocortical brain regions, the ventral tegmental area (VTA), the medial forebrain bundle, nucleus accumbens (nAc) (Dahlstrom and Fuxe, 1964, Koob et al., 1994), amygdala, the extended amygdala and hippocampus (Oades and Halliday, 1987). The system is regulated and modulated through neurons and transmitter substances in the VTA and the nAc, and dopaminergic neurons display both pace maker and burst firing activity (Charley et al., 1991). Numerous ligand-gated and G-protein coupled receptors are expressed in the VTA and nAc, e.g. the NMDA receptor (Thomson, 1989), GABA<sub>A</sub> receptors (Zetterstrom and Fillenz, 1990), nAChR (Nisell et al., 1994, Faure et al., 2014), GlyR (Molander and Soderpalm, 2005), NA receptors (Jones et al., 1985), 5HT<sub>3</sub> receptors (Pazos and Palacios, 1985), and dopamine receptors D1 and D2. GABA-ergic and glutamatergic inputs serve as regulators of the dopaminergic neuronal activity (Beckstead, 1979, Walaas and Fonnum, 1980, Zahm, 1989).

Most drugs of abuse, including alcohol, act on the brain catecholamine systems (Engel and Carlsson, 1977) and have in common that they increase dopamine (DA) levels in the reward neurocircuits and that this action is involved in their reinforcing effects (Di Chiara and Imperato, 1985, Imperato and Di Chiara, 1986, Di Chiara, 2000, Koob, 1992, Faure et al., 2014, Ostroumov et al., 2015, Volkow and Morales, 2015, Soderpalm et al., 2009).

Long-term drug exposure may lead to modulation in receptor numbers, affinity, and responsivity, as well as neurotransmitter synthesis, release, and reuptake. These alterations involve changes in gene transcription and protein expression (Rossetti et al., 1992, Nestler, 2004, Nestler, 1994, Nestler, 1993, Weiss et al., 1996). Chronic alcohol consumption is associated with long-lasting changes in these systems, with changed reactions to both drug reward and non-drug rewards (Volkow and Morales, 2015, Trantham-Davidson and Chandler, 2015, Camarini and Pautassi, 2016).

One of the main criteria for AUD is increased tolerance to alcohol, this is mainly considered a neuronal adaptation (and to a lesser extent changed metabolism of ethanol). Another example of adaptation is sensitization, a paradoxical heightened response to alcohol or other drugs of abuse, after long-term, and especially intermittent, exposure (Kalivas and Stewart, 1991, Kalivas, 1993).

In response to alcohol, dopamine release is increased in several brain regions. Some of the changes are proposed to involve dysregulation of the mesolimbic and mesocortical dopamine systems with e.g. changes in dopamine release in the prefrontal cortex (Trantham-Davidson and Chandler, 2015), but especially the ventral tegmental area (VTA) and projecting dopamine neurons from here to the nAc are believed to play an important part in alcohol-related reward (Volkow and Morales, 2015, Soderpalm et al., 2009). For over four decades dopamine has been viewed one of the most important factors in the development of addiction (Nutt et al., 2015), and to be a key transmitter in reward and reinforcement (Engel and Carlsson, 1977, Wise, 1978). Ethanol increases the firing and burst activity of dopaminergic neurons (Gessa et al., 1985) resulting in dopamine release in the nAc (Imperato and Di Chiara, 1986, Boileau et al., 2003). The modulation of the mesolimbic dopamine levels in nAc is thought to have a crucial role in addiction (Engel et al., 1992, Koob et al., 1994). Thus, increase in limbic and accumbal dopamine is suggested to be a key factor in developing drug dependence (Engel and Carlsson, 1977), and the subsequent decrease in dopamine observed during long-term abstinence may be involved in craving mechanisms (Volkow and Morales, 2015, Koob and Le Moal, 1997).

It is established that alcohol releases dopamine (DA) in the nAc (Nutt et al., 2015, Soderpalm et al., 2009, Soderpalm and Ericson, 2013, Di Chiara, 1997). This increase is closely correlated with blood levels of alcohol and DA seems to increase also in response to alcohol anticipation and could hence be part of the mechanisms of relapse (Soderpalm et al., 2009). The DA signal is thought to be involved both in the positive and the negative reinforcement caused by alcohol consumption, and probably is the driving force in alcohol dependence. After chronic alcohol use, the baseline levels of DA are lowered, however, the dopamine elevation in response to alcohol intake remains high and the discrepancy between baseline level and the peak of DA may actually be larger (Diana et al., 1993). This could explain why alcohol dependent subjects still experience stimulation and euphoria even after long use of alcohol. Thus, the ethanol-induced DA elevation seems to reach the same absolute levels after an acute administration, whether earlier exposed to alcohol or not. The explanation could be that alcohol acts on the mesolimbic DA activity by lifting a brake on the system. After chronic exposure, the brake becomes stronger, resulting in reduced baseline DA levels. However, when the brake is removed, the release of DA is increased, resulting in the same absolute DA levels (Soderpalm and Ericson, 2013).

However, it should be kept in mind that alcohol exerts multiple actions on several neurotransmitter systems in the central nervous system (CNS) and is sometimes described as a “dirty drug” or a “disorganizer”. In different pathways alcohol acts either inhibitory or excitatory. Via the gabaergic (GABA) and glutamatergic (glutamate) pathways alcohol has an effect on sedation, relaxation, sleep-wake

regulations and cognitive/attention functions. In the opioid and serotonergic pathways alcohol influences pleasure, alcohol-seeking and mood (Costardi et al., 2015).

Alcohol has been shown to primarily interact with several receptors, e.g. the cysteine-loop ligand-gated ion channels, such as nicotinic acetylcholine receptors (nAChR), glycine receptors (GlyR), GABA<sub>A</sub> receptors and 5-HT<sub>3</sub> receptors.

The nAChR is a five subunit protein complex situated in the neuronal cell membrane. The nature of the ethanol interaction is determined by the subunit composition of the receptor, with suggested involvement of the  $\alpha_4\beta_2$ ,  $\alpha_3\beta_4$  (Arias et al., 2015, Chatterjee et al., 2011),  $\alpha_6$  and  $\beta_3$  (Hendrickson et al., 2010) and  $\alpha_7$  subunits (Crunelle et al., 2010). Alcohol is thought to potentiate the acetylcholine interaction with the receptor without activating the receptor in itself. Alcohol-induced mesolimbic dopamine activation involving nAChRs in the VTA seems to be secondary to GlyR activation in the nAc. It has been proposed that nAChRs are involved both in alcohol's pharmacological effects and in the dopamine release produced by conditioning (the learned response to alcohol's effects and subsequently the response to anticipation of alcohol intake) (Soderpalm et al., 2009, Lof et al., 2007).

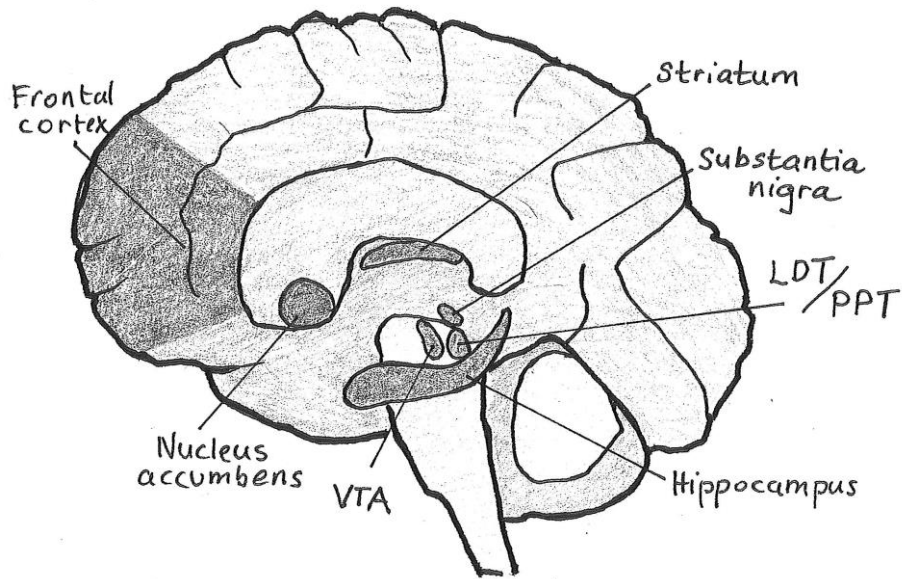
The GlyR is a membrane protein complex composed of five subunits, present in various forms in nAc (Soderpalm et al., 2009). The effects of ethanol on the GlyR could be explained by both direct and indirect interactions with the receptor (Ericson et al., 2013). Modulation of the GlyR is shown to reduce alcohol consumption in animal experiments (Molander et al., 2007, Molander et al., 2005). Expression and regulation of the GlyR in nAc could be associated with the development of AUD where potentiation should result in decreased intake (Burgos et al., 2015). Alcohol facilitates the function of the receptor, and its inhibitory effects in the CNS are potentiated by alcohol (Burgos et al., 2015, Soderpalm et al., 2009).

The dopamine increase due to alcohol has been proposed to involve backward projecting breaks into the VTA, involving GlyRs in the nAc and nAChRs in the VTA. It could be argued that chronic exposure to alcohol could result in a down-regulation of the receptors due to adaptation, which would likely cause a lowered baseline DA activity. It is of importance to further investigate the exact alterations of the neurocircuitry underlying the reduction of baseline DA levels observed after chronic exposure, as this could reveal targets for new pharmacotherapies aimed at reducing alcohol craving and intake (Soderpalm and Ericson, 2013).

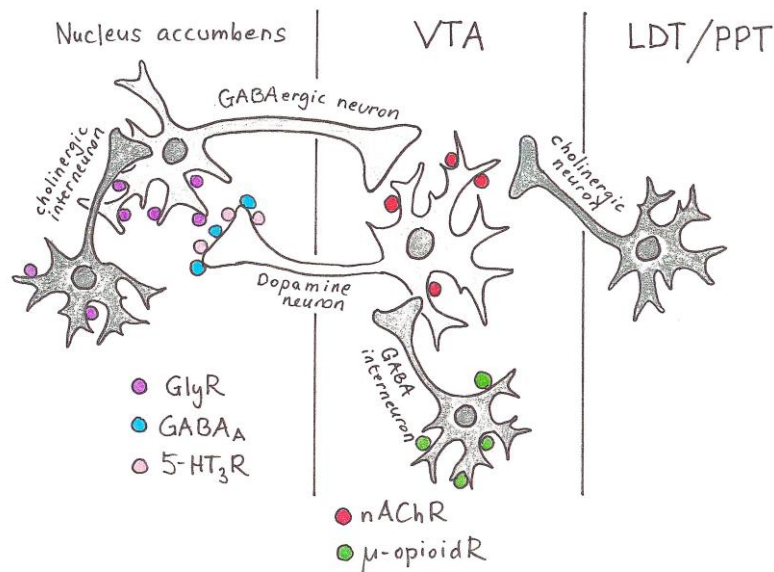
Other mechanisms implied in alcohol-induced DA activity are  $\mu$ -opioid receptors, and NMDA-receptors. Their exact involvement and their internal relationships, however, still are to be revealed. Opiates, for example, both enhance DA release and directly reduce neuronal activity in the nAc. If this is also the case for alcohol, maybe the inhibition of neuronal activity could act via GlyR, present in the nAc. If this inhibition is actually more important for the reward experienced than the DA release, this could explain alcohol's strong rewarding effect in relation to its relatively modest dopamine releasing effects (Soderpalm and Ericson, 2013).



**Figure 1** *Regions in the human brain involved in the effects of alcohol*



**Figure 2** *Receptors involved in mediating the effects of ethanol*



Simplified figure to illustrate the mesolimbic dopamine structure and the receptors involved in the ethanol induced activation. (VTA: ventral tegmental area, LTD: lateral tegmental nucleus, PPT: pedunculo-pontine nucleus, GlyR: glycine receptor, GABA<sub>A</sub>: GABA receptor type A, 5-HT<sub>3</sub>R: serotonin receptor type 3, nAChR: nicotinic acetylcholine receptor, μ-opioid R: opioid receptor of type μ). Adapted from Söderpalm and Ericson. 2013.

## TO SUMMARIZE

Dopamine is a brain neurotransmitter involved in the feeling of euphoria in response to natural rewards as e.g. sex, and food intake.

The dopamine system reaches through many brain regions and is called the "Reward Pathway".

Drugs of dependence release dopamine, resulting in the euphoric feeling of intoxication, via the reward circuits.

Long-term alcohol use may change the way receptors respond to the drug, leading to tolerance and/or sensitization. The effects of these modulations are that a person can drink more before signs of intoxication are obvious, but the system remains sensitive to the rewarding effects of the drug.

Tolerance and sensitization are important components in the development of alcohol dependence, as is the feeling of reward and the craving for more alcohol.

Alcohol use results in a lower level of dopamine in the system when no alcohol is present, but the rise in response to alcohol remains high. This could be explained by alcohol lifting a break on the dopamine system, allowing dopamine release. After long exposure of alcohol, the break becomes stronger and less baseline dopamine is released, but when alcohol is consumed and the break is lifted, even more dopamine is released.

Alcohol has an effect on many systems in the brain, and several receptors are suggested to be involved in the reward neurocircuits. Also, these receptors could be targets for pharmacotherapies of alcohol dependence.

Some of the receptors discussed are nicotinic acetylcholine receptors (nAChR), glycine receptors (GlyR), and 5-HT<sub>3</sub> receptors.

# TREATMENTS

## History of treatments

The negative consequences of excessive alcohol intake have been known for as long as alcohol has been consumed. The ancient Mesopotamian civilization of the Sumerians made the connection between alcohol and birth defects and Hippocrates described symptoms of abstinence (Sternebring, 2016). In the eleventh century, an observant physician practicing in Constantinople reported that drinking wine in excess caused inflammation of the liver (Hanson, 2013).

In America, Anthony Benezet's "Mighty destroyer" published in 1774, is one of the first known essays on the topic, and a decade later Dr. Benjamin Rush catalogued the consequences of high alcohol consumption and argued that alcoholism was a disease that should be treated by physicians (White, 2014). In 1849 the Swedish physician Magnus Huss described the disease of chronic alcohol consumption and named it *Alcoholismus Chronicus* and the term alcoholism was born. The first alcohol institution in Sweden was established in 1885 and the compulsory care act was passed in 1913 (Presjtjan, 2004). In the "Textbook of Alcoholology" ("Lärobok i Alkoholologi" med. Dr. Henrik Berg, 1904) the treatments of chronic alcoholism are described. Formerly the intentions had been to wean slowly, by day by day lowering the alcohol consumed, but now the only correct method was considered to be the "safe and less painful" immediate cessation of alcohol (delirium, however, well known). The cure was performed in an institution and the first day a "wet bandage/pack" was prescribed and at night "kloral" was given for insomnia. The food intake should be coffee, water with lemon, and grain soup, until the patient could leave the bed for meals. After a fortnight the patient could be introduced to a lighter chore. No money was allowed at the institution and the alcoholic spent from 6-12 months inward. Time and hard labor was part of the recovery. Only patients with hope of recovery were accepted and  $\frac{1}{2}$  to  $\frac{2}{3}$  were said to be cured (defined by sustained abstinence). Two institutes were available; "Sans Souci" and "Eolshäll". Alcoholism was considered a disease contracted by intake of liquor (Berg, 1904).

## Less effective experimental treatments

In the early 1900s some scientist had a theory about addicted patients building up antibodies and they decided to create a vaccine called “Equisine” by administering alcohol to horses and then administering their blood to other horses. They claimed the horses receiving the blood did not drink alcohol. A company tried to isolate antibodies and apply to skin cuts on addicts, without success.

