Alcohol consumption and risk of cardiovascular disease and type 2 diabetes

Population-based studies and physiological interventions

Joline WJ Beulens

Promotoren:

Prof. Dr. Ir. G. Schaafsma

Hoogleraar Voeding en Levensmiddelen

Prof. Dr. Ir. F.J. Kok

Hoogleraar Voeding en Gezondheid

Copromotor:

Dr. H.F.J. Hendriks

Product Manager/ Senior Scientist, TNO Kwaliteit van Leven, Zeist

Promotiecommissie:

Prof. Dr. Ir. D. Kromhout

Wageningen Universiteit

Dr. K.J. Mukamal

Beth Israel Deaconess Medical Center/ Harvard Medical School, Boston MA, USA

Prof. Dr. R.J. Heine

VU Universitair Medisch Centrum

Prof. Dr. A.F. Stalenhoef

Radboud Universiteit Nijmegen Medisch Centrum

Dit onderzoek is uitgevoerd binnen de onderzoeksschool VLAG.

Alcoholconsumptie
en risico op cardiovasculaire ziekten
en type 2 diabetes

Populatiestudies en
fysiologische interventies

Ir. Joline WJ Beulens

Proefschrift
Ter verkrijging van de graad van doctor
Op gezag van de rector magnificus
Van Wageningen Universiteit
Prof. Dr. M.J. Kropff,
In het openbaar te verdedigen
Op woensdag 17 januari 2007
Des namiddags te half twee in de Aula

Author: Joline WJ Beulens

Title: Alcohol consumption and risk of cardiovascular disease and type 2 diabetes - Population-based studies and physiological interventions

Thesis Wageningen University, Wageningen, The Netherlands
With abstract – with references – with summary in Dutch

ISBN: 90-8504-571-1

ABSTRACT

Moderate alcohol consumption is associated with a decreased risk of cardiovascular disease and type 2 diabetes compared to both abstainers and heavier drinkers. The inverse association of moderate alcohol consumption with cardiovascular disease is mainly mediated by increased HDL cholesterol concentrations. For type 2 diabetes, the underlying mechanism is not entirely clear, but improved insulin sensitivity is thought to play a role. This thesis aims to further substantiate the evidence for a causal relation of alcohol consumption with cardiovascular disease and type 2 diabetes by investigating consistency of the associations in different populations, an underlying mechanism and effect modification by the alcohol dehydrogenase 1C (*ADH1C*) gene for type 2 diabetes.

As previously shown in the general population, a risk of myocardial infarction of 0.72 (95%-CI: 0.54- 0.97) is observed for consuming two to three alcoholic beverages (15.0- 29.9 grams of alcohol) per day among 11,982 US hypertensive men. Alcohol consumption, however, is not associated with total or cardiovascular mortality, but with coronary heart disease mortality an inverse association is shown. Such inverse association could be explained by increased HDL cholesterol concentrations. Therefore, two randomized controlled crossover trials are focused on functional properties of HDL cholesterol to further explain the underlying mechanism. We show that moderate alcohol consumption increases cholesterol efflux mediated by ABCA1 by 17.5% (95%-CI: 2.3- 34.1), while lipoprotein-associated phospholipase A2 activity remains unchanged (-2.9%; 95%-CI: -7.9; 2.0).

As previously shown for men and younger women, we observe a relative risk of 0.61 (95-% CI: 0.41- 0.92) for consuming two to three alcoholic beverages (20.0 to 29.9 grams of alcohol) per day among 16,330 older women aged 49-70 years. Lifetime alcohol consumption is associated with type 2 diabetes in a U-shaped fashion in this population. In two nested case-control studies from the Nurses' Health Study and Health Professionals Follow-up Study, we show that this inverse association between alcohol consumption and type 2 diabetes is modified by a common polymorphism in the *ADH1C* gene. Among moderate drinkers (>5 g/day for women and >10 g/day for men) carrying of the *ADH1C*2* allele, conferring slower ethanol oxidation, increases risk of type 2 diabetes. Odds ratios are 1.63 (1.02-2.61) for heterozygotes and 2.38 (1.08-5.22) for homozygotes of the

*ADH1C*2* allele. These results suggest that the association between alcohol consumption and type 2 diabetes may be causal. Because slower ethanol oxidation can result in decreased concentrations of downstream metabolites such as acetate, our results indicate that these could mediate the association between alcohol consumption and type 2 diabetes instead of ethanol itself. In three randomized, controlled crossover trials an underlying mechanism for this inverse association between moderate alcohol consumption and type 2 diabetes is investigated. These three studies consistently show that moderate alcohol consumption increases plasma adiponectin concentrations by about 10%. This increase of adiponectin occurs independently from changes of body weight or fat distribution and may be oligomer specific. Particularly high-molecular weight adiponectin is increased by 57.1% ($p= 0.07$) after moderate alcohol consumption. Unfortunately, we do not observe significant improvements of insulin sensitivity after moderate alcohol consumption in any of these trials. Changes of other markers (such as other adipokines and two-hour glucose concentrations), though, are in line with the hypothesis of improved insulin sensitivity.

In summary, research presented in this thesis further substantiates the evidence for a causal association between alcohol consumption and cardiovascular disease and type 2 diabetes. First, it shows consistency of previously observed associations in populations of hypertensive men and older women. Increased cholesterol efflux and adiponectin concentrations are a plausible mechanism to explain the inverse association of moderate alcohol consumption with cardiovascular disease and type 2 diabetes. Finally, the interaction between *ADH1C* and alcohol consumption for type 2 diabetes indicates that this relation may indeed be causal.

Table of Contents

ABBREVIATIONS

ABCA1	ATP binding cassette transporter 1
ADH1C	Alcohol dehydrogenase 1C
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of variance
ApoA-I	Apolipoprotein AI
ApoB	Apolipoprotein B
ApoE	Apolipoprotein E
ASP	Acylation-stimulating protein
AST	Asparagine aminotransferase
A.U.	Arbitrary units
BMI	Body mass index
CE	Cholesterol-ester
CETP	Cholesteryl ester transfer protein
CHD	Coronary heart disease
CRP	C-reactive protein
CVD	Cardiovascular disease
CYP2E	Cytochrome P450 2E
DM2	Type 2 diabetes
EtG	Ethyl glucuronide
FC	Free cholesterol
FFA	Free fatty acids
FFQ	Food frequency questionnaire
GC-MS	Gas-chromatography mass-spectrometry
GGT	Gamma-glutamyltransferase
β-HAD	β-Hydroxyacyl-CoA dehydrogenase
HbA1c	Glycosylated hemoglobin
HDL	High-density lipoprotein
HMW	High-molecular weight
HOMA-index	Homeostasis Model Assessment index

HPFS	Health Professionals Follow-up Study
HR	Hazard ratio
IMTG	Intramyocellular triglycerides
ISI	Insulin sensitivity index
LCAT	Lecithin:cholesterol acyltransferase
LC-MS	Liquid-chromatography mass-spectrometry
LDL	Low-density lipoprotein
LMW	Low-molecular weight
LpA-I	LipoproteinA-I
LpAI:AII	LipoproteinA-I:AII
Lp-PLA2	Lipoprotein-associated phospholipase A2
MI	Myocardial infarction
MMW	Mean-molecular weight
MS	Metabolic syndrome
NASH	Non-alcoholic steatohepatosis
NHS	Nurses' Health Study
OGTT	Oral glucose tolerance test
PL	Phospholipids
PLTP	Phospholipid transfer protein
PON	Paraoxonase
PPAR-γ	Peroxisome-proliferator-activated receptor-γ
RCT	Reverse cholesterol transport
SR-BI	Scavenger receptor-BI
TG	Triglycerides
VLDL	Very-low density lipoprotein

Chapter 1

Introduction

Already since decades it is apparent that moderate alcohol consumption is associated with a decreased risk of cardiovascular disease (CVD) compared with both abstainers and heavier drinkers (1). This risk reduction is mainly mediated by an increase of HDL cholesterol with moderate alcohol consumption (2). More recently a similar risk reduction of moderate alcohol consumption for type 2 diabetes mellitus (DM2) has been observed in several prospective cohort studies (3), but the mechanism by which moderate alcohol consumption may decrease risk of DM2 is not entirely clear. Despite overwhelming evidence for CVD, the debate around causality still continues, while for DM2 the evidence is not yet complete. This thesis therefore aims to further substantiate the evidence for a causal relation between alcohol consumption and CVD and DM2 by investigating consistency of the associations in different populations, an underlying mechanism and effect modification by the alcohol dehydrogenase 1C (*AHD1C*) gene for DM2. This introduction starts by defining the concept of 'moderate alcohol consumption' based on its association with mortality, CVD and DM2. The etiology, genetic and lifestyle risk factors for both CVD and DM2 will be described. The current evidence on the association of alcohol consumption with CVD and DM2 and underlying mechanism is summarized. Finally, the research questions and outline of the thesis is described.

Alcohol consumption: a balanced view

Already in 1969 an inverse association between alcohol consumption and coronary heart disease (CHD) was first reported by St. Leger in an ecological study based on data from 18 developed countries (4). Since then numerous prospective studies confirmed this association (5). More recently a similar protective effect of moderate alcohol consumption has been observed for DM2 (3). Excessive alcohol consumption, however, is associated with an increased risk of aerodigestive cancer, and injury (6). For breast cancer more moderate amounts of alcohol are associated with increased risk. A pooled analysis of cohort studies showed a linear association between alcohol consumption and breast cancer risk up to 60 g/day with a relative risk of 1.09 (95% CI: 1.04-1.13) for each 10 g increment (7). Consequently, alcohol consumption is related to total mortality in a J-shaped manner, reflecting a risk reduction due to cardiovascular mortality with moderate drinking and increases in death from cirrhosis, accidents, violence and cancer in heavier drinkers (6;8). The association of alcohol consumption with total and cardiovascular mortality, and

DM2 is shown in figure 1. This figure indicates that there is a window of benefit for consuming alcohol in moderation.

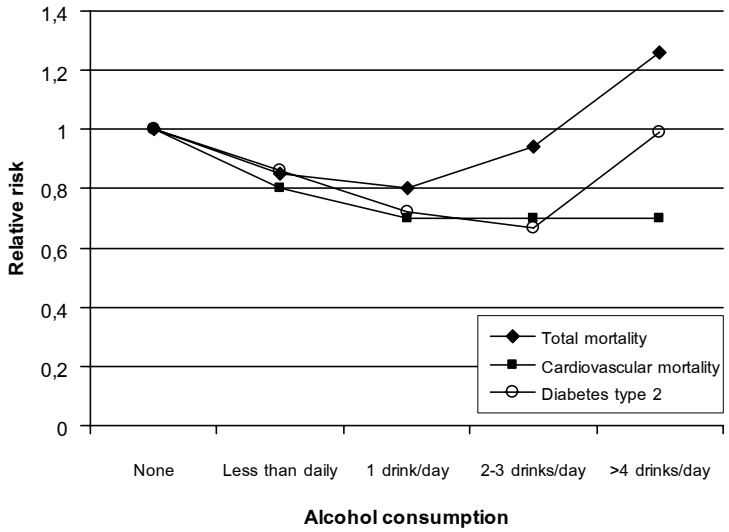


Figure 1: Alcohol consumption and risk of total mortality, cardiovascular mortality and type 2 diabetes. Source: total mortality: Gmel et al. (2003) (132), cardiovascular mortality: Thun et al. (1997) (6) and type 2 diabetes: Koppes et al. (2005) (3).

Definitions of moderate drinking, however, can differ considerably depending on the underlying conceptual basis. The term moderate is used with several different meanings such as a non-intoxicating, statistically normal, non-injurious or problem free drinking, resulting in a range of moderate drinking from 5 g/day up to 60 g/day (9). In this thesis moderate alcohol consumption will be regarded as the amount associated with a reduced overall morbidity and mortality as depicted in figure 1. Using this definition several studies have estimated moderate alcohol consumption to range from 10 to 30 g/day (9). This is consistent with both US and UK guidelines for intake of alcoholic beverages of ~15 g/day for women and 25- 30 g/day for men (10;11) and equals one to three drinks a day based on Dutch units of 10 g alcohol per drink (9).

CARDIOVASCULAR DISEASE

Etiology

CVD includes CHD, cerebrovascular disease, peripheral vascular disease, and sudden death and is the leading cause of morbidity and mortality in the Western world. Worldwide the number of deaths from CVD increased from 14 million in 1990 to more than 16 million in 2000 (12;13). Atherosclerosis is the main cause of CVD. The process of atherosclerosis was initially considered as accumulation of lipids in the arterial wall, but recent research has shown that inflammation plays a key role in atherosclerosis (14;15). The process of atherosclerosis is depicted in Figure 2. It is characterized by accumulation of lipids and fibrous elements in the arteries leading to thickenings of the arterial intima called atherosclerotic lesions. Endothelial dysfunction probably is the first step in the development of these lesions. It can be caused by elevation of LDL cholesterol, oxidative damage by cigarette smoking, diabetes and hypertension. Increased adhesiveness and permeability are key features of endothelial dysfunction, resulting in an increased influx of leucocytes, mainly macrophages and T-cells, and accumulation of LDL in the subendothelial matrix. The trapped LDL undergoes modification, including oxidation, and is taken up by macrophages to form ‘foam cells’. HDL cholesterol can interfere in this process both by removing cholesterol from peripheral tissue and inhibiting lipoprotein oxidation. Secretion of cholesterol by macrophages, mediated by ABCA1, may also protect against atherosclerosis.

This accumulation of foam cells forms the earliest lesions called ‘fatty streaks’. These ‘fatty streaks’ are clinically not significant and they generally appear already during infancy. Finally these foam cells undergo necrosis or apoptosis and are surrounded by a cap of smooth-muscle cells to form the core of the atheroma. Myocardial infarction occurs when the atheromatous process prevents blood flow through the coronary artery. Until recently it was thought that stenosis of the artery was the main cause of ischaemia or infarction, but it is now evident that formation of an occluding thrombus on the surface of the plaque and subsequent rupture of the plaque is the main cause (14;15).

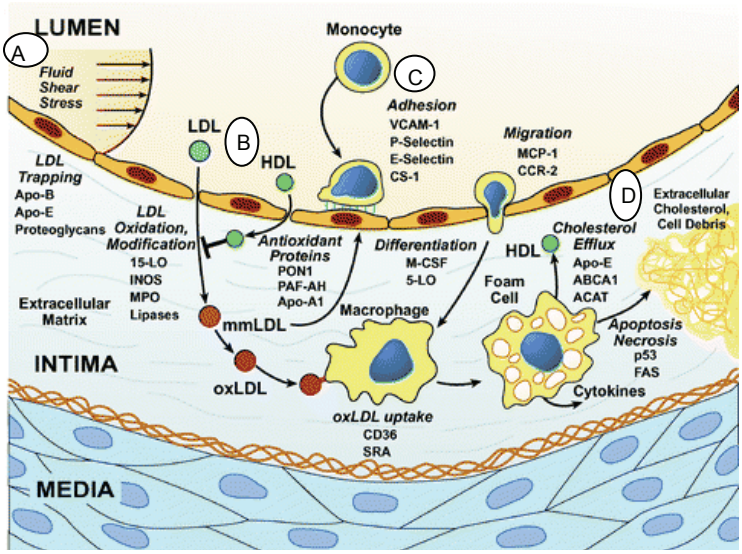


Figure 2: The process of atherosclerosis. Source: Lusis et al. (2004) (25). Moderate alcohol consumption affects the process of atherosclerosis at several points during this process. A. Endothelial dysfunction caused by e.g. smoking, diabetes or hypertension. Alcohol consumption is associated with both decreased risk of diabetes and increased risk of hypertension. B. Accumulation of oxidized LDL in macrophages called foam cells: Alcohol consumption increases HDL cholesterol and its functional properties. Antioxidant proteins PON1 and ApoA1 are increased preventing LDL oxidation. C. Accumulation of oxidized LDL triggers the production of proinflammatory factors, including adhesion molecules. Moderate alcohol consumption is associated with decreased inflammation and adhesion molecules. D. Secretion of cholesterol by macrophages, mediated by ABCA1 and ApoE, may protect against atherosclerosis. Moderate alcohol consumption is shown to increase cholesterol efflux through SRB1 receptor.

Risk factors

Considering this etiology, it seems obvious that smoking, elevated blood pressure, dyslipidemia and diabetes are independently associated with risk of CVD. These traditional risk factors were thought to account for about 50% of CHD cases (16). Recent studies, however, showed that the majority (~90%) of CHD is accounted for by these risk factors (17;18). Apart from these conventional risk factors, several other factors such as obesity (19;20) or inflammation (21) are associated with CVD. Many studies showed that sub-clinical elevations of high sensitive C-reactive protein (CRP) are independently associated with risk of CHD (21). Lipoprotein associated phospholipase A2 (Lp-PLA2), formerly termed

platelet activating factor-acetyl hydrolase, is a novel inflammatory marker. This LDL- and HDL associated enzyme hydrolyses platelet activating factor, but it also effectively hydrolyses oxidized phospholipids (22). Recent studies have shown that increased Lp-PLA2 concentrations (23) or activity (24) are associated with risk of CVD independent from CRP or LDL cholesterol.

Family and twin studies showed that atherosclerosis and its risk factors have a significant genetic component with heritability estimates ranging from 20 to 80% (25). Based on these estimates, many genes should be involved in susceptibility to CVD. Consequently, many association studies for myocardial infarction or coronary artery disease have been performed, but only a few polymorphisms have been convincingly confirmed (25). Examples of these candidate genes are apolipoprotein E (ApoE) and paraoxonase-1 (PON1). Common polymorphisms of ApoE are associated with plasma cholesterol levels (26). Similarly, two polymorphisms of the PON1 gene (M55L and R192Q) are associated with both PON1 activity and risk of CHD (27). Several polymorphisms in ABCA1 gene have been identified and related to both HDL cholesterol levels and CHD, but need further confirmation (28).

Environmental factors, on the other hand, also explain a considerable part of the variation in incidence of CVD between populations (25). Given the risk factors described, it is clear that lifestyle and diet influence the risk for CVD. Physical activity is linearly and inversely associated with risk of CVD (29). High intakes of saturated and trans fatty acids are established risk factors of CVD, while high intakes of polyunsaturated fatty acids such as ω-3 fatty acids and fruits and vegetables are known to be protective (30). Moderate alcohol consumption is also associated with a reduced risk of CVD (1).