Another cure was “The Keely cure” in the late 1800s. The cure included 31 days in a treatment center with injections and tonics of “double chloride gold”. The tonics did in fact contain; coca, morphine, arsenic, strychnine, and extract of nightshade. Dr. Keely claimed a 95 % success rate but soon side effects, like death and insanity, appeared.

Another very common tonic in the late 1800s was “Laudanum”, containing morphine. This tonic was used for many ailments, including on teething children, and a Dr. JR Black, suggested to transfer the alcohol dependence of chronic alcoholics to morphine dependence.

Another attempt to reduce incidence of alcohol dependence in the early 1900s was hot air boxes simulating equatorial weather.

Dysfunction of the endocrine systems became a theory in the 1940-50s and injecting of adrenocorticotrophic hormones was tried. Some results of faster detoxification were reported.

By the 1950s, most of the treatments were handled by prisons and asylums and in the Colorado state penitentiary the “Serum therapy” was proposed. Blisters were created on the addict’s stomach and then fluids were removed by a hypodermic needle and reinjected in the arm of the addict. This was repeated four or five times a day for a week.

In the mid-1900s, LSD and psilocybin was used in psychiatric diseases and also for alcoholism. Actually, a recent meta-analysis from University of science and technology in Trondheim indeed showed that 59 % of the LSD treated patients reported less abuse afterwards, compared to 38 % of patients receiving placebo.

Some very serious abusive procedures were performed on alcohol disease afflicted persons. In 1922, fifteen US states had passed laws on sterilization of institutionalized (in particular female) alcoholics. And between 1948 and 1952, nine cases of frontal lobotomy on the grounds of alcohol addiction were reported in the US.

(Detwiler, 2016, White, 2014)

In the 1930s psychotherapy was the most common approach, however, experiments were conducted with aversive therapy (Zindel and Kranzler, 2014). For example, emetic agents were added to the alcohol and in other attempts patients were injected with apomorphine while consuming alcohol (Zindel and Kranzler, 2014). Also alcoholics were encouraged to drink their favorite drink in a room full of mirrors, and then vomiting was induced using either emetics or electric shocks (Detwiler, 2016).

Experiments were also performed with amphetamine, where reduced desire to drink was reported after administration (Zindel and Kranzler, 2014).

However, by 1935, Alcoholics Anonymous (AA) was organized to address alcoholism and has since spread to about 190 countries around the world. And in the 1940-ies disulfiram was introduced as the first pharmacological treatment (White, 2014).

## TO SUMMARIZE

The harmful effects of alcohol have been known for thousands of years.

Alcoholism was argued a disease in the late 1700s.

In 1849 the Swedish physician Magnus Huss described and named "Alcoholismus Chronicus".

In the turn of the 1900 century alcoholics were treated in institutions.

From the late 1800s up until the 1950-ies, when Alcoholics Anonymous (AA) and disulfiram became established as treatments, alcoholics were exposed to less effective and sometimes invasive treatments.

## Treatments of today

According to European Medicines Agency (EMA), the treatment goal is the achievement of abstinence, reduction in frequency and severity of relapse, and improvement in health and psychosocial functioning. In the present, there are three pharmacotherapies for alcohol dependence approved by both EMA and the Food and Drug Administration (FDA). These are disulfiram, acamprosate and naltrexone (Hughes and Cook, 1997, Kranzler and Van Kirk, 2001). In addition, nalmefene is approved in Europe (Gual et al., 2013, Mann et al., 2013). Several other potential pharmacotherapies are being investigated in both pre-clinical and clinical research, but are of yet not approved for treatment.

## Withdrawal treatments

Withdrawal symptoms occur 4-12 hours after alcohol cessation or reduction, most often the second day of detoxification is the most crucial, with possible severe and potentially deadly complications of seizures, hallucinations, and hyperthermia (DT). Patients are treated with protocolized administration of cross-tolerant agents, typically benzodiazepine, for 5-7 days, possibly in combination with phenobarbital (Schmidt et al., 2016). Also, it is important to treat a possible thiamine deficiency (de la Monte and Kril, 2014, Vedder et al., 2015).

## Disulfiram

The anti-alcohol effect of disulfiram was discovered by serendipity in the 1940s, when Hald & Jacobsen consumed alcohol after an experimental intake of disulfiram. Disulfiram was aimed at treating infections with intestinal worms, but were soon suggested as a therapy for alcohol dependence. Disulfiram inhibits the liver enzyme aldehyde dehydrogenase and a simultaneous alcohol intake results in an accumulation of acetaldehyde, causing tachycardia, facial flushing, nausea, vomiting, hypertension and, rarely, cardiovascular collapse. The drug hence acts as an aversion therapy, due to the unpleasant symptoms, and the treatment needs to be combined with the absolute abstinence goal (Hughes and Cook, 1997). In a meta-analysis on the efficacy of disulfiram, only 11 randomized trials were considered to be of enough quality to be included. Six studies showed a significantly better effect of disulfiram on abstinence when compared to placebo, none, or other treatments (Jorgensen et al., 2011). A retrospective study showed long-term supervised disulfiram treatment to be more effective than acamprosate, in particular when patients had been suffering from dependence for a long time (Diehl et al., 2010). Also, a comparative trial proved supervised disulfiram treatment superior to

acamprosate and naltrexone treatments on several accounts, although all groups reduced their alcohol intake (Laaksonen et al., 2008). Probably disulfiram is under-prescribed both in US and Europe (reports of less than 10 % of alcohol dependent patients receiving the treatment), perhaps due to its hepatotoxicity, although it is extremely rare with side effects leading to deaths due to disulfiram treatment (Diehl et al., 2010).

## **Naltrexone and acamprosate**

**Naltrexone**, an un-selective opioid receptor antagonist, was first approved for treatment of opioid dependence in the 80s. In 1994 it was approved for alcohol dependence treatment (Volpicelli et al., 1992, Zindel and Kranzler, 2014). Its mechanisms are not fully understood, but it may produce its effects by blocking alcohol-induced dopamine activity via blockade of endogenous opioids, triggered by alcohol consumption, thereby reducing the rewarding effects of alcohol. Its main effect should therefore be on craving and reducing heavy drinking, but not in maintaining abstinence (Sinclair, 2001, Spagnolo et al., 2014).

It has also been proposed that naltrexone suppresses craving via activation of the hypothalamo-pituitary-adrenocortical axis (O'Malley et al., 2002). The effect of naltrexone on craving and the stimulatory effect of alcohol seem to be modulated by age, gender and family history of AUD, although heavy drinking was reduced in all subjects (Tidey et al., 2008). There are also possible genetic differences in the treatment response caused by the OPRM1, however the modulation is thought to be modest (Ziauddeen et al., 2016). A tolerance to the effects on alcohol intake has been shown in animal models (Korpi et al., 2016).

**Acamprosate**, a synthetic derivate of homotaurine and related to taurine (Sinclair et al., 2016, Plosker, 2015), was approved for treatment of alcohol dependence in 2004 (Zindel and Kranzler, 2014). Acamprosate is proposed to be an NMDA receptor modulator acting on the glutamate system and is thought to promote abstinence by restoring the imbalance between the excitatory and inhibitory neurotransmitters, glutamate and GABA (Plosker, 2015, Chau et al., 2010, Witkiewitz et al., 2012a). Acamprosate has also been suggested to interact with accumbal GlyRs primarily and secondarily act on nAChRs in the ventral tegmental (Chau et al., 2010), thereby mimicking the effect of alcohol in the nAc. Indeed, the alcohol intake reducing effect of acamprosate can be reversed by blocking of GlyR bilaterally in the nAc (Chau et al., 2010). It has been noted that tolerance develops rather rapidly to the anti-alcohol effect of acamprosate (Lido et al., 2012). Acamprosate is believed to have its main effect on maintaining abstinence rather

than reducing craving and heavy drinking days. Unlike disulfiram and naltrexone, acamprosate is not metabolized in the liver and thereby more suitable to patients with liver conditions (Witkiewitz et al., 2012b).

In a meta-analysis of RCTs between 1970 and 2009 on **oral naltrexone and oral acamprosate**, including 5434 subjects, both naltrexone and acamprosate seem to have best effect if the patient starts treatment after some four days of abstinence. Overall acamprosate had an effect size of Hedges  $g$  correlation= 0.325,  $p < 0.001$  while naltrexone had a  $g$  of 0.160,  $p < 0.001$  (difference outcome  $p = 0.010$ ). However, by separated outcomes, acamprosate had a larger effect size on abstinence while naltrexone had a larger effect size on heavy drinking and craving. Also the goal of treatment seems to have an effect on treatment efficacy where the goal of abstinence benefits the acamprosate treatment. No difference was found on treatment length and little difference between 50 and 100 mg of naltrexone, rather a bit better with the lower dose. Acamprosate seems to do best on “dose by weight” regime (Maisel et al., 2013).

The aggregated Hedges’  $g$  for both treatments were 0.209, small but significantly superior to placebo. The numbers needed to treat (NNT) for acamprosate on abstinence were 7.5 and NNT for naltrexone on preventing return to heavy drinking was 8.6 (Maisel et al., 2013). While a Cochrane review of opioid antagonists for alcohol dependence concludes an NNT of 13 for short-term treatment effect of naltrexone and a relapse risk reduction of 36 % (Rosner et al., 2010).

A meta-analysis from 2014, looking at possible differences between RCT countries in the effect of acamprosate and naltrexone on lapse/relapse compared to placebo, included 22 RCTs on acamprosate and 27 RCTs on naltrexone. The risk for returning to any drinking after 6 months was significantly lower for acamprosate (relative risk (RR) 0.83). For naltrexone there was a reduced risk for returning to any drinking after 3 months (RR 0.92) and reduced risk for heavy drinking (RR 0.85). No between-country differences were identified (Donoghue et al., 2015).



## **Nalmefene and as-needed nalmefene**

Nalmefene is a  $\mu$ -opioid antagonist used as antidote to opioid overdose and to reverse anesthesia. The difference from naltrexone is a methylene group instead of a ketone group at the 6<sup>th</sup> carbon. Nalmefene is partial agonist at the kappa receptor whereas naltrexone is an antagonist. Also, naltrexone has a risk of liver toxicity (although very rare) whereas nalmefene does not. In comparison these two have a minimal difference in efficacy (Swift, 2013).

As-needed nalmefene represents a new treatment paradigm as to treatment goal, dosing and to reach the in many cases untreated population not needing detox or in-ward hospitalization but still in need of reducing their consumption. In RCTs (N=604), mean no of HDD decreased from 19 to 8 days per months and mean consumption in grams decreased from 84 to 33 g per day. Compared to the placebo group, who lowered their consumption from 20 to 11 days per months and decreased from 85 to 45 g per day, the superior effects of nalmefene was statistically significant (HDD  $p=0.021$ , and g per day  $p=0.0003$ ) (Mann et al., 2013).

However, the approval of nalmefene was controversial, as the evidence was considered weak. In a meta-analysis there was no evidence of efficacy of nalmefene on health outcomes and only a slight effect on reduction of monthly HDD. Further, there was no evidence of harm reduction and little evidence of lowered consumption. The EMA approval of nalmefene is for the specific population of alcohol dependent adults consuming more than 60 g per day for men and more than 40 g per day for women. No RCTs were found on this population, only pooled subgroup analyses, which were not defined a priori and hence only possible to regard as exploratory, at the best. Also, no RCTs on nalmefene with another active substance as a comparator were found. Naltrexone on the other hand is not approved for reducing drinking, although this has been shown to be the main effect (Donoghue et al., 2015, Palpacuer et al., 2015).

## **Serotonergic drugs**

Early onset alcoholism (EOA) has been related to a serotonergic dysfunction (Johnson, 2000b, Ait-Daoud and Johnson, 2000). Lower levels of CSF 5-hydroxyindoleacetic acid have been demonstrated in EOAs, especially in those with FHP for alcoholism (Fils-Aime et al., 1996). And there are many studies suggesting impulse control impairment in EOAs potentially due to an impaired function of the 5-HT system (Buydens-Branchey et al., 1989, Linnoila et al., 1989, Virkkunen and Linnoila, 1997, Virkkunen, 1990). Further, there is evidence that the 5-HT<sub>3R</sub> is involved in modulating alcohol consumption and reinforcement (Lovinger, 1999, Machu and Harris, 1994, Liu et al., 2006).

In several RCTs, the 5-HT<sub>3</sub> antagonist ondansetron has been shown to reduce alcohol consumption, especially in subjects with heavier drinking or in EOAs, but not in LOAs (Sellers et al., 1994, Johnson et al., 2000b, Kranzler et al., 2003). Since 5-HT is released by alcohol and since 5-HT<sub>3</sub> receptors may have a role in the rewarding effects of alcohol (Lovinger, 1999), the mechanism for the effects of ondansetron in EOAs may be mediated by a blockade of an expected stimulation of the 5-HT<sub>3</sub>R (Johnson et al., 2002). It has been proposed that EOAs might even have a specific gene variation, rendering them susceptible to the effects of ondansetron (Johnson et al., 2011, Johnson et al., 2000b, Heinz and Goldman, 2000). This could also be an explanation to the different responses to selective serotonergic reuptake inhibitors (SSRIs) found in different subgroups of alcohol dependent subjects. In pre-clinical models and in heavy social drinkers, several SSRIs have been shown to reduce alcohol intake (Naranjo and Sellers, 1989). However, in RCTs the findings have been paradoxical. Fluoxetine had a different effect on type A and type B alcoholism, where type B were actually worsened by the treatment (Kranzler et al., 1996, Kranzler et al., 1995). Sertraline on the other hand showed efficacy in type A alcoholics but not in type B (Pettinati et al., 2000). The same results were seen when age-of-onset subtypes were used, i.e. sertraline was superior to placebo in later-onset individuals but not in early-onset individuals (Kranzler et al., 2012, Kranzler et al., 2011, Pettinati et al., 2013).

## Topiramate

Topiramate was first approved as an anticonvulsant in 1996 (Zindel and Kranzler, 2014). Mechanisms behind its actions may be an interaction with a non-benzodiazepine site on the GABA<sub>A</sub> receptor, facilitating GABAergic transmission. It also may work by antagonizing glutamate activity (Kranzler et al., 2014).

A meta-analysis of 7 RCTs with outcome abstinence, heavy drinking, craving and GGT, showed topiramate to have its largest effect, although moderate in size, on abstinence, followed by heavy drinking and GGT. The effect on craving, however, was not significant. The overall effect size for topiramate ranged from Hedges  $g$  of 0.312 to 0.468 on the three domains abstinence aggregate, heavy drinking aggregate and craving. This exceeded the overall effect size of naltrexone,  $g=0.116$  to 0.189, and acamprosate,  $g=0.034$  to 0.359. Calculated effect size from 3 studies comparing naltrexone and topiramate favored topiramate on all three outcomes, though abstinence aggregate did not reach significance ( $g=0.149$ ,  $p=0.30$ ). The difference in effect size for heavy drinking was  $g=0.284$  ( $p=0.04$ ), and for craving  $g=0.297$  ( $p=0.04$ ). Topiramate was overall well tolerated, with paresthesia, nausea, cognitive impairment, headache, and dizziness as major side effects (Blodgett et al., 2014).