ALCOHOL CONSUMPTION AND CVD

Epidemiological evidence

Since the inverse association between alcohol consumption and cardiovascular mortality was first described in ecological studies, more than 50 prospective cohort studies have confirmed this association. These studies have consistently shown that there is a U-shaped association between alcohol consumption and CVD, including stroke and non-CHD deaths. They also indicate that a similar U-shaped association is present for alcohol consumption

and CHD (1;5). Several studies that considered both CVD morbidity and mortality showed that this association may be slightly stronger for non-fatal than fatal events of CHD (31). Atherosclerosis is associated with alcohol consumption in an L-shape with a substantial lessening of lesions even at high intakes (1). In general the risk reduction with moderate alcohol consumption is estimated to be 20-30% (5;31).

Despite the overall consistency, the association was thought to be biased by the presence of 'sick-quitters' among abstainers and, more recently, unmeasured confounding (32). Several studies separated lifetime abstainers (teetotalers) from former drinkers and observed a similar reduced risk of moderate alcohol consumption comparing to teetotalers (5). Therefore this sick-quitters phenomenon does not account for the higher incidence of CHD among abstainers. A more recent example of such concerns is the study of Naimi et al. (32). This study shows that alcohol consumption is associated with 90% of CVD risk factors in a similar manner which could point to unmeasured confounding. However, their results were unadjusted for confounding by race or education and it was not investigated whether these factors were also associated with CVD (as required for a confounder). Indeed, in a re-analysis of their data Mukamal et al. showed that adjusting for race and education generally attenuated these results and physical activity also showed similar associations with most of the risk factors (33).

Based on ecological studies, red wine was thought to specifically exert cardio-protective effects (4). However, several studies that separately assessed consumption of wine, beer and spirits and provided beverage-specific relative risks for CHD, concluded that the risk reduction is attributable to alcohol rather than other components of each beverage (5). Drinking pattern, however, does seem to be an important determinant of CHD risk. Mukamal et al. have shown that number of drinking days a week is inversely associated with risk of CHD. This risk reduction was similar for those drinking 10 g or more than 30 g alcohol per drinking day (34). A recent study showed that associations with drinking pattern may differ for men and women. Among women, quantity of alcohol intake may be the primary determinant of the inverse association between alcohol consumption and risk of CHD whereas among men, drinking frequency, not quantity, seems more important (134). Binge drinking, 'weekend drinking' or drinking until intoxication, on the other hand, are associated with an increased risk of CHD (35-39), but this is not always consistently reported (40;41).

Recently this association of alcohol consumption with CVD in the general population has been extended to other higher risk populations. CVD is a major complication of DM2. Several studies have shown that moderate alcohol consumption is also associated with a decreased risk of CHD among diabetes patients (42-47, 135). Hypertensive patients are also at increased risk of CHD, but only three previous studies showed a decreased risk of cardiovascular mortality with moderate alcohol consumption among hypertensive men (48-50). The association with incident CHD has so far remained unexamined.

Underlying mechanism

In order to confirm this inverse association of moderate alcohol consumption with risk of CVD, many studies have focused on risk factors of CVD to elucidate an underlying mechanism. Numerous observational studies observed a positive association between alcohol consumption and HDL cholesterol (51;52). Moreover, more than 40 randomized controlled studies have also shown an increase in HDL cholesterol after moderate alcohol consumption compared with a non-alcoholic control condition (2). A meta-analysis of Rimm et al. estimated a mean increase of 0.1 mmol/l (= 3.9 mg/dl) for an intake of 30 g alcohol day, accounting for a 16.8% risk reduction of CHD. Several studies have estimated that this alcohol-induced increase of HDL cholesterol explains 50% of the association between moderate alcohol consumption and risk of CVD (51-55). Effects on other lipoprotein fractions are not thought to be involved in the cardioprotective effect of moderate alcohol consumption. Only three studies among women, have shown a decrease of LDL cholesterol after moderate alcohol consumption (56-58). In addition to increased concentration of HDL cholesterol, moderate alcohol consumption also increases functional properties of HDL cholesterol such paraoxonase activity (59;60) and cholesterol efflux (61;62).

Besides these profound effects of HDL cholesterol, inflammatory and fibrinolytic factors may also partly explain the CVD risk reduction of moderate alcohol consumption. A study of Hendriks et al. showed that moderate alcohol consumption affects the fibrinolytic system by a decreasing blood clot formation and stimulating dissolution of existing clots. This decrease of blood clotting typically occurs in early morning at a time that a large proportion of heart attacks generally take place (63). Other studies have confirmed these results (64;65). Recent observational (66-68) studies and an experimental trial of Sierksma

et al. (69) have observed a decrease in CRP with moderate alcohol consumption. Possibly effects of moderate alcohol consumption on other factors such as arterial stiffness (70) or sex hormones (71) may also be involved. A study of Davies et al. showed that moderate alcohol consumption may also improve insulin sensitivity (72). Combining these potential mediators in statistical models, Mukamal et al. recently showed that HDL cholesterol, hemoglobinA1c and fibrinogen fully explained the association between alcohol consumption and CVD among men and accounted for 75% among women (73).

On the other hand alcohol consumption may increase blood pressure and risk of hypertension at intakes of more than 30 gram per day (1;74;75). However, at moderate intakes of 0- 30 g/day the shape of this association is still discussed. Studies reported a linear, threshold or even J-shaped association between alcohol consumption and blood pressure or hypertension (1;75). Langer et al. showed that increased systolic blood pressure may counterbalance the risk reduction by other mediators such as HDL cholesterol by 17% (54). Effects on systolic blood pressure could thus attenuate the risk reduction caused by increased HDL cholesterol concentrations and effects on other mediators.

Causality

Despite overwhelming evidence from observational studies and short-term randomized trials, causality of the association of moderate alcohol consumption and CHD remains to be established with certainty without conclusive evidence from a long-term randomized controlled trial. Because such a long-term randomized controlled trial may not be feasible and is sometimes even regarded unethical, this has not been performed to date.

Therefore other approaches to answer this question of causality have been adopted. Already in 1984 Marmot reviewed the evidence on alcohol consumption and CHD using the Bradford Hill criteria (figure 3) for a causal association on available evidence (76). He then concluded that the evidence was far from complete, but pointed towards a protective effect of moderate alcohol consumption. Since then data became more solid, particularly for consistency (i.e. extended to several other populations), independence (i.e. not confounded by ‘sick-quitter’ effect or smoking) and plausibility (i.e. more insight in mechanism from short-term trials) (77).

Figure 3: Bradford-Hill criteria for causation (126;133)

Strength	Strong associations are more likely to be causal than weak associations. Weak associations are more likely to be explained by undetected biases, but this does not rule out causation.
Consistency	Consistency refers to similar results emerging from several studies done in different populations.
Specificity	This criteria requires a single cause to produce a single effect.
Temporality	This criterion denotes the sequence of events with regards to time. It is an absolute necessity for a causal association; the cause must precede the effect.
Biological gradient	This implies the presence of a dose-response curve: increasing dose must lead to increasing disease frequency. Absence of a dose-response, again, does not rule out a causal association.
Plausibility	This refers to biological plausibility of the observed association. There should be some biologically acceptable or relevant reason for the cause to produce a certain effect. But biological plausibility is reflection of available knowledge as of now; it may change with time.
Coherence	Coherence implies that the association does not conflict with current knowledge about the disease (its natural history, biology, etc.).
Experimental evidence	The strongest support for causation may be revealed by experimental evidence where introduction or removal of an agent can lead to a change in the effect.
Analogy	A previous experience can be used as an analogy to make a causal inference.

According to the theory of 'Mendelian randomization' (78), an alternative approach to confirm a causal association is to show an association between a polymorphism influencing exposure, thus alcohol metabolism, and outcome. Hines et al. therefore evaluated the influence of a common polymorphism in alcohol dehydrogenase 1C, associated with slower ethanol metabolism in vitro (79), on the association between alcohol consumption and CHD (80). They observed an interaction between alcohol consumption and *ADH1C* polymorphism for risk of CHD. Among moderate drinkers, carrying of the *ADH1C*2*2* allele associated with slow metabolism was associated with decreased risk of CHD. Though less pronounced, these results have been confirmed by others (81-83).

In conclusion, these results show a protective effect of moderate alcohol consumption for cardiovascular disease of about 20-30%. The association seems to be U-shaped for both CHD and CVD. Although it remains difficult to fully exclude confounding, this association is not affected by sick-quitters effect and consistent when adjusted for major confounding factors. Beverage type does not influence the association and the protective effect therefore seems to be caused by ethanol per se instead of other components in the drinks. Drinking pattern does affect risk of CVD. The association between moderate alcohol consumption and CVD is explained by an increase of HDL cholesterol for about 50%. Effect on fibrinolysis, blood clotting and glucose tolerance are other important determinants of this risk reduction.

TYPE 2 DIABETES

Etiology

DM2 is a major disease burden in developed and developing countries. It affects an estimated 170 million persons worldwide and its prevalence is expected to double the next 20 years. The prevalence of DM2 is increasing with age and the majority of type 2 diabetic patients are females (84;85). Diabetes is associated with increased morbidity and mortality. Complications include retinopathy, renal failure and neuropathy. Peripheral artery disease, stroke and CHD are macrovascular complications of diabetes. These complications may affect about 1/3 of diabetes patients. Poor glycemic control and glycated end products are thought to play an important role in the development of these complications (86;87).

Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia. Type 1 diabetes is characterized by destruction of pancreatic β-cells, resulting in an absolute deficiency of insulin. Type 2 is the most common form of diabetes and accounts for approximately 90% of the cases. It is characterized by insulin resistance and abnormal insulin secretion, either of which may predominate. Peripheral insulin resistance is considered as the first step in the development of diabetes. Normally the β-cells respond to peripheral insulin resistance by increasing insulin secretion to maintain normal blood glucose concentrations. Defects in insulin secretion were only thought to occur when β-cells are no longer able to compensate for insulin resistance (88-90). Recent studies, however, showed that β-cell dysfunction is already present before glucose intolerance appears supporting the concept that β-cell failure is a primary defect in the development of diabetes (91). Although this concept is still subject to debate, it is apparent that both insulin resistance and β-cell dysfunction are major risk factors for DM2 and are present already by the time hyperglycemia occurs (91).

Although insulin resistance was initially regarded as a defect in glucose metabolism, recent studies showed that insulin resistance may be caused by defects in fat metabolism (Figure 4). It is shown that decreased muscle fatty acid oxidation and increased intramyocellular fatty acid content interferes with insulin signaling and glucose transport (92). In addition, decreased expression of genes involved in oxidative phosphorylation may also contribute to insulin resistance and DM2 (93;94). Finally, adipose tissue synthesizes several proteins that may be involved in the development of insulin sensitivity (95;96). The

most abundant of those proteins is adiponectin, which is thought to improve insulin sensitivity by suppression of hepatic glucose production, or increased glucose uptake and fatty acid oxidation in muscle tissue (97).

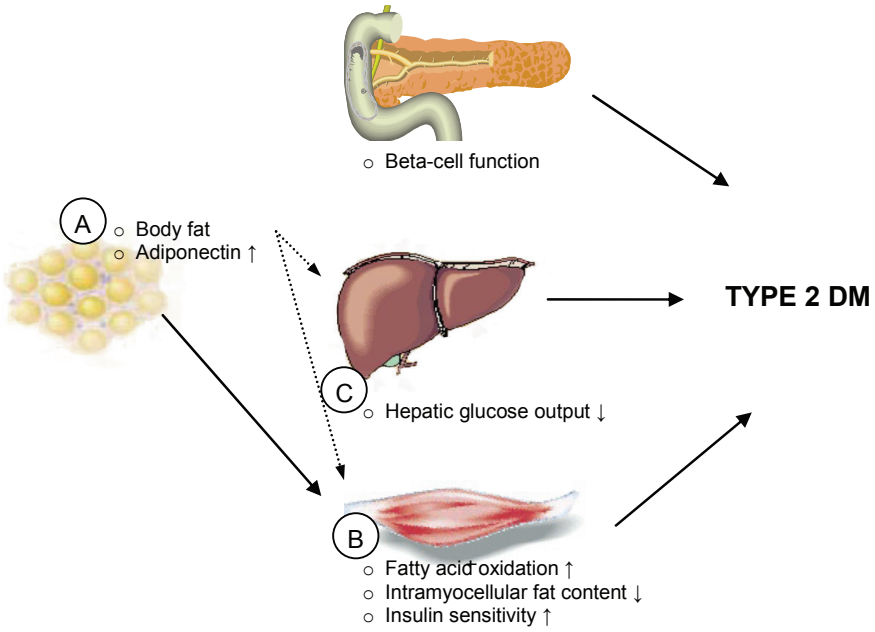


Figure 4: The process of type 2 diabetes. Moderate alcohol consumption may affect the development of DM2 at several points. A: moderate alcohol consumption may increase adiponectin concentrations. B: increased adiponectin concentrations may in turn increase muscle fat oxidation and decrease intramyocellular fat content. This could result in an increase of insulin sensitivity. C: increased adiponectin concentrations may decrease hepatic glucose output.

Risk factors

It is apparent that both beta-cell dysfunction and insulin resistance are major risk factors of DM2. Data from twin studies indicate that genetic susceptibility plays a major role in particularly β-cell dysfunction, while for insulin resistance genetic susceptibility is involved to a lesser extent and seems to be mostly due to environmental factors such as obesity (98;99). Altogether, fenetic susceptibility is an important determinant in the development of DM2 (100). Despite this, only the Pro12Ala polymorphism in the peroxisome proliferator-

activated receptor-γ (PPAR-γ) is consistently associated with a decreased risk of DM2. A meta-analysis of over 3000 individuals showed a modest (1.25 fold), but significant increased risk of DM2 with the more common (~85% frequency) proline allele (101). Another promising candidate gene associated with risk of DM2 is KCNJ11. This encodes for Kir6.2, an ATP-dependent potassium channel associated with the sulfonylurea receptor, that is the target for sulfonylurea drugs, such as tolbutamide and glibenclamide, used to treat DM2 (100).

Apart from these genetic determinants of DM2, studies have shown that a large proportion of cases of DM2 can be attributed to lifestyle factors (102;103). As previously mentioned, obesity is the most important determinant of DM2 (102). In addition, distribution of body fat may contribute to risk of DM2. Abdominal adiposity (waist circumference) is more strongly associated with insulin resistance and risk of DM2 than peripheral adiposity (hip circumference) (104). Independent from obesity, a sedentary lifestyle is associated with an increased risk of DM2 (102). Dietary factors associated with decreased risk of DM2 include high intakes of fiber, polyunsaturated fatty acids and low intakes of trans fatty acids and glycemic load (102). Habitual coffee consumption is also associated with a decreased risk of DM2 (105). In comparison to both abstainers and heavier drinkers, moderate consumers of alcohol also have a decreased risk of DM2 (3).

ALCOHOL CONSUMPTION AND TYPE 2 DIABETES

Epidemiological evidence

The first prospective study to investigate the association between alcohol consumption and DM was performed by Stampfer and colleagues among 85,051 women aged 34 to 59 years (106). They observed an inverse association between alcohol consumption and DM2, which was largely explained by BMI. This inverse association has been confirmed by approximately 15 prospective studies and a recent meta-analysis of Koppes et al. concluded that alcohol consumption is associated to DM2 in a U-shaped fashion (3). This relation has, however, been investigated mainly among male populations (3) as only three studies included both men and women and two included younger women up to 55 years of

age (102;107). Koppes et al. estimated that moderate drinking is associated with a risk reduction of about 30% (3;108).

Similar to CVD, it is shown that beverage type does not influence the association of alcohol consumption with DM2 (107;109). Therefore the risk reduction of DM2 also seems attributable to alcohol per se instead of other components in alcoholic beverages. Drinking pattern also influences risk of DM2. Consuming moderate amounts of alcohol on more than four days a week is also associated with a decreased risk (109) and risk seems to be lowest for those drinking 15 to 30 g alcohol/day on four to seven days a week (107).

As previously described, moderate alcohol consumption is related to a lower incidence of diabetes-related CHD. However, both the association of alcohol consumption with DM2 and CHD appear to be independent. Prospective studies show an inverse association of moderate alcohol consumption with CHD while adjusting for or excluding people with pre-existing DM2 (1). The other way around, two prospective studies observed a reduced risk of DM2 with moderate drinking while adjusting for pre-existing CHD (109;110). However, this does not exclude that a risk reduction of DM2 with moderate alcohol consumption could, at least to some extent, mediate the association with CHD.

For other complications of DM2, however, insufficient data are available to draw conclusions (111). Limited data suggest that moderate alcohol consumption has no acute effect on glycemic control and is not associated with complications of medical therapy among diabetes patients (111).

Underlying mechanism

The underlying mechanism of this decreased risk of DM2 for moderate alcohol consumers is not yet elucidated, but it seems likely that improved insulin sensitivity after moderate alcohol consumption is involved. Several cross-sectional studies reported an inverse association between alcohol consumption and insulin sensitivity (112;113). These observations were confirmed by a randomized controlled trial of Davies et al. that showed a dose-dependent increase of insulin sensitivity after eight weeks of consuming 15 or 30 g alcohol/day among 63 postmenopausal women (72). Other studies, however, observed no change of insulin sensitivity after moderate alcohol consumption (114-116) or only a borderline significant increase in an insulin-resistant subgroup (117). To what extent and by

which mechanism moderate alcohol consumption may improve insulin sensitivity is not yet known, but possibly changes of adipokines such as adiponectin may be involved (117).

Because chronic subclinical inflammation is a risk factor of DM2 (118), anti-inflammatory effects of moderate alcohol consumption may also explain the risk reduction of DM2 (69). Furthermore, alcohol consumption is a recognized risk factor for (delayed) hypoglycemia in patients with type 1 diabetes (119). It seems plausible that also in healthy persons moderate alcohol consumption may decrease postprandial glucose responses. Studies, however, have only investigated this in non-habitual situations of alcohol consumption such as intravenous infusion (120-122). Only a recent study of Greenfield et al. indeed showed a decrease of postprandial glucose concentrations after a meal consumed with moderate amounts of alcohol (123). Whether these potential mediators actually explain the association between alcohol consumption and DM2 has not been investigated to date.

BMI is the most important predictor of DM2 and alcohol consumption is generally considered to increase body weight. However, a positive association between alcohol consumption and BMI is not reported consistently (124). Moreover, among women an inverse association between alcohol consumption and BMI may be present (125). Therefore BMI could confound or even mediate the association between alcohol consumption and DM2.

Causality

Causality of the risk reduction for DM2 with moderate alcohol consumption cannot be assessed with certainty, as no long-term randomized controlled trials with incidence of DM2 as outcome have been performed. The Bradford Hill criteria for causality (126) could be applied to draw a valid conclusion based on currently available evidence, but a summary of the evidence according to those criteria has not been described to date.