## Baclofen

Baclofen, a GABA<sub>B</sub> receptor agonist, originally developed as a treatment for spasticity, has been proposed as a possible pharmacotherapy for alcohol dependence (Myzuk). GABA<sub>B</sub> is highly expressed in the limbic system and has been implicated in anxiety control (Brennan et al., 2013). A suggested mechanism for baclofen's potential effects on alcohol intake would be by local inhibition of surrounding mesolimbic dopamine neurons (Brennan et al., 2013, Kiefer, 2009). However, reports on the effects on alcohol consumption are inconsistent. Two RCTs, by one team, have shown benefit over placebo regarding abstinence, craving and anxiety (Addolorato et al., 2002), while two other trials have failed to show treatment effect (Garbutt et al., 2010). Additionally a French physician treated himself with high doses, claiming success (Ameisen, 2011), and in France baclofen can be prescribed according to a "temporary recommendation for use" (Gorsane et al., 2012, Brennan et al., 2013). Since the numbers of trials are small and includes small sample sizes, and selected patients, there is yet not enough evidence for baclofen being an effective treatment for alcohol (Brennan et al., 2013, Muzyk et al., 2012, Kiefer, 2009, Gorsane et al., 2012).

## Sodium oxybate

Sodium oxybate is a liquid formulation of the sodium salt of  $\gamma$ -hydroxybutyric acid (GHB). As GHB it is an illegal drug of abuse but as sodium oxybate it has been used for withdrawal treatments for over 20 years in Italy and Austria to prevent severe symptoms as delirium tremens and seizures and for maintenance of abstinence after detoxification (Busardo et al., 2015, Keating, 2014). As of today sodium oxybate is the only pharmacotherapy approved both for alcohol withdrawal syndrome and abstinence maintenance. It is generally well tolerated and at least as effective as benzodiazepines and clomethiazole. The alleviation of symptoms has a rapid onset and in patients with severe alcohol withdrawal syndrome it seems to be even more effective than benzodiazepines for treatment of the withdrawal syndrome (Keating, 2014, Skala et al., 2014). Recent studies on craving have shown divergent results and also the issue of correct dosing has been discussed. Too low doses seem to be ineffective but at the same time sodium oxybate clearly has an addiction potential. Patients that have shown a tendency to develop a craving for sodium oxybate are mostly individuals with an axis II diagnose or a former opiate dependence. Misuse has been reported in 12 % while ca. 80 % underwent successful rehabilitation and 78 % were still abstinent after 6-12 months (Nava, 2013). Approx. 30-40 % does not respond to monotherapy with sodium oxybate and hence a combination with naltrexone has been shown to be superior to either treatment alone (Keating, 2014). A solid form of sodium oxybate is under development.

## Individualized treatment

Recent findings have indicated that treatment response is dependent on polymorphisms in certain genes (Kranzler and McKay, 2012, Enoch, 2014). For example, the effect of topiramate seem to be modulated by the GRIK1 polymorphism (Kranzler et al., 2014), treatment response to acamprosate may be influenced by GATA4 polymorphism (Kiefer et al., 2011), naltrexone's treatment effect could be modulated by the OPRM1 gene (Oslin et al., 2003), and 5-HTTLPR has been suggested to play a role in the treatment response to ondansetron and sertraline (Kenna et al., 2014) (please see also section subtyping of a heterogeneous disease).

## TO SUMMARIZE

There are currently four treatments for alcohol dependence approved in Europe; disulfiram, naltrexone, acamprosate and nalmefene.

Effect sizes are small to moderate.

Disulfiram is an aversive therapy, acting by blockade of a liver enzyme. If alcohol is ingested, this results in an accumulation of acetaldehyde causing i.a. vomiting and headache.

Naltrexone is an opioid receptor antagonist with main treatment effect on reducing craving and reducing heavy drinking.

Acamprosate is thought to restore imbalance between excitatory and inhibitory neurotransmitters and have its main treatment effect on maintaining abstinence.

Nalmefene is a  $\mu$ -opioid receptor antagonist and related to naltrexone. Nalmefene can be prescribed as-needed, which represents a new treatment approach.

Several potential pharmacotherapies have been suggested to be effective in lowering alcohol consumption, e.g. topiramate, baclofen, sodium oxybate and ondansetron. However, results from studies have been inconsistent.

Recent research suggests that treatment response could be modulated by polymorphisms in certain genes, possibly allowing for personalized treatment strategies.

# BIOMARKERS

Whether state or trait a biomarker should be sensitive, i.e. be accurate for most if not all alcohol consumers, and specific, i.e. linked to alcohol use only. It should be non-invasive, easy-to-perform, inexpensive, rapid, and show high validity and reproducibility. A state marker should be related to recent drinking and could be used for, establishing consumption pattern, time for last drink, maintenance of abstinence, legal aspects of soberness and monitoring progress of treatment. State and also trait markers can also be used for screening, prognosis, severity of disease, choice of treatment, and personalizing treatment. A reliable biomarker for alcohol with a well-defined time range would be of great value and could provide valuable information for the clinician about the extent of alcohol use. As of date, several markers for alcohol consumption are available. The most commonly used are carbohydrate-deficient transferrin (CDT), Gamma-glutamyl transferase (GGT), Mean corpuscular volume (MCV) and liver enzymes aminotransferase (AST) and alanine aminotransferase (ALT) (Hashimoto et al., 2013). However, these indirect markers have lower specificity than the newer tests measuring direct ethanol metabolites, such as ethyl glucuronide (EtG), ethyl sulphate (EtS), and PEth (Hashimoto et al., 2013, Jatlow et al., 2014). The correspondence between alcohol markers and self-reported data are in some studies quite high (Niemela, 2007, Anton and Youngblood, 2006, Litten et al., 2010). The direct alcohol markers show better correspondence with self-reported consumption compared to indirect markers (Crunelle et al., 2014, Hartmann et al., 2007).

A review of the use of objective markers in RCTs in-between 1985-2001 concludes that biomarkers should be included in clinical trials. At the time the markers were not considered very accurate but albeit of some value for treatment goal as liver function and for estimating drug safety. Biomarkers and self-report integrated might enhance statistical power of the outcome. However, in 2001 biomarkers were not recommended as measurement of abstinence for criteria for inclusion. The objective markers should be measured as soon as possible and analyzed by a central laboratory. Time since last drink should however still be documented (Allen et al., 2001).

## Indirect markers

Indirect state markers such as GGT, MCV and CDT are often influenced by e.g. age, gender, and non-alcoholic diseases; also they do not represent the different time frames necessary to mirror acute to short-term to long-term alcohol consumption (Wurst et al., 2015).

Of the indirect markers **carbohydrate-deficient transferrin (CDT)** is the most sensitive and specific single test for recent moderate to heavy drinking. Transferrin is the most important iron-transport protein in humans and is synthesised and secreted by the liver (Hashimoto et al., 2013). The amount of the deficient isoforms of transferrin increases in response to heavy drinking, creating a measurable marker in blood. However, the mechanisms behind the increase are still largely unknown (Stibler, 1991). In early studies both high sensitivity and specificity were suggested (Stibler, 1991, Stibler et al., 1978). With a half-life reported in-between 7-16 days, the most reliable time frame of CDT is between 7-10 days (Hashimoto et al., 2013).

Although a simple basis structure, the variety of human transferrin is complex, High ethanol intake could affect synthesis and secretion as well as membrane assembly and enzyme activity for transferrin modulation. The most important forms for alcohol diagnostics are asialotransferrin and disialotransferrin, the first form not found in abstainers or moderate drinkers (Niemela, 2007, Arndt, 2003).

Also, the exact amounts of alcohol required to elevate CDT are not known, although an intake of 50-80 g per day during 2-3 weeks probably is sufficient, at least in alcohol dependent individuals (Stibler, 1991, Mikkelsen et al., 1998, Winkler et al., 2013). It could be that CDT is more sensitive as a relapse marker, i.e. changes in alcohol intake in alcohol dependent patients (Burke et al., 1998), rather than a marker of actual consumption levels (Anton et al., 1996, Mikkelsen et al., 1998).

CDT will thus increase after drinking levels of more than 50-80 g per day for 2-3 weeks and has been shown to have a higher sensitivity in alcohol dependent individuals than in non-dependent (52 % vs 5 %) (Mikkelsen et al., 1998). However, when monitoring change, CDT may be of help even at a lower daily intake of 20-60 g per day where change in alcohol intake had a  $R^2=0.6$  correlation with change in CDT levels (Burke et al., 1998).

As the methodology to measure CDT has not been standardized, the exact sensitivity and specificity of CDT is still not fully established, with figures ranging from 34 % to 64 %. Patients reporting over 150 g ethanol intake per day reached 64 % sensitivity while only 34 % in patients with a mean intake of 100 g per day

(Helander 1997 WHO ISBRA). In the first day of detoxification sensitivity was 69.2 % for CDT (Wurst et al., 2010). And the sensitivity seems to be dose-dependent with levels measured of approx. 40 % at 40 g per day, 60 % at 40-60 g per day, 80 % at 80-120 g per day, and 90 % at levels over 200 g per day (Aradottir et al., 2006). The discrepancies in reported sensitivity are as wide as 26 to 83 % (Hashimoto et al., 2013), and even wider in women (19-86%) (Allen et al., 2000).

It appears that percent CDT of total transferrin is a better option than crude concentrations of CDT, especially for women prone to iron deficiency and for patients with liver disease, which also influences transferrin (Helander and Tabakoff, 1997).

It seems that CDT is a more sensitive marker in men than women (Sillanaukee et al., 1998, Niemela, 2007). Women seem to have higher basal levels of CDT but increase less in response to alcohol. Also during pregnancy levels are heightened but after menopause levels are lower. Influence of smoking, anorexia and blood loss also need to be further evaluated (Niemela, 2007).

In combination with other variables, such as GGT and liver enzymes, sensitivity of CDT increased with intact specificity (Hietala et al., 2006). In Sweden, analyzing CDT levels is still the method of choice to validate abstinence in legal issues of driver's license (Englund, 2016). Apart from being used in research and for legal issues, CDT is often used for detecting at risk for monitoring high risk patients in type 2 diabetes and hypertension patients in primary care and in surgical risk evaluations (Fleming et al., 2004, Miller et al., 2006, Hashimoto et al., 2013). However, as CDT has also been associated with hypertension, the relation to alcohol in these patients could be confused. CDT levels have also been influenced by rheumatoid arthritis and asthma, common diseases which should be taken into consideration when interpreting CDT results (Sillanaukee et al., 2001).

Taken together, although CDT is not entirely standardized, and its value as a marker of alcohol consumption is limited due to influences of gender, smoking, age and several medical conditions, such as BMI, and iron deficiency (Fagerberg et al., 1994, Whitfield et al., 1998, Stauber et al., 1996) and CVD risk factors (Nikkari et al., 2001), it is inexpensive and still the most commonly used marker (Hashimoto et al., 2013).

**Gamma-glutamyl transferase (GGT)** is a membrane-bound glycoprotein enzyme. GGT transfers the gamma-glutamyl component of glutathione to peptide acceptors. Serum GGT increases with chronic alcohol intake. Although widely used, the specificity and sensitivity of GGT vary in different studies, e.g. showing elevated GGT in 52 % of alcohol dependent subjects, 55 % for patients reporting over 150 g ethanol intake per day, and 47 % for patients with a mean of 100 g per day (Helander and Tabakoff, 1997). Sensitivity has been reported reaching as high as 61 % (Hashimoto et al., 2013) and even 73.1 % in the first day of abstinence (Wurst et al., 2010). As in the case of CDT, GGT seems to be dose-related with essentially the same sensitivity of approx. 40 % at 40 g per day, 60 % at 40-60 g per day, 80 % at 80-120 g per day, and 90 % at levels over 200 g per day (Aradottir et al., 2006).

Sensitivity is higher for men than women and apart from the influence of gender, obesity and age increase while coffee decreases GGT levels. Abstainers however show decreased GGT with older age and the sensitivity is especially poor among young people. It is suggested that GGT might be an indicator of oxidative stress. There are also differences due to ethnicity and GGT is increased in all forms of liver diseases (Niemela, 2007). A number of other diseases may also affect GGT levels, as hepatic, biliary, hepatic congestion in heart failure, diabetes, hypertension and pancreatitis (Sillanaukee et al., 2001, Conigrave et al., 2003).

A daily alcohol intake of 80-200 g for several weeks is needed to detect activity of GGT in blood (Winkler et al., 2013). GGT has a half-life of approx. 4 weeks (Hashimoto et al., 2013) and increased activity returns to normal in 2-5 weeks after discontinued alcohol intake (Niemela, 2007, Winkler et al., 2013).

With insufficient sensitivity and many variables influencing blood levels, GGT is a poor marker for alcohol intake, but could have a place in distinguishing patients with liver disease (Niemela, 2007).



The third of the most commonly used indirect alcohol markers is **mean corpuscular volume (MCV)**. The mechanism behind the elevation in response to alcohol intake might be hematotoxicity, but is largely unknown. As ethanol can permeate cell membranes and also interfere with metabolism, this could possibly affect erythrocytes' stability and red blood cell size (Hashimoto et al., 2013).

MCV has a half-time of 2-3 months (Hashimoto et al., 2013) and has in several studies shown approximately 40 % sensitivity (Niemela, 2007); 39 % for MCV in patients reporting over 150 g ethanol intake per day and 34 % for MCV in patients with a mean of 100 g per day (Helander and Tabakoff, 1997). In some studies sensitivity reached 47 % (Hashimoto et al., 2013). Increase of MCV is dose-dependent in response to alcohol intake, stable in nature and reversed to normal levels within 2-4 months. Somewhat higher levels are measured in women (Niemela, 2007, Hashimoto et al., 2013).

All in all, MCV has a low sensitivity and also a poor specificity in conditions such as B-vitamin deficiency, liver, blood diseases, and hypothyreosis (Niemela, 2007) and is not satisfactory as an alcohol marker.

The liver-derived enzymes, aspartate **aminotransferase (AST)** and **alanine aminotransferase (ALT)** are elevated in 39-47 % of alcohol dependent patients (Hietala et al., 2006), but are also increased in liver diseases. They are less sensitive and specific of heavy drinking than are CDT and GGT (Hashimoto et al., 2013) (hashimoto). An AST/ALT ratio over 2 suggests alcohol etiology, as most non-alcohol liver diseases stay below 1 (Niemela, 2007), and is more indicative of alcoholic liver disease than heavy drinking (Hashimoto et al., 2013).

Gender **influences the levels** of several markers and in a analysis in heavy drinkers sensitivity reached 39 % in males and 29 % in females for CDT, 28% in males and 40 % in females for MCV, 12 % in males and 20 % in females for AST, 28 % in males and 29 % in females for ALT and 33 % in males and 34 % in females for GGT. However, CDT, MCV and GGT in combination showed to be positive in 69 % in males and 70 % in females, increasing sensitivity and eliminating the gender difference. Except from gender, also smoking and age influenced the results (Sillanaukee et al., 1998).

A **combination of different markers** increases sensitivity at the expense of specificity, and price. The GGT-CDT combination, called **gamma-CDT** ( $\gamma$ -CDT), has been shown to have a higher sensitivity than either GGT or CDT alone. An alcohol intake of 40 g per day is required for detecting an increase in  $\gamma$ -CDT (Hietala et al., 2006). The mathematical equation of the  $\gamma$ -CDT combination is  $(0.8 \cdot \ln(\text{GGT}) + 1.3 \cdot \ln(\text{CDT}))$  (Sillanaukee and Olsson, 2001, Hietala et al., 2006) or rather  $(0.8 \cdot \ln(\text{GGT}) + 1.3 \cdot \ln(\% \text{CDT}))$  using the % CDT, which is shown to be better than crude CDT (Hietala et al., 2006). With the combined markers, correlations to consumed alcohol increased from 0.71 for GGT and 0.59 for CDT to 0.76 for gamma-CDT (Hietala et al., 2006).

Markers can also be **combined with screening instruments** as alcohol use disorder identification test (AUDIT). The PPV (positive predictive value) for predicting withdrawal for AUDIT alone has been shown to be 17.3 % for scores, but when combined with at least two biomarkers (of MCV, AST, ALT and GGT) the PPV increased to 47.1 % (Dolman and Hawkes, 2005).

Also **other variables shown to be associated with alcohol consumption**, but not specific to alcohol consumption, such as high blood pressure, could be combined with alcohol markers for further information of the alcohol component in the condition. Sillanaukee et al have shown an association between high blood pressure (BP) and the combination of GGT and % CDT (Sillanaukee et al., 2001).

Other indirect and quite un-specific measurements that can indicate ethanol intake are **low platelet counts**, which occur in over 30 % of patients with heavy alcohol intake, normalizing in 1-3 weeks, and **mean corpuscular haemoglobine (MCH)** (Niemela, 2007). Another possible marker is **NtBNP (N-terminal pro-BNP)**, a circulating neuro-hormone. NtBNP is a marker of cardiac dysfunction but has also been found in alcoholic patients and been shown to decrease after withdrawal therapy, indicating a correlation to alcohol intake (Hofer et al., 2011).

## TO SUMMARIZE

An alcohol state marker should be related to recent alcohol intake and only to alcohol and also, ideally, have a well-defined time-frame, be non-invasive, easy to perform, inexpensive and rapid.

Indirect alcohol markers are not a direct product of alcohol and are influenced by numerous other variables, i.a. gender, age and hypertension.

The most used indirect alcohol markers are CDT, GGT, MCV and liver-enzymes AST and ALT.

CDT measures consumption over approx. 2 weeks, GGT over approx. 1 month and MCV over 2-3 months.

AST and ALT are primarily indicative of alcohol-induced liver damage.

Combinations of markers, e.g. CDT and GGT ( $\gamma$ CDT), increase sensitivity at the expense of specificity.

## Direct markers

In contrast to indirect markers, ethanol, or ethanol metabolites i.e. direct markers, are only formed in the presence of alcohol. They typically have a short half-life of a few hours, which creates a shorter time-frame than of indirect markers. They are widely used to assess acute alcohol intake but are slowly gaining ground also in research (Hashimoto et al., 2013, Wurst et al., 2010, Wurst et al., 2015, Cabarcos et al., 2015).

The most commonly used direct markers, besides measuring ethanol itself, are; 1) EtG in serum, whole blood, urine, and hair 2) EtS in serum, whole blood, and urine 3) FAEs in hair and 4) PEth in whole blood. To generalize, ethanol metabolites are detectable in blood for hours, in urine for a couple of days, and in hair over months. An exception is PEth, that has a longer time-frame of detection in blood (Wurst et al., 2015).

**The molecule of ethanol** is practically insoluble in fat however, like water, easily diffuses through biological membranes and distributes from blood to all tissue and fluids in proportion to their water content (Cederbaum, 2012). Ethanol contains approx. 7 kcal per gram, compared to carbohydrates with 4 kcal per gram and fat with 9 kcal per gram. The structure formula of ethanol is  $C_2H_6O$  with a density of 0.7893 at 20 °C. Ninety percent of alcohol is removed by oxidation, mainly in the liver, less than 10 % is excreted in breath, sweat and urine (Cederbaum, 2012).

**Ethanol measured** in blood, breath or urine is mostly used for intoxication controls but can also be a tool for long-term drinking where 1.5 pro mille without signs of intoxication or 3 pro mille at any time are indications of tolerance (Niemela, 2007). Ethanol elimination rate in heavy drinkers could be 1.5 fold faster than in non-heavy drinkers (10-35 mg/mL/h, approx. 15 mg/mL/h in social drinkers and approx. 19 mg/mL/h in binge drinkers) (Jones, 2010). Ethanol concentration is highly specific and an easy to perform screening, but the short half-life makes it inappropriate as an alcohol intake marker over time (Niemela, 2007).

**Ethyl Glucuronide (EtG)** is metabolite of ethanol, a non-volatile, water-soluble substance with the molecular weight of 222 g/mol, formed by UDP-glucuronosyl transferase, which is a minor pathway for ethanol elimination (less than 0.1 %) (Wurst et al., 2003).

In urine, EtG has been detected up to 90 hours after alcohol intake. *Nota bene*, mouth wash and other small amounts of alcohol yielded positive EtG of no more than 0.1 mg/l in urine for up to 11 h. As a result, this is suggested as cut-off. In Sweden, however, the cut-off limit is 0.5 mg/L (personal communication, Anders Isaksson).

Hair EtG allows for cumulative and retrospective analysis of alcohol intake. Hair grows approx. 1 cm/month and a concentration of 30 pg/mg in 0-3 cm up to 0-6 cm of hair, is indicative of chronic excessive consumption. However, cosmetic treatments could render false negative results and should be documented (Wurst et al., 2003).

The in vitro formation and degradation has been discussed as bacteria could degrade EtG, also renal function and gender, age and e.g. cannabis ingestion have been shown to influence EtG concentration. However, the positive predictive value of patients reporting 3 days of abstinence was as high as 81 % and the negative predictive value was 91 % (Stewart et al., 2013, Wurst et al., 2003, Wurst et al., 2015).

**Ethyl sulfate (EtS)**, with a molecular weight of 126 g/mol, is formed in the secondary elimination pathway for alcohol catalyzed by the enzyme sulfo-transferase and the breakdown of sulfatases. A Cut-off for lowest level of detection of 0.05 mg/L has been proposed. EtG is detectable in many tissues and is stable but could be degraded by microbes. It has been shown to vary in inter-individual concentrations and renal function influences elimination rate. The positive predictive values (PPV) for 3 days of abstinence were 70 % and the negative predictive value was 93 % (Wurst et al., 2003, Wurst et al., 2015, Stewart et al., 2013).

Both EtG and EtS have short detection windows but have been reported to be detectable for up to 4 days in blood and urine, even after trace amounts of alcohol (Winkler et al., 2013, Gnann et al., 2014). EtS can detect small amounts of alcohol up to 80 hours after intake (Wurst et al., 2003, Hashimoto et al., 2013). EtG and EtS have a high correlation (Spearman's 0.886) and both are mainly used for abstinence monitoring (Winkler et al., 2013, Gnann et al., 2014).

**Fatty acid ethyl esters (FAEE)** are non-oxidative metabolites of ethanol, formed in the presence of ethanol from free fatty acids, triglycerides, lipoproteins, or phospholipids in tissue with reduced capacity to oxidize ethanol. The formation is catalyzed by several enzymes; acyl-coenzyme a-ethanol o-acyltransferase and FAEE-synthase, but also pancreatic lipase and glutathione transferase. Four of the 15 FAEEs (ethyl stearate, ethyl oleate, ethyl myristate, and ethyl palmitate) in hair function as a marker for chronic excessive alcohol consumption. In hair the detection level proposed for a segment of 0-3 cm is 0.5 ng/mg, this renders a specificity and sensitivity of 90 %. Hair tonic and cosmetic hair treatments could influence results. FAEE can be detected in hair and skin with a maximum after 7-9 days after intake (Gonzalez-Illan et al., 2011). In blood FAEEs are detected for at least 24 h after ingestion (Wurst et al., 2015). A combination of EtG and FAEEs could increase validity (Wurst et al., 2015).

FAEEs in meconium have also been suggested as a reliable, detectable marker for gestational ethanol exposure in new-born (Cabarcos et al., 2012, Bearer et al., 1999). Also EtG and EtS in hair in combination with AUDIT have been proposed to detect fetal alcohol syndrome (FAS) (Wurst et al., 2008, Hashimoto et al., 2013). The specificity of FAEEs has been shown to be 94.4 % and 90 % in hair and blood respectively (Hashimoto et al., 2013, Wurst et al., 2004).

**Transdermal alcohol sensors** have shown reliable data on alcohol consumption, highly correlated to breath alcohol measurements (although with a time lag of at least an hour) (Leffingwell et al., 2013, Dougherty et al., 2012). A mathematical model to estimate number of standard drinks has been developed and the method could be of use in clinical research (Dougherty et al., 2015). As it includes wearing a sensor and is limited to acute (not chronic) consumption, it could be seen as a complement to rather than a replacement for alcohol markers.

## TO SUMMARIZE

Direct alcohol markers are products of ethanol and its metabolites.

The most commonly used are EtG, EtS and FAEE.

Direct markers can be measured in different tissues, as blood, urine and hair.

They typically have a detection span of hours to days, thereby limiting the use to measuring acute alcohol intake.

# Phosphatidylethanol (PEth)

## PEth Formation

Phosphatidylethanol (PEth) is formed in cell membranes from the precursor phosphatidylcholine homologues in the presence of ethanol by phospholipase-D (PLD) (Alling et al., 1984, Gustavsson, 1995, Gustavsson and Alling, 1987). PEth is representing several glycerophospholipid homologues, with phosphoethanol as head group and 2 fatty acids chains that differs in length (typically containing 14-22 carbon atoms) and with 0-6 double bonds as substituents. Different homologues of PEth are created with variations of the fatty acids and they are named "PEth A:B/C:D", where A is the number of carbons in the chain at the first position on the glycerol backbone and B is the number of double bonds, while C is the number of carbons in the chain at the second position and D the number of double bonds (Isaksson et al., 2011). Forty-eight homologues have been detected among which 16:0/18:1 and 16:0/18:2 are the most prevalent in humans (Gnann et al., 2010, Gnann et al., 2014, Isaksson et al., 2011). The combined sum of the most prevalent homologues correlates well with total PEth levels and hence is considered a good proxy for total PEth (Zheng et al., 2011). The formation of PEth starts directly after alcohol consumption but has a relatively slow half-life of 4.5-12 days (Hannuksela et al., 2007, Gnann et al., 2012, Helander et al., 2012). PEth samples are stable in human blood (Aradottir et al., 2004, Gnann et al., 2014).

## PEth homologues and elimination

In a PEth elimination study, 11 healthy volunteers consumed alcohol to a BAC level of 1 g/kg within 1 hour for 5 consecutive days after an abstinence period of 3 weeks (BAC was calculated by Widmark's formula from 1932, including sex, height and weight (please see section calculation formulas). PEth homologue 16:0/18:1, CDT and GGT are measured. Max BAC was reached within 1-3 hours; mean degradation rate of 0.11-0.17 g/kg/h was measured. CDT and GGT levels were within normal range. There was a continuous rise in PEth levels from day to day, with the peak reached on different days. PEth was accumulated over days, but then probably the elimination rate surpassed the formation rate and a decrease was registered, even before the last drinking session. Half-life ranged from 4.5-10 days in the first week, and between 5-12 days in the second week (Gnann et al., 2012).

Also, in alcohol dependent patients during withdrawal PEth 16:0/18:1 levels had a steep decrease from day one to day two, and thereafter a slow elimination. It could however still be measured/detected after the full 19 days. This suggests a non-linear elimination of PEth (Winkler et al., 2013).

## **Sensitivity and specificity**

No known false positive results have been reported and in most studies PEth has shown sensitivity close to 100 % (94.5-100) (Aradottir et al., 2004, Aradottir et al., 2006, Hartmann et al., 2007, Wurst et al., 2010, Isaksson et al., 2011) and also a specificity of 100 % (Hartmann et al., 2007).

In a study of PEth's normalization during detoxification, no false negatives were detected at the first day of detoxification, yielding a sensitivity of 100 %. However, the sensitivity decreased over time showing 92.5 % at day 7, 76 % at day 14, and 64.3% at day 28. No gender difference was found (Wurst et al., 2010). Neither liver disease nor hypertension has been shown to influence PEth results (Stewart et al., 2014).

## **Correlations and detection levels**

A single dose of alcohol rendering a blood alcohol concentration (BAC) of 0.1 g/dl resulted in PEth 16:0/18:1 levels of 120 ng/ml (0.17 micromole/l) in whole blood (Schrock et al., 2014). Four days of alcohol levels of BAC 0.1 g/dl gave PEth concentrations of 237 ng/ml (0.32 micromole/l) (Gnann et al., 2012, Isaksson et al., 2011) showed that 500 ng/ml (0.7 micromole/l) of total PEth levels were typical for chronic alcohol misuse. However, in alcohol dependent individuals the concentrations could possibly be much higher (6 micromole/l) (Helander and Zheng, 2009). A cut-off of 210 ng/ml (0.3 micromole/l) is suggested to separate alcohol misuse from moderate use (Aradottir et al., 2004).

PEth has also been validated in patients with chronic liver disease and in subjects with quantifiable PEth values (using a cut-of level at 20 ng/ml) the correlation between PEth and alcohol use did not depend on gender, age or liver disease. PEth outperformed % disialo CDT in heavy drinkers but they found an overlap of PEth concentrations between heavy and moderate drinkers and a cut-off of 80 ng/ml has been suggested, averaging for at least 4 drinks (Stewart et al., 2014).

In repeated intake of mean daily alcohol of 48-102 g subjects tested positive for PEth, but below this level of drinking they did not, suggesting a cut-off detection level of 50 g per day (Varga et al., 1998). However, in recent literature PEth has also been detected in social drinkers, with a lower consumption than 50 g per day (Nalesso et al., 2011, Zheng et al., 2011).



In some studies, strong correlations have been found between PEth levels and self-reported alcohol consumption in certain situations. For example, in young drug-abusers, PEth tested negative in 94 % of subjects reporting no consumption. However, in subjects reporting consumption heavy drinking, only 61 % tested positive. The correlating figure for subjects classified as dependent was 88 %. The strongest correlation was found between PEth levels and number of drinking days in the preceding month ( $r=0.7$ ) (Jain et al., 2014). PEth's correlation with self-report was also studied in an HIV-infected population in Uganda. Among individuals that reported drinking, a similarly high correlation with total number of drinking days the last 30 days ( $r=0.73$ ), as well as with total number of drinks the last 30 days ( $r=0.72$ ). In this sample men were more likely to under-estimate (Bajunirwe et al., 2014). Also in healthy volunteers, a prospective randomized study yielded correlations between PEth levels and alcohol consumption over 3 months of  $r=0.56$  and  $r=0.61$ , in habitual consumers and subjects randomized to daily red wine consumption of 15 cl for women and 30 cl for men, respectively (Kechagias et al., 2015).

Taken together, PEth is considered to have a theoretical sensitivity and specificity of 100 % and in many studies have indeed reaches almost 100 %. PEth is shown to be able to detect lower consumption levels than the most commonly used alcohol markers, e.g. CDT. In some studies a quite strong correlation has been shown to self-reported alcohol consumption and the rather long detection window could allow for detection of PEth up to three weeks after withdrawal (Hannuksela et al., 2007). This intermediate time-frame contrasts to other direct markers with much shorter detection span.

## TO SUMMARIZE

Phosphatidylethanol (PEth) is formed in the cell only in the presence of alcohol and is shown to have 100 % specificity (no false positive tests).

The homologues 16:0/18:1 and 16:0/18:2 are the most prevalent in humans, and serves as a proxy for total PEth levels.

PEth is a direct alcohol marker with a slower half-time of 4.5-12 days, and therefore is suitable for detecting alcohol intake in an intermediate time frame of approx. 3 weeks.

Sensitivity is close to 100 % (no false negative tests), but decreases after 7 days.

PEth has shown a relatively high correlation with self-reported alcohol intake and can detect alcohol consumption at social drinking levels (below 50 grams of pure alcohol per day and maybe as low as 15-20 grams).