When applying these criteria to the current evidence on the association between alcohol consumption and DM2 the following conclusions can be drawn:

1. *Strength*: a recent meta-analysis of 15 prospective studies showed that the relative risk for DM2 of moderate alcohol consumption is 0.7 (3). Although we cannot rule out

that this association is accounted for by an unmeasured confounder, it is unlikely that a factor accounting for this association would have so far remained undetected.

2. *Consistency*: the inverse association between alcohol consumption and risk of DM2 has been consistently shown in case-control or prospective studies in different populations, providing a strong argument for causality (3;111).
3. *Specificity*: alcohol consumption is associated with CVD and DM2 in a very similar fashion. This could argue against causality. However, CVD is a composite endpoint and alcohol consumption shows differing associations with different forms of CVD such as CHD and stroke. In addition, several forms of cancer show a positive, association with alcohol consumption. These findings support a specific relation of alcohol consumption with DM2 and related disorders.
4. *Temporality*: a number of prospective studies have consistently shown a reduced risk of DM2 with moderate alcohol consumption (3).
5. *Biological gradient*: a meta-analysis of 15 prospective studies shows a dose-dependent risk reduction of DM2 up to intakes of 24-48 g/day, while heavier drinking was related to an increased risk of DM2 (3). A randomized controlled trial of Davies et al. showed a dose-dependent increase of insulin sensitivity with moderate alcohol consumption (72).
6. *Plausibility*: several cross-sectional studies and one randomized controlled trial showed increased insulin sensitivity with moderate alcohol consumption (72;112;113), providing a plausible explanation for this association. Results of other trials are, however, not consistent (114-117). A decrease of inflammatory factors (69) and improvement of components of the metabolic syndrome (127) could provide a plausible mechanism as well, but need to be further confirmed in trials and related to DM2.
7. *Coherence*: literature on the association of alcohol consumption with DM2 and CVD and its underlying mechanism so far show coherent results. Global trends of alcohol consumption and attributable risk for CVD and DM2 provide a coherent picture, showing the risk reduction for DM2 and CVD in countries with regular moderate drinking patterns in contrast to countries where drinking patterns are characterized by irregular and heavy drinking (128).
8. *Experimental evidence*: apart from short-term randomized controlled trials for intermediate endpoints, no experimental evidence is currently available.

9. *Analogy*: the association between alcohol consumption and CVD provides a good analogy for DM2.

In summary, the evidence for the association between alcohol consumption and DM2 is not complete, but points towards a causal relation. Particularly with regard to plausibility and experimental studies more evidence is needed. Alternatively, evidence of effect modification of the association between alcohol consumption and DM2 by a functional polymorphism of enzymes in alcohol metabolism could support a hypothesis of causality, but this has not been investigated to date.

In conclusion, similar to CVD moderate alcohol consumption is associated with a decreased risk of DM2. This risk reduction of about 30% seems attributable to alcohol per se instead of other components of alcoholic drinks. Drinking pattern influences risk of DM2. The underlying mechanism is not entirely clear, but it seems likely that improvement of insulin sensitivity is involved. The underlying mechanism for increased insulin sensitivity is not clear, but is potentially mediated by adiponectin. Despite this, evidence for this association is less robust than for CVD and needs further confirmation, particularly with regard to plausibility of the association.

RATIONALE

As described in this chapter, moderate alcohol consumption is associated with both CVD and DM2 in a U-shaped fashion. For CVD this association has been described extensively in the general population, while for DM2 the association with alcohol consumption has been described in less detail. Beverage type influences neither of the associations and thus risk reduction seems attributable to alcohol itself. Drinking pattern, however, is an important determinant of both CVD and DM2 risk.

For CVD the association has been confirmed in populations at increased risk for CVD such as diabetes patients. Hypertensive patients are also at increased risk of CVD and high intakes of alcohol (>30 g/day) increase risk of hypertension. Guidelines on alcohol consumption for hypertensive patients are, however, contradictory (129-131). This could be due to lack of data on the association of alcohol consumption with CVD among hypertensive patients. Similarly, most studies for DM2 included male populations or younger women and data among older women are therefore limited, while the majority of DM2 patients are females and DM2 risk increases with age.

We thus aimed to further quantify the association of alcohol consumption with both CVD and DM2. For CVD we investigated this association among hypertensive men and for DM2 we chose a population of women aged 50 to 70 years. In both studies the influence of beverage type and several measures of drinking pattern on the association were explored. We also investigated the influence of changes of alcohol consumption over time (i.e. changes after diagnosis of hypertension for CVD and lifetime alcohol consumption for DM2) in both analyses.

The risk reduction of CVD with moderate alcohol consumption is largely explained by an increase of HDL cholesterol. In addition, functional properties of HDL cholesterol such as cholesterol efflux and PON activity are improved with moderate alcohol consumption. For DM2 the underlying mechanism of the risk reduction is not yet elucidated. Based on cross-sectional studies it seems likely that an increase of insulin sensitivity is involved, but results from randomized trials are inconsistent. Observational studies showed that the risk reduction of DM2 was dependent on body weight, suggesting that body weight or fat distribution may confound or mediate this association. Furthermore, fat tissue secretes

proteins associated with insulin sensitivity (eg. adiponectin), that could also play a role in the protective effect of moderate alcohol consumption for DM2.

The second aim of this thesis therefore is to investigate the underlying mechanism for the protective effect of moderate alcohol consumption for CVD and DM. For CVD we focused on functional properties of HDL cholesterol that are of particular importance in the etiology of atherosclerosis. Firstly, the effect of moderate alcohol consumption on cholesterol efflux mediated by ABCA1 was investigated. Similar to PON, Lp-PLA2 hydrolyses platelet-activating factor and may be a novel inflammatory marker for CVD. Therefore the effect of moderate alcohol consumption on Lp-PLA2 activity was also investigated. In both studies we also assessed whether BMI modified these relations.

For DM2, we aimed to further elucidate whether moderate alcohol consumption increases insulin sensitivity. A first study investigated whether adipokines are involved in an increase of insulin sensitivity after moderate alcohol consumption. Secondly, we investigated whether changes of body weight and fat distribution are involved in secretion of adipokines and changes of insulin sensitivity. In a third trial we focused on the relation of muscle oxidative capacity and oligomers of adiponectin with insulin sensitivity.

Despite overwhelming evidence from observational and experimental studies, causality of the association between alcohol consumption and both CVD and DM2 remains to be established with certainty. Because polymorphisms are randomly distributed throughout the population, the finding that genotype modifies the association of alcohol intake with diabetes would support the hypothesis that the association is causal (78). This alternative approach was applied by Hines et al. showing a significant interaction between alcohol consumption and a common polymorphism influencing ethanol oxidation for CVD (80). Effect-modification of genetic variation in ethanol oxidation for DM2 has not been studied to date.

Therefore we aimed to investigate the interaction between alcohol consumption and ADH1C polymorphism for DM2 in 2 case-control studies nested in the Nurses' Health Study and Health Professionals Follow-up Study.

OUTLINE OF THESIS

In this thesis, two studies are presented that investigate the association of alcohol consumption with risk of CVD or DM2 in a prospective cohort study. Two chapters describe results of randomized controlled crossover trials on the effect of moderate alcohol consumption on functional properties of HDL cholesterol and three chapters present data from randomized crossover trials investigating the effect of moderate alcohol consumption on insulin sensitivity, fat distribution and adipokines. The results of two case-control studies on the influence of *ADH1C* polymorphism on the association between alcohol consumption and DM2 are presented in another chapter. The thesis is divided in two parts as follows:

Cardiovascular disease

In **chapter 2** we evaluate the association between alcohol consumption and CVD in a prospective cohort of hypertensive men. **Chapter 3 and 4** describe results from randomized controlled crossover trials investigating the effect of moderate alcohol consumption on functional properties of HDL cholesterol. Chapter 3 focuses on cholesterol efflux mediated by ABCA1, while chapter 4 is focused on Lp-PLA2 activity.

Type 2 diabetes

Chapter 5 presents an analysis of the association of alcohol consumption with DM2 in a prospective cohort study of women aged 50 to 70 years. **Chapter 6** describes results from two case-control studies that investigate whether ADH1C polymorphism modifies the association between alcohol consumption and DM2. In **chapter 7, 8 and 9** results from randomized controlled trials investigating the effect of moderate alcohol consumption on insulin sensitivity, fat distribution and adipokines are reported. Chapter 7 focuses on insulin sensitivity and adipokines, while chapter 8 is focused on fat distribution. Chapter 9 evaluates the effect of moderate alcohol consumption on muscular fatty acid metabolism, insulin sensitivity and adiponectin.