**Table 2a Indirect biomarkers**

	half-time	consumption level	consumption type	sensitivity	influenced by i.a.
<b><i>CDT</i></b>	7-16 days detection	(20)-50-80 g/d for 2-3 weeks	heavy drinking	16-83 % 34 % for 100 g/d	gender hypertension liver disease
<b><i>GGT</i></b>	4 weeks detection 2-5 weeks after alcohol stop	ca 100 g/d	chronic use	47-61 %	gender, age CVD liver disease
<b><i>γ-CDT</i></b>		40 g/d		higher than CDT and GGT alone	
<b><i>MCV</i></b>	2-3 months	ca 100 g/d	heavy use	34-47 % the lower for 100 g/d	Gender, B-vitamin deficiency liver disease blood disease
<b><i>AST/ALT ratio</i></b>		Ratio of over 2 = alcohol etiology of liver disease	chronic use	39-47 %	liver disease

CDT; carbohydrate deficient transferrin, GGT; gamma glytamytransferase, MCV; mean corpuscular volume, AST; aspartate aminotransferase, ALT; alanine aminotransferase

**Table 2b** *Direct biomarker*

	<b>Detection time</b>	<b>consumption type</b>	<b>Sensitivity/ PPV</b>	<b>specificity</b>
<b><i>EtG</i></b>	(11)-80-90 h (for small amounts) -4 days	recent use, monitoring abstinence	81 % PPV	
<b><i>EtS</i></b>	80 h (for small amounts) -4 days	recent use, monitoring abstinence	70 % PPV	
<b><i>FAEE</i></b>	max7-9 days in hair 24 h in blood	Recent use in blood Chronic excessive use in hair		90 %
<b><i>PEth</i></b>	Half-life 4.5-12 days detection 3 weeks after withdrawal	4 drinks /day	Sensitivity 94.5-99 %	100 %

EtG; ethyl glucuronide, EtS; ethyl sulfate, FAEE; fatty acid ethyl esters, PEth; phosphatidylethanol, PPV; positive predictive value

# STUDY DESIGN AND ANALYSIS

## Study design

To develop new effective treatments for alcohol disorders, there is a need to improve quality in study design and reporting of studies (Witkiewitz et al., 2015). Using state-of-the-art methods can hopefully decrease the number of negative studies in the field.

The EMA is a guidance in the definition of treatment goals, study design, outcome measures and data analysis. The main focus is on developing products for aiding to achieve and maintain abstinence in alcohol dependence. The full abstinence goal is also the proposed ultimate goal (defined as relapse prevention after detoxification) and hence also the goal for the primary endpoint in RCTs. The harm reduction goal (defined as significant moderation without prior detoxification) is described as a valid, although only intermediate, treatment goal, for patients unable or unwilling to become abstinent immediately. The EMA recommendation states that it is necessary to aim at maintaining abstinence as soon as the patient gets ready for it (EMA, 2010).

In the case of harm reduction studies, efficacy outcome should be change to baseline in total consumption (pure alcohol/day) and reduction in HDD defined as daily intake of over 60 g for males and over 40 g for females (Witkiewitz et al., 2015).

## Exclusion criteria

Extensive exclusion criteria may reduce external validity and the generality to out-patient settings (Kazdin, 2008). In the reporting of RCTs, the effect of exclusion must be considered and be reported thoroughly in the publication (Witkiewitz et al., 2015).

## Randomization procedure

The stratified permuted-block randomization is the recommended procedure. However, the stratified co-variate must be considered in the statistical analyses and also in the power calculation (Lachin et al., 1988, Matts and Lachin, 1988).

In the case of stratified randomization procedures, the variables need to be addressed in the analyses (Witkiewitz et al., 2015). Randomization and stratification as well as co-variates must be correctly performed (Grouin et al., 2005).

In the case of sub-group analysis, other than exploratory, these need to be specified in the statistical analytical plan (SAP) and the numbers need to be sufficient for statistical power. Exploratory sub-group analysis for scientific reasons should be described as performed based on results from the primary and secondary outcome analyses described in the SAP (Freemantle, 2001). Four criteria are suggested for higher validity of sub-group analyses; 1, chance should not be able to explain the sub-group effect; 2, the effect should be consistent across studies; 3, the sub-group hypothesis should be one of a small number of hypotheses defined a priori; 4, there should ideally be strong pre-existing biological support (Sun et al., 2014).

## Study retention

To clearly state the study goal and the importance of the study, both individual and for others, and to explain the importance of medication adherence is important for keeping subjects in the study and minimizing missing data Flexible dosing of the medication could be used to tackle possible side-effects. Follow-up by phone in-between visits, information about a significant others to contact and incentives are also possible ways to increase study retention (Davis et al., 2002). Some of these efforts to maintain subjects in the study may also have an impact on outcome, however, with blinding the impact can be reduced (Clifford et al., 2007, Maisto et al., 2007). The number of assessments also affect drop-out rate and should be considered in the design (Gastfriend et al., 2005), and the number of completed assessments should be analyzed in relation to treatment effect (Clifford and Davis, 2012).

## Missing data

Attrition analyses to find any systematical difference in the study variables such as demographics and baseline variables collected prior to attrition may be performed. However, many studies lack enough power to show any effects on these relatively small numbers. An alternative is a sensitivity analysis, examining the impact of missing data on the outcome variables (Jackson et al., 2014, Baraldi and Enders, 2010).

Also, the least biased estimate should be chosen in the missing data model. Among the most commonly used methods are last observation carried forward, baseline observation carried forward, placebo mean imputation, poor outcome imputation, full information maximum likelihood and multiple imputation. Full information maximum likelihood and multiple imputations are the best choices (Hallgren and Witkiewitz, 2013, Witkiewitz et al., 2014), while last observation carried forward and poor outcome imputation (i.e. assuming heavy drinking) produced the most biased estimates.

## Alcohol consumption data

As of today most researchers rely on self-reported data. The current recommendation is to use self-reported consumption data collected daily by a calendar and from there calculate various outcomes (Hallgren and Witkiewitz, 2013, Witkiewitz et al., 2015).

The most commonly used method to ascertain subjective alcohol consumption is the Time Line Follow Back method (TLFB) (see section study design) that relies upon a subject's recollection of alcohol consumption over the last 30 days (or longer or shorter), TLFB (Sobell and Sobell, 1992, Robinson et al., 2014) or "Form 90" (Miller, 1996). Both Form 90 and TLFB are considered to produce reliable data on consumption (Del Boca and Darkes, 2003).

The TLFB has been shown to corroborate to approx. 85% with data from transdermal alcohol sensors (TAS) (Simons et al., 2015) and is generally considered to be a sufficiently reliable source of alcohol consumption. However, comparisons between 7-days and 30-days of TLFB showed the shorter recall period to have a significantly higher number of total drinks and days of HDD, and also a lower number of abstaining days. The discrepancies increased with the length of recall time (Ekholm, 2004, Hoepfner et al., 2010). Giving a list of drinks and addressing special occasion drinking increased accuracy of self-reported alcohol intake (Muggli et al., 2015). In a 28-days prospective diary study, validated with TAS, providing information of drink size and strength to calculate alcohol consumption resulted in

a 22 % higher total consumption compared to only documenting number of “drinks” (estimated at 14 g) (Bond et al., 2014). Especially in restaurant and bar settings the drinks, apart from bottled beers and shots, typically contain more alcohol than the standard drink (Kerr et al., 2008). These results show that pattern of drinking; the preferred beverage and the setting could influence the accuracy of self-report. Individuals with lower income earning drank stronger and bigger drinks, but gender age and ethnicity did not influence size and strength adjustments to the number of drinks consumed (Bond et al., 2014).

The sensitivity of TAS to the diary was approx. 85% (Bond et al., 2014), hence the same as for TLFB. However, in a comparison between a 28-day daily diary and TLFB the diary fared moderately better (Carney et al., 1998). The subjects in neither comparison were patients in RCTs.

Both diary and TLFB seem to be more accurate than quantity-frequency and graduated-frequency methods (Stockwell et al., 2004, Bond et al., 2014), but in a short time perspective a close-ended beverage-specific day-by-day typical week intake method was similar to the 7-day TLFB method (Ekholm et al., 2008).

One way to control for validity is by retrieving information from a family member. However, this method corresponds only moderately with self-reported consumption (Donovan et al., 2004).

Daily alcohol consumption could also be aggregated to create outcome variables i.e. continuous outcome and binary outcome. Continuous variables are e.g. percent of abstinent days, drinks per drinking day, drinks per day and percentage of heavy drinking days (Anton and Randall, 2005). Binary outcome include e.g. any drinking and no heavy drinking (Falk et al., 2010). Continuous variables usually need smaller samples and allow for more powerful statistical analyses (Bakhshi et al., 2012).

Objective alcohol markers have traditionally been indirect and of low sensitivity and specificity, whereas the direct markers have not been suitable for evaluation/monitoring consumption over long time periods, but rather of acute intake (please see biomarker section).

In clinical trials biomarkers have mostly been used to confirm consumption, but to a lesser extent to detect real consumption, as the capacity to detect alcohol consumption over time is not yet fully described (Jatlow et al., 2014) (please see section biomarkers for details).

A new approach to monitoring alcohol consumption is the TAS, collecting real time drinking data (Barnett et al., 2011, Barnett et al., 2014, Dougherty et al., 2015, Roache et al., 2015). TAS data is highly correlated with breath alcohol measurements (Leffingwell et al., 2013), but it may not be able to detect low grade consumption (Barnett et al., 2014).



## **Craving**

In secondary analyses cravings are commonly explored, often by the Obsessive compulsive drinking scale (OCDS) (Anton et al., 1995, Anton, 2000). The validity of OCDS has been discussed and the total score has been shown to have poor ability to predict drinking (Kranzler et al., 1999). Other means to measure craving is a visual analogue scale (VAS) (Yen et al., 2016, Sinha and O'Malley, 1999, Volpicelli et al., 1992). The VAS is a single-question item asking patients to grade their craving from “none” to “overwhelming” on a 10 cm long line.

## **Monitoring adherence to IMP**

Monitoring adherence to investigational medicinal product (IMP) is usually done by pill count, self-report, blood or urine analysis (Witkiewitz et al., 2015). The degree of monitoring and the quality could both have an impact on the ability to detect an IMP effect and should always be reported in publications (Baros et al., 2007, Swift et al., 2011).

## **Follow-up**

When addressing the long-term treatment effect by follow-up, usually 6-12 months post treatment, the natural cause of the AUD disease must be considered (Collins and Graham, 2002, Maisto et al., 2014). A natural (i.e. untreated) recovery rate was calculated in a nationally representative sample in the US. Natural recovery had occurred in 24.4 % during the past year in adults with prior-to-past-year alcohol dependence (Dawson et al., 2005).

## **Pretreatment changes**

Also the drinking in the period before study start must be considered, as the greatest reduction in alcohol may occur even before IMP starts. Patients with a quick change of alcohol intake also had fewer drinks per drinking day 3 months after end of treatment, compared to patients with a more gradual change (Stasiewicz et al., 2013). In an RCT on ondansetron, the participants' consumption levels nearly halved in between enrollment and end of run-in period (single placebo period) (Johnson et al., 2000b). Information about alcohol consumption before study start should be collected, in a way comparable with study outcome variables (Witkiewitz et al., 2015).

## Statistical analyses

Site should be incorporated in the analyses as fixed variables i.e. co-variables (Kraemer and Robinson, 2005) and large differences between sites should be avoided, which could otherwise lead to biased standard errors and make the interpretations of results difficult. Multisite, multi-therapist studies are recommended to be analyzed by mixed effect models (linear models and multilevel models). Such models incorporate site and/or other variables as random effect, allowing for estimation of the variability in outcome due to the variable in question. This produce less biased estimates and less biased standard deviation (SD) and allow for more statistical power (Chu et al., 2011).

## Intention-to-treat

The primary outcome should be pre-specified and analyzed as intention-to-treat (ITT) (Del Re et al., 2013). The ITT label is sometimes used differently by different authors, but most commonly refers to including all available randomized subjects, regardless of the treatments actually received and regardless of missing value management (Gravel et al., 2007). The ITT approach is a complete strategy for design, conduct, and analyses. Ignoring non-compliance, drop-out, and protocol violations is considered to maintain the prognostic balance the randomization originally generated and give an unbiased estimate. In the ITT, treatment effect estimates are conservative and avoids over-optimistic efficacy estimates, which could be the result of excluding non-completers. As could the removal of non-completers introduce differences between treatment groups. This mimics the practical clinical scenario, where noncompliance and protocol violations are common. The ITT approach minimizes type I errors and allows for generalizability and preserve the sample size the power calculation was based on. However, ITT analysis is criticized for being more susceptible for type II errors and for being too cautious and thereby missing the true efficacy of the IMP (Gupta, 2011). Modified ITT populations may not eliminate subjects due to missing data or low treatment adherence (Del Re et al., 2013). Excluding randomized subjects from ITT (in a modified ITT) has been accepted when randomized on incorrect eligibility criteria or when subject did not receive any of the interventions (Fergusson et al., 2002). As much outcome data as possible is of importance for the best application of the ITT approach. Per protocol (PP) analysis is a pre-defined data subset of subjects completing the study (Gupta, 2011).

However, the ITT as golden standard has been questioned and in a simulation of non-compliance, based on a real study, Ye et al. compared alternative analysis strategies (i.a. per protocol (PP) and as-treated (AT)) with the ITT approach. For large or moderate treatment effect, the ITT was biased, while the PP and AT were unbiased if when non-compliance was random. By defining type of non-compliance, the most correct analyze approach could be chosen (Ye et al., 2014).

## TO SUMMARIZE

Studies can have an abstinence goal or be harm-reduction studies, depending on prior detoxification or not.

Exclusion criteria should always be reported in publications

The recommended form of randomization is the stratified permuted block randomization.

Sub-groups need to be specified in the SAP.

Flexible dosing and phone follow-up are two ways to enhance retention in the study, which is of great importance to minimize missing data.

Multiple imputations is the best method for handling missing data and a sensibility analysis can be performed to examine the impact of missing data on results.

Alcohol consumption outcome is most often measured by self-report in TLFB method. This method has shown adequate correlation with e.g. breath alcohol, but also to be less accurate the longer the recall time.

Alcohol markers have mostly been used to confirm consumption, not as outcomes *per se*.

Craving, usually measured by OCDS is also used as alcohol outcome variable.

IMP adherence should be monitored and the method reported in publications.

Follow-up should be carried out after 6-12 months.

Alcohol consumption prior to inclusion should be thoroughly documented.

For multi-center studies, mixed effect models are recommended for statistical analysis. Site should be included as a co-variate.

Outcome should be pre-specified in SAP and results should be analyzed according to ITT.

# METHOD

## The Study Design of the RCTs

All three studies, which the four papers in the present thesis are based on are double-blind, two-armed, randomized, placebo-controlled clinical trials.

The first study conducted by the Addiction Biology Unit - Clinical Trials was the mirtazapine study (Paper I). This single-center investigator-initiated RCT was launched based on clinical observations of reduced alcohol consumption in patients receiving mirtazapine for depression (Bo Söderpalm, personal communication). The study had a harm reduction approach and included only men with high alcohol consumption rather than alcohol dependent subjects. Alcohol intake was documented by diary self-report and main outcome was measured as total intake of alcohol units per week.

The Org 25935 study was an international multi-center study coordinated by the pharmaceutical company MSD (former Sheerig Plough, former Organon) (Paper II). As the concept derived from pre-clinical data from the Addiction Biology Unit, we were advisors in the development of the protocol, but were not ultimately responsible and could not choose the outcome, the method for measuring outcome or the goal of treatment. This study was, according to EMA guidelines, detoxifying subjects before enrollment and using total abstinence as treatment goal. Main outcome was maintenance of abstinence measured by TLFB.