REFERENCES

1. Grobbee DE, Rimm EB, Keil U, Renaud S. Alcohol and the Cardiovascular System. In: MacDonald I, ed. Health issues related to alcohol consumption. Bodmin: Blackwell Sciences Ltd 1999:125-79.
2. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523-8.
3. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
4. St Leger AS, Cochrane AL, Moore F. Factors associated with cardiac mortality in developed countries with particular reference to the consumption of wine. Lancet 1979;1:1017-20.
5. Maclure M. Demonstration of deductive meta-analysis: ethanol intake and risk of myocardial infarction. Epidemiol.Rev. 1993;15:328-51.
6. Thun MJ, Peto R, Lopez AD et al. Alcohol consumption and mortality among middle-aged and elderly U.S. adults. N.Engl.J.Med. 1997;337:1705-14.
7. Smith-Warner SA, Spiegelman D, Yaun SS et al. Alcohol and breast cancer in women: a pooled analysis of cohort studies. JAMA 1998;279:535-40.
8. Bagnardi V, Zambon A, Quatto P, Corrao G. Flexible meta-regression functions for modeling aggregate dose-response data, with an application to alcohol and mortality. Am.J.Epidemiol. 2004;159:1077-86.
9. Kalant H, Poikolainen K. Moderate drinking: concepts, definitions and public health significance. In: MacDonald I, ed. Health issues related to alcohol consumption. Bodmin: Blackwell Sciences Ltd. 1999:1-25.
10. Inter-Departmental Working Group. Sensible Drinking. 1995. Wetherby, England, Department of Health.
11. U.S.Department of Agriculture and U.S.Department of Health and Human Services. Dietary Guidelines for Americans, 2005. 6th edition. 2005. Washington DC, US Government Printing Office.
12. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. Lancet 1997;349:1498-504.
13. World Health Organization. World Health Report 2002: Reducing Risks, Promoting Healthy Life. 2002. Geneva, Switzerland, World Health Organization.
14. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N.Engl.J.Med. 2005;352:1685-95.
15. Ross R. Atherosclerosis--an inflammatory disease. N.Engl.J.Med. 1999;340:115-26.
16. Magnus P, Beaglehole R. The real contribution of the major risk factors to the coronary epidemics: time to end the "only-50%" myth. Arch.Intern.Med. 2001;161:2657-60.
17. Greenland P, Knoll MD, Stamler J et al. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. JAMA 2003;290:891-7.
18. Khot UN, Khot MB, Bajzer CT et al. Prevalence of conventional risk factors in patients with coronary heart disease. JAMA 2003;290:898-904.
19. Ajani UA, Lotufo PA, Gaziano JM et al. Body mass index and mortality among US male physicians. Ann.Epidemiol. 2004;14:731-9.
20. Rexrode KM, Carey VJ, Hennekens CH et al. Abdominal adiposity and coronary heart disease in women. JAMA 1998;280:1843-8.
21. Danesh J, Collins R, Appleby P, Peto R. Association of fibrinogen, C-reactive protein, albumin, or leukocyte count with coronary heart disease: meta-analyses of prospective studies. JAMA 1998;279:1477-82.
22. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology,

epidemiology, and possible therapeutic target. Arterioscler.Thromb.Vasc.Biol. 2005;25:923-31.

23. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation 2004;110:1903-8.
24. Oei HH, van dM, I, Hofman A et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation 2005;111:570-5.
25. Lusis AJ, Mar R, Pajukanta P. Genetics of atherosclerosis. Annu.Rev.Genomics Hum.Genet. 2004;5:189-218.
26. Lusis AJ, Fogelman AM, Fonarow GC. Genetic basis of atherosclerosis: part I: new genes and pathways. Circulation 2004;110:1868-73.
27. Durrington PN, Mackness B, Mackness MI. Paraoxonase and atherosclerosis. Arterioscler.Thromb.Vasc.Biol. 2001;21:473-80.
28. Singaraja RR, Brunham LR, Visscher H, Kastelein JJ, Hayden MR. Efflux and atherosclerosis: the clinical and biochemical impact of variations in the ABCA1 gene. Arterioscler.Thromb.Vasc.Biol. 2003;23:1322-32.
29. Williams PT. Physical fitness and activity as separate heart disease risk factors: a meta-analysis. Med.Sci.Sports Exerc. 2001;33:754-61.
30. Hu FB, Willett WC. Optimal diets for prevention of coronary heart disease. JAMA 2002;288:2569-78.
31. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction 2000;95:1505-23.
32. Naimi TS, Brown DW, Brewer RD et al. Cardiovascular risk factors and confounders among nondrinking and moderate-drinking U.S. adults. Am.J.Prev.Med. 2005;28:369-73.
33. Mukamal KJ, Ding EL, Djousse L. Alcohol consumption, physical activity, and chronic disease risk factors: a population-based cross-sectional survey. BMC.Public Health 2006;6:118.
34. Mukamal KJ, Conigrave KM, Mittleman MA et al. Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. N.Engl.J.Med. 2003;348:109-18.
35. Kauhanen J, Kaplan GA, Goldberg DE, Salonen JT. Beer binging and mortality: results from the Kuopio ischaemic heart disease risk factor study, a prospective population based study. BMJ 1997;315:846-51.
36. McElduff P, Dobson AJ. How much alcohol and how often? Population based case-control study of alcohol consumption and risk of a major coronary event. BMJ 1997;314:1159-64.
37. Mukamal KJ, Maclure M, Muller JE, Mittleman MA. Binge drinking and mortality after acute myocardial infarction. Circulation 2005;112:3839-45.
38. Suhonen O, Aromaa A, Reunanen A, Knekt P. Alcohol consumption and sudden coronary death in middle-aged Finnish men. Acta Med.Scand. 1987;221:335-41.
39. Trevisan M, Dorn J, Falkner K et al. Drinking pattern and risk of non-fatal myocardial infarction: a population-based case-control study. Addiction 2004;99:313-22.
40. Hammar N, Romelsjo A, Alfredsson L. Alcohol consumption, drinking pattern and acute myocardial infarction. A case referent study based on the Swedish Twin Register. J.Intern.Med. 1997;241:125-31.
41. Murray RP, Connett JE, Tyas SL et al. Alcohol volume, drinking pattern, and cardiovascular disease morbidity and mortality: is there a U-shaped function? Am.J.Epidemiol. 2002;155:242-8.
42. Ajani UA, Gaziano JM, Lotufo PA et al. Alcohol consumption and risk of coronary heart disease by diabetes status. Circulation. 2000;102:500-5.
43. Diem P, Deplazes M, Fajfr R et al. Effects of alcohol consumption on mortality in patients with Type 2 diabetes

mellitus. Diabetologia. 2003;46:1581-5.
44. Solomon CG, Hu FB, Stampfer MJ et al. Moderate alcohol consumption and risk of coronary heart disease among women with type 2 diabetes mellitus. Circulation. 2000;102:494-9.
45. Tanasescu M, Hu FB, Willett WC, Stampfer MJ, Rimm EB. Alcohol consumption and risk of coronary heart disease among men with type 2 diabetes mellitus. J.Am.Coll.Cardiol. 2001;38:1836-42.
46. Tanasescu M, Hu FB. Alcohol consumption and risk of coronary heart disease among individuals with type 2 diabetes. Curr.Diab.Rep. 2001;1:187-91.
47. Valmadrid CT, Klein R, Moss SE, Klein BE, Cruickshanks KJ. Alcohol intake and the risk of coronary heart disease mortality in persons with older-onset diabetes mellitus. JAMA 1999;282:239-46.
48. Malinski MK, Sesso HD, Lopez-Jimenez F, Buring JE, Gaziano JM. Alcohol consumption and cardiovascular disease mortality in hypertensive men. Arch.Intern.Med. 2004;164:623-8.
49. Palmer AJ, Fletcher AE, Bulpitt CJ et al. Alcohol intake and cardiovascular mortality in hypertensive patients: report from the Department of Health Hypertension Care Computing Project. J.Hypertens. 1995;13:957-64.
50. Renaud SC, Gueguen R, Conard P, Lanzmann-Petithory D, Orgogozo JM, Henry O. Moderate wine drinkers have lower hypertension-related mortality: a prospective cohort study in French men. Am.J.Clin.Nutr. 2004;80:621-5.
51. Gaziano JM, Buring JE, Breslow JL et al. Moderate alcohol intake, increased levels of high-density lipoprotein and its subfractions, and decreased risk of myocardial infarction. N.Engl.J.Med. 1993;329:1829-34.
52. Suh I, Shaten BJ, Cutler JA, Kuller LH. Alcohol use and mortality from coronary heart disease: the role of high-density lipoprotein cholesterol. The Multiple Risk Factor Intervention Trial Research Group. Ann.Intern.Med. 1992;116:881-7.
53. Criqui MH, Cowan LD, Tyroler HA et al. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. Am.J.Epidemiol. 1987;126:629-37.
54. Langer RD, Criqui MH, Reed DM. Lipoproteins and blood pressure as biological pathways for effect of moderate alcohol consumption on coronary heart disease. Circulation 1992;85:910-5.
55. Marques-Vidal P, Ducimetiere P, Evans A, Cambou JP, Arveiler D. Alcohol consumption and myocardial infarction: a case-control study in France and Northern Ireland. Am.J.Epidemiol. 1996;143:1089-93.
56. Baer DJ, Judd JT, Clevidence BA et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am.J.Clin.Nutr. 2002;75:593-9.
57. Clevidence BA, Reichman ME, Judd JT et al. Effects of alcohol consumption on lipoproteins of premenopausal women. A controlled diet study. Arterioscler.Thromb.Vasc.Biol. 1995;15:179-84.
58. van der Gaag MS, Sierksma A, Schaafsma G et al. Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. J.Womens Health Gend.Based.Med. 2000;9:607-16.
59. Sierksma A, van der Gaag MS, van TA, James RW, Hendriks HF. Kinetics of HDL cholesterol and paraoxonase activity in moderate alcohol consumers. Alcohol Clin.Exp.Res. 2002;26:1430-5.
60. van der Gaag MS, van Tol A, Scheek LM et al. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. Atherosclerosis 1999;147:405-10.
61. Sierksma A, Vermunt SH, Lankhuizen IM et al. Effect of moderate alcohol consumption on parameters of reverse cholesterol transport in postmenopausal women. Alcohol Clin.Exp.Res. 2004;28:662-6.