The second investigator-initiated study was the varenicline study (Paper III). This multi-center study was a post-doc project coordinated by Elin Löf and the substance was cordially provided by Pfizer. Pre-clinical data from the Addiction Biology Unit was the foundation of the initiative. The mirtazapine study was used as a template in the planning but as the varenicline study was conducted at three Swedish sites and included more subjects and more personnel, the recruitment and subject flow as well as the case report file (CRF) were adapted and improved. Although we kept the strategy of harm reduction over total abstinence, the main outcome variables were adjusted according to EMA guidelines, choosing proportion of HDD rather than total consumption of alcohol as main outcome. Subjects documented self-reported alcohol consumption in a diary and AUDIT and craving measured by OCDS was used as secondary outcomes. Also, alcohol markers were introduced as objective measures of alcohol intake.

The study on correlations of alcohol markers and self-reported alcohol consumption data (Paper IV) is based on data from the varenicline study and represents a collaboration with Anders Isaksson and Lisa Walther at the Division of Clinical Chemistry and Pharmacology, Department of Laboratory Medicine, University Hospital, Lund University.

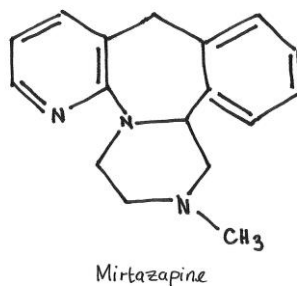
## Statistics

In Paper I and III and IV the statistical analytical plan (SAP) and statistical analyses were performed in collaboration with external statisticians (please see papers, sections PAPER I & PAPER III, and Appendix 1 for details).

In Paper II the pharmaceutical company MSD was responsible for the statistical analyses (please see paper and the section PAPER II for details).

# PAPER I

## The Effects of Mirtazapine versus Placebo on Alcohol Consumption in Male High Consumers of Alcohol; a Randomized, Controlled Trial.



**The aim** of the study was to investigate mirtazapine's effect on alcohol consumption in males with a high alcohol intake,

with the **hypothesis** that mirtazapine reduces drinking and that Family History of alcoholism (FHP) influences the results.

The **main outcome** was total alcohol consumed as a weekly mean, measured by self-reported consumption documented in a diary. The secondary outcome was the influence of FHP on treatment effect.

In the **statistical analysis** of covariance, the dependent variable was the difference between mean weekly consumption during the active treatment period and consumption at baseline. Treatment group, FHP and consumption category were used as factors. Also the interaction between treatment and FHP was analyzed. Missing data was handled by LOCF. Results were analyzed according to ITT and completers.

**The substance**, mirtazapine, originally called ORG 3770, was developed by NV Organon in the late 80ies as an antidepressant drug (de Boer et al., 1988, Mattila et al., 1989).

Mirtazapine increases noradrenergic transmission by blocking pre-synaptic  $\alpha_2$ -noradrenergic auto-receptors. This secondarily leads to stimulation of serotonergic neurons via activation of  $\alpha_1$  noradrenergic receptors. The serotonin released stimulates i.a. post synaptic 5-HT<sub>1A</sub> receptors, but not 5-HT<sub>2</sub> or 5-HT<sub>3</sub> receptors, which are blocked by mirtazapine. Mirtazapine also blocks histamine H<sub>1</sub> receptors (de Boer, 1996, de Boer, 1995).

Fifty-nine male **subjects** with high alcohol consumption, recruited via advertising, were included. After a 2-week single-blind placebo run-in period, subjects received either placebo or mirtazapine 30 mg daily for 10 weeks.

**The results** of the analysis of the main outcome showed no treatment effect of mirtazapine over placebo. However, in the secondary outcome the interaction between treatment and FHP was close to significant (0.079) in the ITT population and significant (0.032) in completers, with FHP showing a decrease in alcohol consumption.

**To conclude**, these results indicate that mirtazapine reduces alcohol intake in men with heredity for AUD.

And **what to make of it**. The serotonergic components of mirtazapine, both the indirect stimulation of 5-HT<sub>1A</sub> and the blockade of 5-HT<sub>3</sub> receptors have been shown to reduce alcohol intake in pre-clinical studies (LeMarquand et al., 1994). The effect of the 5-HT<sub>3</sub> antagonist ondansetron on alcohol intake has also been studied in humans and the treatment response has been shown to be correlated to genetic variables (Sellers et al., 1994, Johnson et al., 2000b, Johnson et al., 2002, Kranzler et al., 2003). Mirtazapine has also been suggested as adjuvant to withdrawal therapies (Liappas et al., 2004, Liappas et al., 2005). The antidepressant effects could help both co-morbid depression and the depressed mood often caused by the AUD (Cornelius et al., 2013).

The noradrenergic component of mirtazapine is also of interest as noradrenergic neurons have been suggested to interact with e.g. the dopaminergic system (Shelkar et al., 2015). The selective noradrenergic reuptake inhibitor (SNRI) atomoxetine has been shown to reduce HDD, although not time to relapse, in ADHD subjects with co-morbid AUD. The dose was 100 mg, which is the highest recommended daily dose for ADHD treatment (Wilens et al., 2008).

The third component of the pharmacological profile of mirtazapine is a blockade of the H<sub>1</sub> receptors, which possibly could add to the effects of mirtazapine on alcohol intake as it is suggested that brain histamine could influence alcohol-related behavior (Panula and Nuutinen, 2011).

Mirtazapine enhances nocturnal melatonin secretion and influences sleep (Palazidou et al., 1989, Antonioli et al., 2012). Possibly the endoneurocrine effects of antidepressants, suggested to be involved in their effects on depression, where high levels of corticosteroids and pro-inflammatory cytokines are found (Antonioli et al., 2012), could also be involved in effects on alcohol dependence.

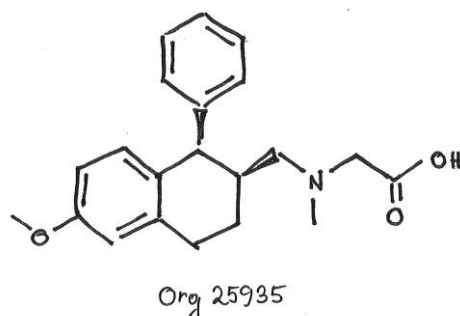
The dose of mirtazapine used in the study was 30 mg, which is the lowest dose recommended in treatment for depression (30-60 mg). Possibly, a higher dose could enhance the alcohol reducing effect found in the study. Studies on naltrexone, for example, have lately used the high-dose naltrexone (150 mg) rather than standard dose of 50 mg (Yoon et al., 2016, Yoon et al., 2011).

Furhtermore, mirtazapine in combination with other pharmacotherapies for alcohol dependence, e.g. naltrexone (Adamson et al., 2015, Pettinati et al., 2013) could be of interest for further research.



## PAPER II

### Efficacy and Safety of the Glycine Transporter-1 Inhibitor Org 25935 for the Prevention of Relapse in Alcohol-Dependent Patients: A Randomized, Double-Blind, Placebo-Controlled Trial.



**The aim** of the study was to investigate the effect of Org 25935 on relapse in detoxified alcohol-dependent subjects,

with the **hypothesis** that the Glycine transporter 1 inhibitor Org 25935 prolongs abstinence after detoxification and reduces drinking in alcohol dependent subjects.

The **main outcome** was percentage of HDD, as a mean of 2 weeks interval.

In the **statistical analysis** a mixed model repeated measurement was used on biweekly periods (repeated measure) longitudinal data. This model does not include imputations of adjustment for missing values. Continuous variables were analyzed with ANCOVA, including treatment, centers, and baseline as fixed factors. The efficacy analysis was performed on modified ITT population. An interim analysis was performed half-way and study inclusion was arrested due to futility.

**The substance**, Org 25935, is a glycine uptake inhibitor acting on glycine transporter 1 (GlyT1) causing a raise in extracellular glycine levels. Originally the substance was developed by Organon as a treatment for schizophrenia (Schoemaker et al., 2014). In preclinical studies Org 25935 has been shown to prevent mesolimbic dopamine release caused by alcohol and to powerfully lower ethanol intake in animal models.

One hundred and forty-one detoxified **subjects** with alcohol dependence were recruited either via advertising or via in-ward recruitment from 18 centers in 8 countries and included in the study. Subjects received either placebo or Org 25935, 12 mg daily for 84 days.

**The results** of the analysis of the main outcome showed no treatment effect of Org 25935 over placebo.

**To conclude**, the results of the 141 subjects included before the premature end of the study showed no effect of Org 25935 over placebo on relapse prevention.

And **what to make of it**. Glycine transporter 1 inhibitors have shown promising results in preclinical studies, but the therapeutic window is narrow and doses used in humans might not be high enough for treatment effects before subjects experience side effects. Since the preclinical and clinical data show discrepancies, there might be translational difficulties. However, in the animal models, alcohol and Org 25935 needed to be co-administered for several days to produce the alcohol reducing effect (Molander et al., 2007, Vengeliene et al., 2010). Since Org 25935 blocks the ethanol induced dopamine elevation, the repeated co-administration could result in an extinction of the in-learned behavior of the alcohol rewarding effect. In the design of the RCT this initial prolonged co-occurrence of alcohol and Org 25925 was not present since the subjects were detoxified before entering the study. Both groups also maintained low relapse rates throughout the study. Hence, the study design could be a possible explanation for the lack of treatment effect in the RCT despite the promising pre-clinical data.

The results of the study are interesting in the aspect of the relapse frequency. Although no superiority of Org 25935 over placebo was detected, both groups had a lower frequency of relapse than would have been expected (Anton et al., 2006, Weisner et al., 2003, Jin et al., 1998).

Unfortunately, no alcohol markers were sampled for outcome analyses. Possibly, alcohol marker analyses could have detected differences between Org 25935 and placebo, as was the case in the varenicline study (Paper III).

**Figure 3 Relapse rate in the Org 25935 study**

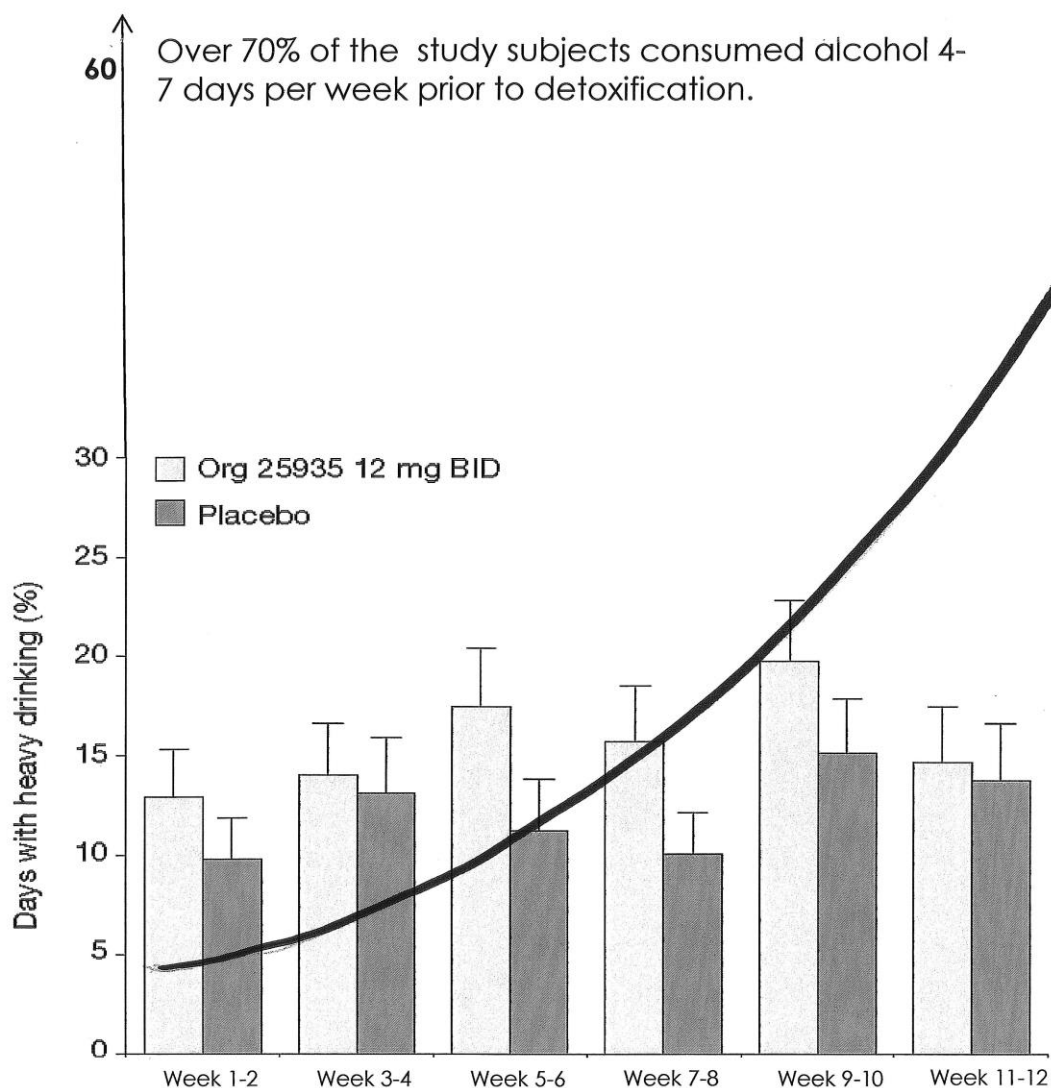
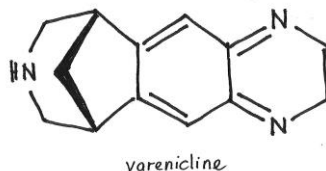


Figure illustrating expected relapse rates.

Both groups in the Org 25935 RCT displayed a low relapse frequency during the study period. In e.g. the COMBINE study, comparing naltrexone and combined behavioral interaction (CBI), approx. 30 % remained abstinent after 12 weeks (Anton et al., 2006). Estimated relapse over a one year period has been shown to be 60 % for treated individuals (Weisner et al., 2003) ranging from 20 % to 80 % (Moos and Moos, 2006, Jin et al., 1998), and almost 80 % for untreated individuals (Weisner et al., 2003).

## PAPER III

### Varenicline for Treatment of Alcohol Dependence: a Randomized, Placebo-Controlled Trial.



**The aim** of the study was to investigate the effect of varenicline on alcohol consumption in alcohol dependent subjects,

with the **hypothesis** that varenicline reduces alcohol drinking.

The **main outcome** was proportion of HDD as a weekly mean, measured by self-reported consumption documented in a diary. Alcohol consumption was also measured by the direct alcohol marker phosphatidylethanol (PEth). The secondary outcomes were AUDIT and craving measured by OCDS.

In the **statistical analysis** of covariance, the dependent variable was the proportion of HDD over the 10 week steady state active treatment period. Treatment group, smoking, heredity and site were used as covariates as well as baseline HDD. Missing data was handled by imputation. PEth analysis was conducted without imputations. Results were analyzed according to ITT and PP completers.

**The substance**, varenicline, originally developed from cytisine (derived from the plant *Cytisus laburnum* 1865) and used since mid-1900s in Eastern Europe as Tabex<sup>®</sup> as substitute for nicotine (Coe et al., 2005, Prochaska et al., 2013). Varenicline, (Chantix<sup>®</sup>/Champix<sup>®</sup> by Pfizer) is a smoking cessation drug that acts on the nicotinic acetylcholine receptor as a partial agonist at  $\alpha 4\beta 2$  nAChR. The receptor activation is thought to slightly increase dopamine levels while inhibiting further release of dopamine, induced by nicotine and possibly by ethanol (Feduccia et al., 2014, Ericson et al., 2008).

One hundred and seventy-one **subjects** with alcohol dependence from three sites in Sweden, recruited via advertising, were included in the study. After a 2-week single-blind placebo run-in subjects received either placebo or varenicline tartrate 2 mg daily for 12 weeks.

**The results** of the analysis of the main outcome failed to show treatment effect of varenicline over placebo. However, the outcome analysis on PEth showed a significant reduction of PEth levels in the varenicline group ( $p=0.02$ ). OCDS showed treatment effect in the PP population ( $p=0.05$ ) and almost in the ITT ( $p=0.06$ ). End of treatment (EoT) AUDIT score showed a significant difference between treatment groups (ITT  $p=0.02$ , PP  $p=0.01$ ). There was no effect of treatment on nicotine use (ITT  $p=0.37$ ).

**To conclude**, Varenicline reduces alcohol consumption in alcohol dependent subjects. Self-reported data failed to show efficacy, while the objective alcohol marker PEth revealed an effect.

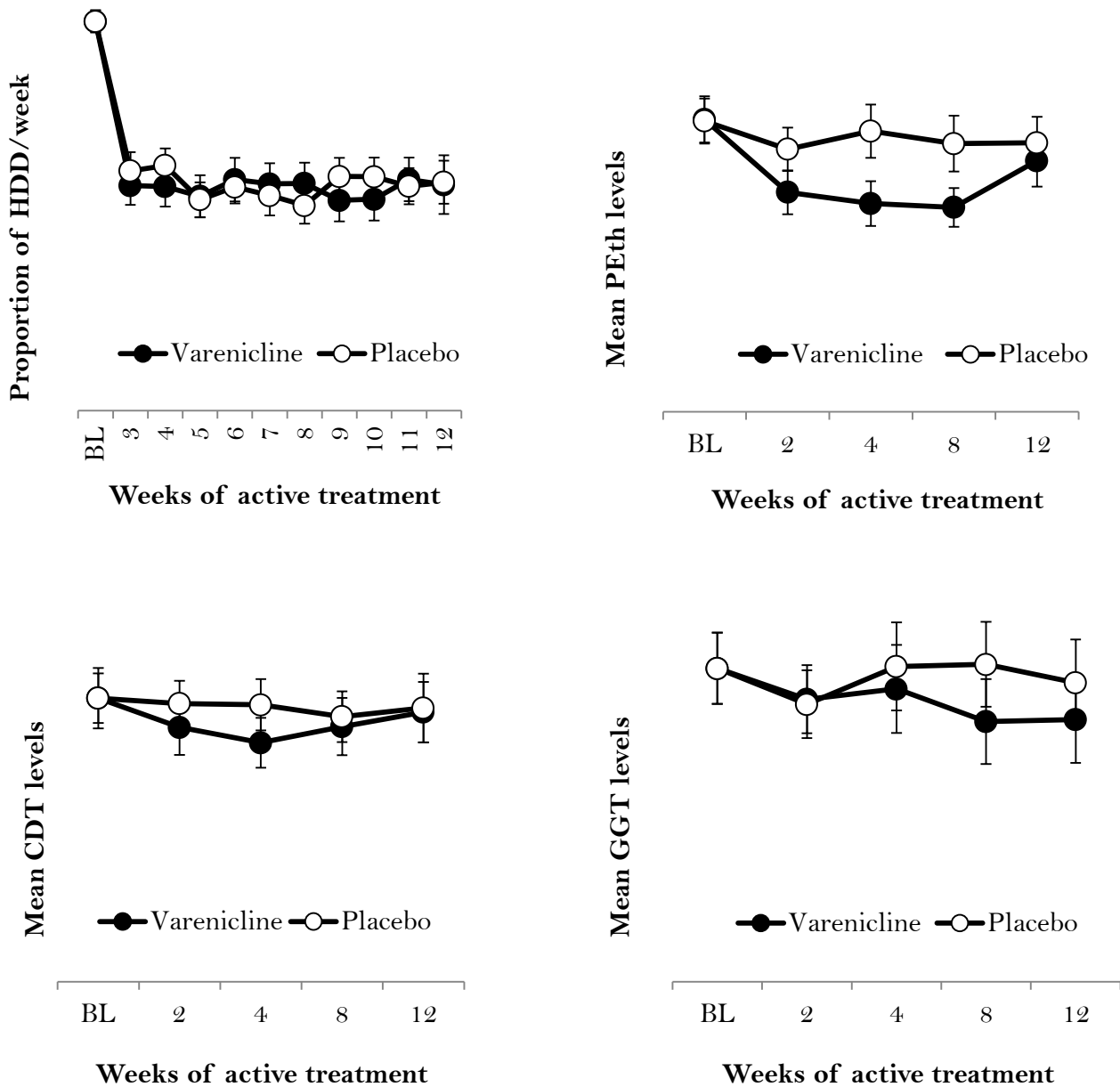
And **what to make of it**. It appears that both nicotine and alcohol produce a dopamine increase in the brain reward system via the nAChR receptor (Blomqvist et al., 1993, Larsson and Engel, 2004, Soderpalm et al., 2000, Tizabi et al., 2007, Balfour, 2009, Katner and Weiss, 2001). Preclinical data indicate that varenicline modulates the increase of dopamine due to alcohol as well as the increase due to nicotine (Ericson et al., 2009), and that varenicline reduces alcohol intake in rats (Steensland et al., 2007). In heavy drinking smokers varenicline reduced both alcohol craving and intake (McKee et al., 2009, Fucito et al., 2011, Mitchell et al., 2012), this was also the case in a recently published RCT on alcohol dependent subjects (Litten et al., 2013). The results of the present study support the effect of varenicline as a mean to reduce alcohol craving and alcohol intake. However, as the self-reported data failed to show the difference between treatment groups, while the objective alcohol marker did, the study results also have a methodological implication as to the choice of main outcome variable in studies of interventions aimed at reducing alcohol consumption.

The theory of the mechanisms underlying varenicline's alcohol intake reducing effect thus derives from pre-clinical data, and is based on the presumption that basal dopamine levels are slightly elevated while the further increase of dopamine, caused by alcohol, is blocked (Rollema et al., 2007, Ericson et al., 2009). However, recent animal studies have shown that the blockade of the dopamine elevation was not evident when varenicline was administered to the VTA of rats, and that the dopamine release in nAc was actually additively enhanced following systemic administration of varenicline and alcohol (Feduccia et al., 2014). Knock-out mouse models have given further evidence for the involvement of  $\alpha 4^*$  nAChRs in the alcohol reducing effects of varenicline (Hendrickson et al., 2010), which then could be an effect of the slight dopamine elevation, caused by the substance via this receptor (Rollema et al., 2007).

On the other hand, varenicline analogues with selective interaction on the  $\alpha_4\beta_2^*$  nAChRs failed to reduce alcohol intake while analogues with interaction at  $\alpha_3\beta_4$  nAChRs decreased ethanol intake (Chatterjee et al., 2011).

Taken together, these findings could indicate that the alcohol reducing effects here observed are due to the slight dopamine elevation caused by varenicline and not to a tentative blockade of ethanol-induced dopamine release. If analogues with a combined action at  $\alpha_4\beta_2$  and other receptor subtypes that have been implicated in ethanol's dopamine activating effects in the VTA, i.e.  $\alpha_3$  and  $\alpha_6$  (Hendrickson et al., 2010), could be developed, this could be a way to enhance the effects on alcohol consumption of treatments aiming at the nAChRs.

**Figure 4** *Mean levels of HDD per week, mean PEth, CDT and GGT levels during active treatment in the varenicline study, adjusted graphs*



Graph illustrating the difference in the shapes of the two treatments curves for the biomarkers, the data points for active treatment have been adjusted to meet the baseline levels of the placebo curve. The shape of the curve for CDT is similar to that of PEth, which is logical considering the high correlation between the two markers. Also GGT shows the same tendency, however possibly with a delay. The GGT marker is a slower marker of consumption over months rather than weeks. In this figure one outlier (of GGT=50) in the GGT data set has been removed. (HDD: Heavy drinking days, BL: baseline, PEth: phosphatidylethanol, CDT:carbohydrate-deficient transferrin, GGT:gamma glytamyltransferase).

**Table 3 Effect size**

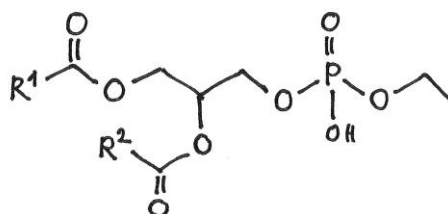
	<i>Cohen's d</i>	<i>ITT</i>	<i>PP</i>
<b>GGT</b>	Mean for treatment period	0.152	0.304
<b>CDT</b>	Mean for treatment period	0.049	0.023
<b>PEth</b>	Mean for treatment period	0.259	0.329
<b>GGT</b>	Visit 1 screening	0.079	0.208
<b>CDT</b>	Visit 1 screening	-	-
<b>PEth</b>	Visit 1 screening	-	0.081
<b>GGT</b>	Visit 2 randomization	0.115	0.196
<b>CDT</b>	Visit 2 randomization	-	-
<b>PEth</b>	Visit 2 randomization	0.080	-
<b>GGT</b>	Visit 4, 2 weeks of treatment	0.064	0.248
<b>CDT</b>	Visit 4, 2 weeks of treatment	0.039	-
<b>PEth</b>	Visit 4, 2 weeks of treatment	0.228	0.272
<b>GGT</b>	Visit 5, 6 weeks of treatment	0.126	0.370
<b>CDT</b>	Visit 5, 6 weeks of treatment	0.107	0.061
<b>PEth</b>	Visit 5, 6 weeks of treatment	0.347	0.447
<b>GGT</b>	Visit 6, 8 weeks of treatment	0.155	0.356
<b>CDT</b>	Visit 6, 8 weeks of treatment	-	0.053
<b>PEth</b>	Visit 6, 8 weeks of treatment	0.332	0.341
<b>GGT</b>	Visit 7 end of treatment	0.215	0.374
<b>CDT</b>	Visit 7 end of treatment	-	-
<b>PEth</b>	Visit 7 end of treatment	0.089	0.165

Table of effect size, calculated by Cohen's  $d$ , for all sample time-points for biomarkers GGT, CDT and PEth. ITT and PP populations. Missing values represents a slight negative treatment effect, i.e. where the value for varenicline treatment group was lower than the value of the placebo treatment group not applicable, i.e. value for active treatment higher than value for placebo. Effect sizes are generally greater for PP than for ITT. PEth and GGT detect greater effect size and more consistent values than CDT. (ITT: intention-to-treat, PP: per protocol, PEth: phosphatidylethanol, CDT:carbohydrate-deficient transferrin, GGT:gamma glytanyltransferase).



## PAPER IV

### Phosphatidylethanol is Superior to Carbohydrate-Deficient Transferrin and $\gamma$ -Glutamyltransferase as an Alcohol Marker and is a Reliable Estimate of Alcohol Consumption Level.



Phosphatidylethanol

**The aim** of the study was to investigate the correlation between self-reported alcohol consumption and the objective direct alcohol marker phosphatidylethanol (PEth) and to compare PEth to the indirect alcohol markers CDT and GGT

with the **hypothesis** that the direct alcohol marker PEth was superior to the indirect markers CDT and GGT in estimating alcohol consumption.

The **main outcome** was levels of PEth, CDT and GGT in blood.

In the **statistical analysis** associations between self-reported consumption and alcohol marker levels were calculated with scatterplot and linear regression. Correlations were examined using Spearman's rank correlation coefficients ( $r_2$ ) at week 6. Comparisons were made by Mann-Whitney U-test and Wilcoxon signed rank test. Descriptive statistics were used for baseline values of consumption data and alcohol markers at 5 occasions.

One hundred and fifteen **subjects** from the varenicline RCT (Paper III) that completed treatment and provided samples for measuring alcohol markers.

**The results** of the analysis showed that all subjects had elevated PEth levels at baseline while 42 % had elevated CDT levels and 15 % had elevated GGT levels. At baseline retrospective self-reported consumption showed significant ( $p < 0.05$ ) correlation with PEth ( $r_2 = 0.23$ ) and CDT ( $r_2 = 0.22$ ) and GGT showed significant correlation to consumption category of AUDIT (AUDIT-C) ( $r_2 = 0.24$ ,  $p < 0.05$ ). At week 6 PEth showed the strongest correlation to self-reported consumption ( $r_2 = 0.52-0.56$  for week 1-4 before sampling). The strongest correlation was however between PEth and CDT ( $r_2 = 0.63$ ).

**To conclude**, PEth is superior to CDT and GGT in estimating alcohol consumption.

And **what to make of it**. Subjects reported a quite stable alcohol consumption level 4 weeks before the week 6 sampling; hence the correlations were calculated for this time point, examining the correlation for 1-4 weeks of consumption before the sampling. At baseline alcohol consumption data was collected retrospectively while at samplings during the study alcohol marker levels were correlated with self-reported diary data. The latter showed higher correlations, indicating that diary-based self-report is more reliable. Women showed a slightly higher PEth level for the same reported alcohol intake. When translating PEth levels in this study to consumption levels a PEth value of  $0.32 \mu\text{mol/l}$  was defined as a consumption level of 0-49 grams per day, 0.51 represents 50-99 grams per day and 0.77 represents 100-142 grams per day.

# DISCUSSION

## The Placebo paradox

The concept of “placebo” originates from a (mistranslated) passage in the Bible; “*Placebo Domino in regione vivorum*” (“I will please the lord in the land of the living”, 9<sup>th</sup> line, psalm 116). In the 13<sup>th</sup> century hired mourners chanted the 9<sup>th</sup> line while waiting for the “Vesper for the Dead”, and to describe the fake mourning they were called “placebos”. To use fake procedures controls to separate real effects from imaginations was used by the Catholic Church to undermine exorcism. Possessed individuals were given false holy relics and when they reacted to these it was concluded that the possession was in their own imagination. The idea of placebo control was transferred into medical experiments in 1748 with the Franklin commission. The meaning of placebo as a treatment to make patients comfortable came later in the end of the 18<sup>th</sup> century and the modern use of placebo effects began with the wider use of RCTs after World War II (Finniss et al., 2010). In 1955 Henry Beecher posed the question if the perception of pain could be influenced by belief in a non-active treatment and found a 35 % placebo effect. However, he also raised questions about improvement over time and how the placebo effect differs from the natural course (Beecher, 1955, Best and Neuhauser, 2010). An important distinction in the entangling of the placebo concept is “placebo/treatment response” vs “placebo/treatment effect”. These are often used interchangeably; however, treatment response should be seen as the change observed following treatment administration (*post hoc* “after this”) and the treatment effect should be seen as the change **produced** by the treatment (*propter hoc* “because of this”). The role of placebo controls in RCTs is to be able to tell these apart. Hence, the placebo **effect** is the difference between the placebo response and the changes that would have occurred even without administration of placebo (natural progression and regression towards the mean). And to prevent the logical fallacy of “*Post hoc ergo propter hoc*” (“after this, therefore because of this”) where you attribute causality to events just because they happen in sequence (Kirsch, 2013).

In the self-reported diary outcome analysis in the varenicline study, all subjects lowered their consumption with about 40 % from baseline. Both the placebo group and the varenicline group reported a very similar reduction in alcohol consumption (p=0.73). However, in the analysis with the objective alcohol marker PEth as outcome variable, this initial reduction, interpreted as the placebo effect, was not evident at all. Neither had the active group this initial drop in consumption level (measured by PEth). But the active group did show a reduction in PEth levels during the study period creating a significant difference between treatment groups and proving varenicline superior to placebo. It seems that the true efficacy of

varenicline was obscured by the under-reporting of consumption by the placebo group.