62. van der Gaag MS, van Tol A, Vermunt SH, Scheek LM, Schaafsma G, Hendriks HF. Alcohol consumption stimulates early steps in reverse cholesterol transport. J.Lipid Res. 2001;42:2077-83.
63. Hendriks HF, Veenstra J, Velthuis-te Wierik EJ, Schaafsma G, Kluft C. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. BMJ 1994;308:1003-6.
64. Pikaar NA, Wedel M, van der Beek EJ et al. Effects of moderate alcohol consumption on platelet aggregation, fibrinolysis, and blood lipids. Metabolism 1987;36:538-43.
65. Sierksma A, van der Gaag MS, Kluft C, Bakker M, Hendriks HF. Moderate alcohol consumption and fibrinolytic factors of pre- and postmenopausal women. Nutr.Res. 2001;21:171-81.
66. Albert MA, Glynn RJ, Ridker PM. Alcohol consumption and plasma concentration of C-reactive protein. Circulation 2003;107:443-7.
67. Imhof A, Froehlich M, Brenner H, Boeing H, Pepys MB, Koenig W. Effect of alcohol consumption on systemic markers of inflammation. Lancet 2001;357:763-7.
68. Imhof A, Woodward M, Doering A et al. Overall alcohol intake, beer, wine, and systemic markers of inflammation in western Europe: results from three MONICA samples (Augsburg, Glasgow, Lille). Eur.Heart J. 2004;25:2092-100.
69. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur.J.Clin.Nutr. 2002;56:1130-6.
70. Sierksma A, Muller M, van der Schouw YT, Grobbee DE, Hendriks HF, Bots ML. Alcohol consumption and arterial stiffness in men. J.Hypertens. 2004;22:357-62.
71. Sierksma A, Sarkola T, Eriksson CJP, Gaag MS, Grobbee DE, Hendriks HFJ. Effect of moderate alcohol consumption on plasma dehydroepiandrosterone sulfate, testosterone, and estradiol levels in middle-aged men and postmenopausal women: A diet-controlled intervention study. Alcohol Clin.Exp.Res. 2004;28:780-5.
72. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
73. Mukamal KJ, Jensen MK, Gronbaek M et al. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406-13.
74. Fuchs FD, Chambless LE, Whelton PK, Nieto FJ, Heiss G. Alcohol consumption and the incidence of hypertension: The Atherosclerosis Risk in Communities Study. Hypertension 2001;37:1242-50.
75. Thadhani R, Camargo CA, Jr., Stampfer MJ, Curhan GC, Willett WC, Rimm EB. Prospective study of moderate alcohol consumption and risk of hypertension in young women. Arch.Intern.Med. 2002;162:569-74.
76. Marmot MG. Alcohol and coronary heart disease. Int.J.Epidemiol. 1984;13:160-7.
77. Klatsky AL. Commentary: could abstinence from alcohol be hazardous to your health? Int.J.Epidemiol. 2001;30:739-42.
78. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet 2001;358:1356-60.
79. Bosron WF, Lumeng L, Li TK. Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. Mol.Aspects Med. 1988;10:147-58.
80. Hines LM, Stampfer MJ, Ma J et al. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N.Engl.J.Med. 2001;344:549-55.
81. Djousse L, Levy D, Herbert AG et al. Influence of alcohol dehydrogenase 1C polymorphism on the alcohol-

cardiovascular disease association (from the Framingham Offspring Study). Am.J.Cardiol. 2005;96:227-32.
82. Hines LM, Hunter DJ, Stampfer MJ et al. Alcohol consumption and high-density lipoprotein levels: the effect of ADH1C genotype, gender and menopausal status. Atherosclerosis 2005;182:293-300.
83. Younis J, Cooper JA, Miller GJ, Humphries SE, Talmud PJ. Genetic variation in alcohol dehydrogenase 1C and the beneficial effect of alcohol intake on coronary heart disease risk in the Second Northwick Park Heart Study. Atherosclerosis 2005;180:225-32.
84. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care 1998;21:1414-31.
85. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
86. Nathan DM. Long-term complications of diabetes mellitus. N.Engl.J.Med. 1993;328:1676-85.
87. Williams R, Van GL, Lucioni C. Assessing the impact of complications on the costs of Type II diabetes. Diabetologia 2002;45:S13-S17.
88. Grundy SM. What is the contribution of obesity to the metabolic syndrome? Endocrinol.Metab Clin.North Am. 2004;33:267-82.
89. Nijpels G. Determinants for the progression from impaired glucose tolerance to non-insulin-dependent diabetes mellitus. Eur.J.Clin.Invest 1998;28 Suppl 2:8-13.
90. Saltiel AR, Olefsky JM. Thiazolidinediones in the treatment of insulin resistance and type II diabetes. Diabetes 1996;45:1661-9.
91. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. Diabetologia 2003;46:3-19.
92. Petersen KF, Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. Am.J.Cardiol. 2002;90:11G-8G.
93. Mootha VK, Lindgren CM, Eriksson KF et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat.Genet. 2003;34:267-73.
94. Patti ME, Butte AJ, Crunkhorn S et al. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc.Natl.Acad.Sci.U.S.A 2003;100:8466-71.
95. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 2004;53 Suppl 1:S143-S151.
96. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin.Chem. 2004;50:1511-25.
97. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat.Med. 2001;7:941-6.
98. Lehtovirta M, Kaprio J, Forsblom C, Eriksson J, Tuomilehto J, Groop L. Insulin sensitivity and insulin secretion in monozygotic and dizygotic twins. Diabetologia 2000;43:285-93.
99. Poulsen P, Levin K, Petersen I, Christensen K, Beck-Nielsen H, Vaag A. Heritability of insulin secretion, peripheral and hepatic insulin action, and intracellular glucose partitioning in young and old Danish twins. Diabetes 2005;54:275-83.
100. Florez JC, Hirschhorn J, Altshuler D. The inherited basis of diabetes mellitus: implications for the genetic analysis of complex traits. Annu.Rev.Genomics Hum.Genet. 2003;4:257-91.
101. Altshuler D, Hirschhorn JN, Klannemark M et al. The common PPARgamma Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes. Nat.Genet. 2000;26:76-80.

102. Hu FB, Manson JE, Stampfer MJ et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N.Engl.J.Med. 2001;345:790-7.
103. Knowler WC, Barrett-Connor E, Fowler SE et al. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N.Engl.J.Med. 2002;346:393-403.
104. Frayn KN. Visceral fat and insulin resistance--causative or correlative? Br.J.Nutr. 2000;83 Suppl 1:S71-S77.
105. van Dam RM, Hu FB. Coffee consumption and risk of type 2 diabetes: a systematic review. JAMA 2005;294:97-104.
106. Stampfer MJ, Colditz GA, Willett WC et al. A prospective study of moderate alcohol drinking and risk of diabetes in women. Am.J.Epidemiol. 1988;128:549-58.
107. Wannamethee SG, Camargo CA, Jr., Manson JE, Willett WC, Rimm EB. Alcohol drinking patterns and risk of type 2 diabetes mellitus among younger women. Arch.Intern.Med. 2003;163:1329-36.
108. Carlsson S, Hammar N, Grill V. Alcohol consumption and type 2 diabetes Meta-analysis of epidemiological studies indicates a U-shaped relationship. Diabetologia 2005;48:1051-4.
109. Conigrave KM, Hu BF, Camargo CA, Jr., Stampfer MJ, Willett WC, Rimm EB. A prospective study of drinking patterns in relation to risk of type 2 diabetes among men. Diabetes 2001;50:2390-5.
110. Wannamethee SG, Shaper AG, Perry IJ, Alberti KG. Alcohol consumption and the incidence of type II diabetes. J.Epidemiol.Community Health 2002;56:542-8.
111. Howard AA, Arnsten JH, Gourevitch MN. Effect of alcohol consumption on diabetes mellitus: a systematic review. Ann.Intern.Med. 2004;140:211-9.
112. Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB, Jr., Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care 2000;23:1630-6.
113. Kiechl S, Willeit J, Poewe W et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). BMJ 1996;313:1040-4.
114. Cordain L, Bryan ED, Melby CL, Smith MJ. Influence of moderate daily wine consumption on body weight regulation and metabolism in healthy free-living males. J.Am.Coll.Nutr. 1997;16:134-9.
115. Cordain L, Melby CL, Hamamoto AE et al. Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. Metabolism 2000;49:1473-8.
116. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. Diabetes Care 2003;26:608-12.
117. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
118. Festa A, D'Agostino R, Jr., Tracy RP, Haffner SM. Elevated levels of acute-phase proteins and plasminogen activator inhibitor-1 predict the development of type 2 diabetes: the insulin resistance atherosclerosis study. Diabetes 2002;51:1131-7.
119. Turner BC, Jenkins E, Kerr D, Sherwin RS, Cavan DA. The effect of evening alcohol consumption on next-morning glucose control in type 1 diabetes. Diabetes Care 2001;24:1888-93.
120. Avogaro A, Watanabe RM, Gottardo L, de Kreutzenberg S, Tiengo A, Pacini G. Glucose tolerance during moderate alcohol intake: insights on insulin action from glucose/lactate dynamics. J.Clin.Endocrinol.Metab 2002;87:1233-8.
121. Svartberg J, Holst JJ, Gutniak M, Adner N. The ethanol augmentation of glucose-induced insulin secretion is abolished by calcium antagonism with nifedipine: no evidence for a role of glucagon-like peptide-1 (GLP-1).