Clinically meaningful changes in patients' reported pain have been examined and the patient-perceived satisfactory improvement (PPSI) was shown to be a reduction of 55 % (ten Klooster et al., 2006), but figures between 30 % improvement (Dworkin et al., 2005), and 50 % improvement have also been suggested (Scott et al., 1990, Moore et al., 1996). This is not directly applicable to alcohol consumption changes but a similar mechanism of PPSI could be discussed, where patients may consider a 50 % reduction in alcohol intake as being satisfactory, maybe to both themselves and to society/study staff. This could be an explanation to the peculiar fact that both the placebo and the active group underestimated their consumption to the same level, i.e. a reduction by approximately 40 % (unadjusted means Paper III). Also other studies on alcohol dependence have shown an initial reduction of alcohol consumption in both groups of approximately the same magnitude. The varenicline RCT by Litten showed a reduction from baseline to study week two of 44 % in the active group and 39 % in the placebo group. Although in this study subjects reducing their drinking with more than 50 % before randomization were excluded, and the numbers presented are least square (LS) means and not unadjusted data (Litten et al., 2013). In studies on nalmefene the reduction of HDD from baseline to randomization was substantial in both groups with a 46 % reduction in placebo and 53 % reduction in the nalmefene group (derived from graph, LS means) (Gual et al., 2013), 46 % placebo and 50 % nalmefene (derived from graph, LS means) (van den Brink et al., 2014), 32 % placebo, 45 % nalmefene (derived from graph, LS means) (Mann et al., 2013). Also ondansetron studies describe an almost 50 % reduction of alcohol consumption in the sample between enrollment and the end of the lead-in single blind placebo period (Johnson et al., 2000b). In these studies IMP was superior to placebo in the self-reported outcome analysis. However, there might be an even greater efficacy hidden in the results, if the same mechanisms of under-reporting as in the varenicline study are in play.

The placebo effect in alcohol dependence RCTs was examined in an exploratory analysis of 51 naltrexone and acamprosate studies (Litten et al., 2013). Only studies with abstinence at randomization were included, as to be able to exactly quantify the change in placebo response from randomization to treatment. Although there was no description of the method of collection of the outcome variable, it is logical to assume it is self-reported data. A comparison was made with depression and schizophrenia studies, also showing placebo effects but not in the magnitude of alcohol trials. Neither of these conditions is usually measured by objective biomarkers.

The naltrexone studies and the acamprosate studies differed in mean placebo response, but both showed a substantial reduction, 77.5 % for naltrexone and 39.1 % for acamprosate. The placebo response was negatively correlated with treatment response, hence, the greater the placebo response the less likely to detect a treatment effect. One explanation given is the ceiling effect, i.e. for a variable as abstaining days 100 % is the maximum and could be reached by the placebo effect, which could totally hide a potential treatment effect. Another explanation discussed is that placebo could use the same mechanisms as the IMP, as shown in e.g. pain reduction studies where placebo appeared to activate the opioid receptors (Finniss et al., 2010). In depression studies, severity has been shown to be inversely correlated with placebo response, however, not enough studies were considered to report this variable in the alcohol trials, and hence it was not analyzed. Year of publication and younger age was associated with a greater placebo response but not with treatment response (Litten et al., 2013).

No correlation was found between number of sites and efficacy of IMP. This however, was the case in a meta-analysis of 19 naltrexone studies, where multi-center studies showed a smaller effect size, as was the case with later studies vs earlier studies (Feinn and Kranzler, 2005).

Possibly changes in outcome variable measurements could be part of the explanation for the greater placebo responses in more recent publications.

The severity aspect in the depression case could be that the subjects were too sick for the psychological effect of under-reporting to be present. Although CDT and GGT are not the optimal biomarkers, if they were measured in the 51 studies it would be interesting to re-analyze the data set to examine if the placebo response was still present.

## Negative studies or false negative studies

When developing a pharmacotherapy, there are often both positive and negative studies to take into consideration. Even well-established treatments for alcohol dependence as naltrexone and acamprosate have shown diverse results. Another example is studies on the efficacy of SSRIs in the treatment of depression. In this case clinicians and researchers are quite convinced of the benefits of treating depression with SSRIs, but still many studies show negative results. The lingering question is if these studies are false negative and fail to show superiority over placebo due to methodological short-comings. The group of Elias Eriksson managed to collect data from 32 RCTs and reassessed them on the hypothesis that the negative results may be due to the main outcome not really measuring depression. In the original publications, when the full Hamilton depression rating scale was used for depression outcome, 18 out of 32 SSRI failed to prove superiority to placebo. However, when only the item depressed mood was used as outcome variable only 3 remained negative (Hieronymus et al., 2016).

This demonstrates the urgency of choosing the correct outcome variable. Also many RCTs on alcohol dependence come out negative and in fact the varenicline study showed no benefit over placebo when self-reported alcohol consumption was used as outcome. The importance of using variables that correctly and accurately mirror the intended efficacy outcome is thus illustrated, as false negative studies could lead to disregard of potentially effective treatments.

A biomarker with high specificity and sensitivity, as PEth has been shown to be, is superior to self-reported data in this aspect and we argue that at the present, PEth should be the main outcome efficacy measure used in RCTs on alcohol dependence.

## **Intention-to treat (ITT) vs. per protocol (PP)**

The approach of analysis of outcome data could influence the possibility to detect a treatment effect. ITT is considered the golden standard and is a complete strategy for trial design rather than just an analysis strategy. It is a cautious analysis and minimizes type 1 error, but is more likely to create false negative results (Gupta, 2011). While ITT is making studies more comparable it also needs high compliance. In studies with non-compliance other approaches, like PP and As-treated (AT), should be considered (Shrier et al., 2014, Ye et al., 2014). ITT is thought to mimic the real patient setting and is more generalizable, but does not correctly mirror the true pharmacological effect of the study treatment. With the possibility to analyze data according to both ITT and PP, more questions could be answered and fewer studies would be false negative. In paper I and paper III, results are analyzed firstly by the ITT approach and secondly by the PP approach. In most cases, the PP analyses strengthen the ITT results.

## **The predictive value of animal models**

Many substances have shown promising results in animal studies but in human trials fail to show superiority over placebo, e.g. Org 25935 in paper II. Preclinical models are crucial in developing new pharmacotherapies. However, several factors may influence the ability to translate results to humans. Different strains of animals may have different treatment response, the concentration of the administered ethanol may influence alcohol intake and the manner of administration may not mirror the human setting. As animals are not susceptible to placebo effect, the signal strength is often stronger in animal models, which could lead to an over-estimation of the effect of the drug. Also, publication bias towards positive results also could overestimate the potential of a drug (Yardley and Ray, 2016). Although no animal models of today is fully reproducing alcohol dependence in humans, it is still the best option to find new pharmacological targets for developing new treatments (Mason and Higley, 2013).

## Harm reduction

All-cause mortality in a population has been shown to decrease with a reduction in *per capita* alcohol consumption (Her and Rehm, 1998, Norstrom, 2001, Norstrom and Ramstedt, 2005). Indeed, 77 % of the overall alcohol-attributable mortality in Europe is due to heavy drinking (men >60 g/day, women >40 g/day) (Rehm et al., 2013). Natural experiments due to drastic changes in alcohol policies, e.g. by the Gorbachev government (Leon et al., 1997), have indicated the causal association and that reduced consumption leads to decreased mortality rates (Rehm and Roerecke, 2013). The importance of harm reduction applied to alcohol consumption has been discussed as a political necessity (McCambridge et al., 2014), and the UK has recently redefined their alcohol goals and lowered the advised weekly consumption levels (Nature, 2016).

Harm reduction is also discussed as a treatment goal, as of now the EMA guidelines of total abstinence as treatment goal seem to have a substantial impact both on individual treatment and on research (Marlatt and Witkiewitz, 2002). EMA recommendations have a great impact on the study design of RCTs on alcohol dependence and also perhaps on the substances developed, as the major focus is on abstinence and not on reduction in consumption. However, a reduction in consumption has been shown to have a major impact on mortality and also, indisputably, on social costs and problems due to alcohol (Her and Rehm, 1998, Rehm et al., 2013, Leon et al., 1997, McCambridge et al., 2014, Norstrom, 2001, Norstrom and Ramstedt, 2005).

In a calculation from 3 RCTs on as-needed nalmefene vs placebo, reduction in risk levels was defined as the shift in drinking risk levels (EMA, 2010). The overall treatment effect on 9-year mortality risk of subjects with a high drinking risk-level at baseline was estimated at a reduction of 8 % (Roerecke and Rehm, 2014).

Nutt et al 2014 proposes a harm reduction approach to reduce the alcohol inflicted harm to individual health and to society. It is rather a prevention strategy to imply on alcohol consulting dialog with patients and political measures to be taken for lowering alcohol consumption in the population. The policy changes should include; minimum price setting for alcoholic beverages, labelling of grams alcohol per bottle to enable people to easier calculate this intake and a limitation of selling (in the direction of a monopoly as Systembolaget in Sweden), encourage the use of evidence based treatments, support research and development of safer alcohol alternatives (in the line of buccally-activated tobacco (snus)). In the personal approach the patients should be encouraged to; "know their number", i.e. calculate their daily intake; set an upper limit of 20 (men) or 15 (women) g per day; take pride in the health effects of reducing their intake and; take two white days a week (Nutt and Rehm, 2014).



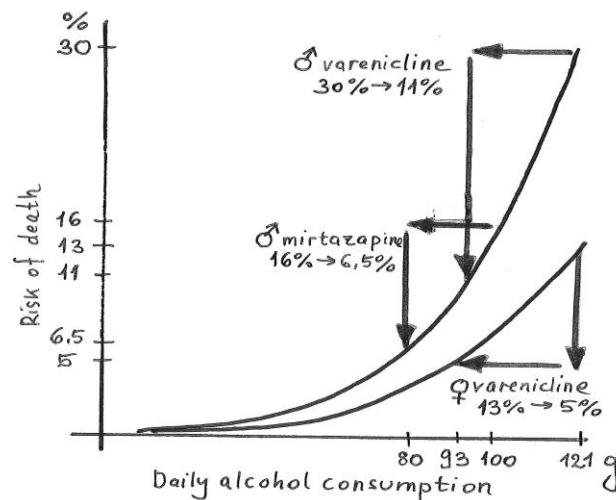
The life-time risk of death due to alcohol injury could be described as an exponential rise in percent death in relation to grams of daily consumption (sustained changes) (Nutt and Rehm, 2014). The effect of reduction in alcohol consumption on life-time risk of death due to alcohol-related injury is illustrated by Nutt et al. (Nutt and Rehm, 2014) adapted from (Rehm et al., 2011), showing that a 50 % reduction from 100 g daily to 50 g daily produces an eight-fold reduction in risk of death. With the exponential curve of increased risk with increased alcohol intake, the greatest risk reduction will be for the higher levels of alcohol consumption. This suggests the importance of not disregarding the harm-reduction policy. Total abstinence might not always be an achievable goal, but a sufficient reduction in alcohol intake, shifting from high-intake to medium or even low intake levels, might be possible. In light of this, pharmacotherapies with the ability to reduce alcohol consumption with medium efficacy could be of substantial value.

Applying this approach to the PEth reduction in the varenicline data, with estimated figures, the benefits from varenicline treatment could be described in similar risk-reduction terms.

With the assumption that the consumption categories calculated in Paper IV, with PEth levels of 0.77  $\mu\text{mol/l}$  representing consumption levels of 100-142 g/day, are more correct than the retrospectively documented self-report baseline alcohol, PEth levels may be used for estimation of baseline consumption. The mean baseline level of PEth of 0.79 would then represent a mean baseline consumption level of 121 g/day. The reduction in alcohol intake would be 23 % (calculated as a mean over the 4 points of data of PEth levels during the active treatment period;  $0.791 - 0.609 = 0.180$ ; 23 % reduction), representing a reduction from 121 g/day to approx. 93 g/day (28 g reduction). Extracted from the life-time risk of death due to alcohol-related injury graph by Nutt and Rehm (Nutt and Rehm, 2014), the reduction in life-time risk of death for men would be decreased from 30 % to 11 %, which constitutes a risk-reduction of 63 %. For women the equivalent number would be a reduction from 13 % to 5 %, which would represent a risk-reduction of 62 %.

In the mirtazapine study, subjects with family history of alcoholism receiving active substance reduced their drinking from 102 grams per day to 81 g/day in the ITT population (75.5 g/day in the PP completer population). This would reduce life-time risk of death due to alcohol-related injury from approx. 16 % to 6.5 % (5 % for completers), a risk-reduction of nearly 60 % (69 % for completers).

**Figure 4** *life-time risk of death from alcohol injury in relation to grams alcohol consumed*



This exercise shows that even though subjects retain quite high alcohol consumption in spite of the treatments and the mean effect sizes seem modest (Cohen's  $d= 0.26$  for PEth outcome on varenicline), the benefit calculated as harm-reduction is substantial.

Additionally, in a study on risk reduction by brief intervention, the same relationship is evident; the greatest impact on risk of death comes at the highest drinking levels. The curve shows a reduction of almost 50 % in heavy drinkers (approx. 100 g per day) with 20 g/day drinking reduction (Rehm and Roerecke, 2013).

Many physicians seem to be open to the harm reduction goal, as measured by a survey of the treatment goal of over 500 French alcohol specialists. Patient preference was the main criterion to choose treatment goal. Controlled drinking was practiced as a treatment goal by over 60 %. These specialists' opinion is not mirrored in research and this could complicate the translation of research findings into the every-day practice of treating patients with alcohol dependence (Luquiens et al., 2011).

## CONCLUSION

Mirtazapine could have a role in treatment of alcohol dependence in patients with heredity for AUD. More research is needed to examine the possible effects of Glycine-transporter inhibitors on alcohol dependence. Varenicline seems to reduce alcohol consumption by at least 23 % in alcohol dependent subjects, as measured by the objective alcohol marker PEth. Such a reduction could represent an over 60 % reduction in life-time mortality for both men and women. Harm reduction as a goal for treatment and for RCT outcome should be considered over total abstinence goal. The diary consumption reported by the subjects is underestimated and failed to prove varenicline efficacy. Traditional indirect alcohol markers have as yet not been sufficiently high in sensitivity and specificity to be used as outcome, and direct markers have too short time-frames. PEth, a direct marker with high specificity and sensitivity and with a time frame of approx. 3 weeks was superior to the indirect alcohol markers GGT and CDT in estimating alcohol consumption and should be the marker of choice. Also, the placebo response almost always prominent in alcohol studies, often at 40 % or more, obscuring the prospect of IMP efficacy, was not present in the PEth outcome analysis. It was evident that the placebo group had a greater underestimation of their actual alcohol intake than did the active group. Possibly the many negative studies in the field could be due to this skewness in underreporting. Taken together these results strongly suggest a shift in study design to introduce objective markers as main outcome and not rely on self-report in studies of interventions aimed at reducing alcohol consumption.

## FUTURE PERSPECTIVES

PEth should be further validated against known alcohol consumption levels as to create a correct PEth level to gram of consumption relationship. Also a more certain time-frame of PEth needs to be established. In a study performed at Addiction Biology Unit in collaboration with Lund, PEth will be analyzed in subjects starting detoxification and weekly for 6 weeks, with recent alcohol intake collected by TLFB. PEth could also be validated with direct alcohol markers and instruments, such as transdermal alcohol sensors.

Glycine-transporter inhibitors, other than Org 25935, should be studied in experimental and clinical trial settings as to establish possible effects on alcohol consumption.

The effect of mirtazapine could be investigated for higher doses and also in combination with other substances, e.g. mirtazapine and naltrexone as combination therapy.

Varenicline could be co-administered with other nAChRs modulators to enhance the tentative effect of blockade of ethanol-induced dopamine release.

With the advances in the genetics of alcohol disorder and the knowledge about genes modulating effects of pharmacotherapies, the individualized therapy approach could be investigated also for noradrenergic drugs, GlyR transporter inhibitors and drugs acting on nAChRs.

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