Pancreas 1998;16:66-71.

122. Yki-Jarvinen H, Koivisto VA, Ylikahri R, Taskinen MR. Acute effects of ethanol and acetate on glucose kinetics in normal subjects. Am.J.Physiol 1988;254:E175-E180.
123. Greenfield JR, Samaras K, Hayward CS, Chisholm DJ, Campbell LV. Beneficial postprandial effect of a small amount of alcohol on diabetes and cardiovascular risk factors: modification by insulin resistance. J.Clin.Endocrinol.Metab 2005;90:661-72.
124. Macdonald I, Derby G, and Westerterp K. Alcohol and overweight. Heatlth isssues related to alcohol consumption. 263-279. 1993. Brussels, ILSI Europe.
125. Wannamethee SG, Field AE, Colditz GA, Rimm EB. Alcohol intake and 8-year weight gain in women: a prospective study. Obes.Res. 2004;12:1386-96.
126. Hill AB. The environment and disease: association or causation? Proc.R.Soc.Med. 1965;58:295-300.
127. Djousse L, Arnett DK, Eckfeldt JH, Province MA, Singer MR, Ellison RC. Alcohol consumption and metabolic syndrome: Does the type of beverage matter? Obes.Res. 2004;12:1375-85.
128. Rehm J, Room R, Graham K, Monteiro M, Gmel G, Sempos CT. The relationship of average volume of alcohol consumption and patterns of drinking to burden of disease: an overview. Addiction 2003;98:1209-28.
129. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J.Hypertens. 2003;21:1011-53.
130. Chobanian AV, Bakris GL, Black HR et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA 2003;289:2560-72.
131. Goldberg IJ, Mosca L, Piano MR, Fisher EA. AHA Science Advisory: Wine and your heart: a science advisory for healthcare professionals from the Nutrition Committee, Council on Epidemiology and Prevention, and Council on Cardiovascular Nursing of the American Heart Association. Circulation 2001;103:472-5.
132. Gmel G, Gutjahr E, Rehm J. How stable is the risk curve between alcohol and all-cause mortality and what factors influence the shape? A precision-weighted hierarchical meta-analysis. Eur.J.Epidemiol. 2003;18:631-42.
133. Rothman KJ, Greenland S. Modern Epidemiology. Philadelphia, USA: Lippincott-Raven, 1998.
134. Tolstrup J, Jensen MK, Tjonneland A, Overvad K, Mukamal KJ, Gronbaek M. Prospective study of alcohol drinking patterns and risk of coronary heart disease in women and men. BMJ 2006; 332:1244-8.
135. Koppes LL, Dekker JM, Hendriks HF, Bouter, LM, Heine RJ. Meta-analysis of the relationship between alcohol consumption and coronary heart disease and mortality in type 2 diabetic patients. Diabetologia 2006;49(4):648-52.

Chapter 2

Alcohol consumption and risk of coronary heart disease among hypertensive men

Joline WJ Beulens

Eric B Rimm

Alberto Ascherio

Henk FJ Hendriks

Kenneth J Mukamal

Accepted in revised form Ann Intern Med

ABSTRACT

Background

Heavy alcohol consumption increases risk of hypertension, itself a strong risk factor for cardiovascular disease (CVD). However, data on the association between alcohol consumption and CVD among individuals with hypertension are scarce.

Methods

We prospectively determined the association between alcohol consumption and risk of coronary heart disease (CHD) and stroke among 11,982 hypertensive men from the Health Professionals Follow-up Study. Alcohol consumption was assessed every four years using a semi-quantitative food-frequency questionnaire. Incident cases of non-fatal myocardial infarction (MI), fatal CHD, and stroke were documented from 1986 to 2002.

Results

During follow-up, we documented 683 cases of MI. Compared to abstainers, the hazard ratios for MI were 1.12 (95% confidence interval (CI): 0.89-1.41) for men consuming 0.1-4.9 g/day, 0.84 (0.63-1.12) for 5.0-9.9 g/day, 0.69 (0.52-0.92) for 10.0-14.9 g/day, 0.76 (0.57-1.02) for 15.0-29.9 g/day, 0.68 (0.49-0.95) for 30.0-49.9 g/day and 0.42 (0.23-0.78) for ≥50.0 g/day (ptrend <0.001). This association was similar for fatal and non-fatal MI. Alcohol consumption was not associated with total or cardiovascular mortality among this subpopulation of hypertensive men. Risks of total or ischemic stroke for consuming 10-29.9 g/day were 1.37 (0.91- 2.05) and 1.50 (0.88- 2.55) respectively, compared with abstention.

Conclusion

In this population of hypertensive men, moderate alcohol consumption was associated with a decreased risk of MI, but not with risks of total or cardiovascular mortality. As in the general population, hypertensive men who drink moderately and safely may not need change their drinking habits.

INTRODUCTION

Hypertension is a major public health burden in the United States, with 65 million adults affected in 1999, an approximately 30% increase compared to 1991 (1). Hypertension is associated with a doubled risk of cardiovascular disease (CVD) and may account for 30% of CVD events (2).

Moderate alcohol consumption is inversely associated with CVD and total mortality (3, 4). However, intake of more than 30 g alcohol/day (i.e., more than two drinks per day) is associated with an increased risk of hypertension (5, 6). Guidelines for alcohol consumption among patients with hypertension range from a limit of two to three drinks per day to complete abstention (7-9). These contradictory recommendations may reflect the paucity of information on the relation between alcohol consumption and CVD risk among hypertensive patients.

To our knowledge, three studies have addressed the relation of alcohol intake and cardiovascular mortality among hypertensive patients (10-12). Although all reported lower cardiovascular mortality with moderate alcohol consumption, none assessed specific incidence of MI. Importantly, all were limited to alcohol consumption assessed after diagnosis of hypertension and were unable to account for changes of alcohol consumption after the diagnosis of hypertension.

To address these issues, we investigated whether alcohol consumption is inversely associated with CVD and mortality among 11,982 hypertensive participants of the Health Professionals Follow-up Study, a prospective cohort of male health professionals in the United States.

METHODS

The Health Professionals Follow-up Study enrolled 51,529 US male dentists, veterinarians, optometrists, pharmacists, osteopathic physicians, and podiatrists 40 to 75 years of age who returned a mailed questionnaire regarding diet and medical history in 1986. Follow-up questionnaires are sent biennially to update information on exposures and newly diagnosed illnesses.

Hypertensive status was determined from biennial questionnaires. In 1986, participants were asked if they had ever been diagnosed with hypertension and, if so, when. On subsequent questionnaires, participants reported if they had been diagnosed with hypertension in the past two years and, if so, the year of diagnosis. For this analysis, we included 9057 hypertensive men diagnosed during follow-up (i.e. incident hypertensives) and 2925 men with hypertension at baseline that were diagnosed between 1975 and 1986 and reported not changing their alcohol consumption during that time (i.e. prevalent hypertensives). From the full cohort of 51,529 men, we excluded 4397 men reporting a baseline history of cardiovascular disease, stroke or cancer (other than nonmelanoma skin cancer), 81 diagnosed with hypertension after having a CVD event, 1653 who had missing or implausible nutritional information at baseline (including missing alcohol consumption, ≥70 missing food items, or estimated daily energy intake ≤800 or ≥4200 kcal; only 438 of these were actually hypertensive), and 95 whose initial questionnaire had other technical problems. Because we could not determine their prior drinking history, we also excluded 6548 men with hypertension at baseline who were diagnosed before 1975 or reported substantially changing their alcohol consumption within ten years before enrollment. From the remaining men, we identified 11,982 hypertensive men at baseline or during follow-up eligible for analysis. Although our sample size was set by the size of the original HPFS cohort assembled in 1986, a sample size calculation showed that 1700 subjects in abstaining and moderate-drinking categories would detect a relative risk of 0.6 as observed by Malinski et al. (10) with 80% power and accepting a false-positive rate of 0.05.

We assessed alcohol consumption using a 131-item semi-quantitative food-frequency questionnaire with separate items for beer, white wine, red wine and liquor. We specified standard portions as a glass, bottle, or can of beer; a four-ounce glass of wine; and a shot

of liquor. For each beverage participants were asked how often, on average over the past year, they consumed that amount. We calculated ethanol intake by multiplying the frequency of consumption of each beverage by the alcohol content of the specified portion size (12.8 g for beer, 11.0 g for wine, and 14.0 g for liquor) and summing across beverages (13). This process was repeated in 1990, 1994, and 1998, with an added item about light beer in 1994. In 1986, men also reported the number of days per week that they typically drank any form of alcohol. We examined associations between alcohol consumption estimated with the food-frequency questionnaire with consumption estimated with two one-week dietary records collected approximately six months apart among 136 participants residing in eastern Massachusetts (14, 15); the Spearman correlation coefficient between these two measures was 0.86. Estimated mean alcohol intake was 12.8 g/day using dietary records and 12.5 g/day with the food-frequency questionnaire, with no evidence of systematic bias. The correlation coefficient for drinking frequency between our questionnaire and diet records was 0.79.

Our primary endpoints were incident non-fatal MI and fatal coronary heart disease or stroke. MI and stroke were first identified by self-reports on biennial questionnaires and then confirmed by medical records with World Health Organization or National Survey of Stroke criteria (16). Fatal MI and stroke were confirmed by medical records or autopsy reports. All endpoints were confirmed by a physician blinded to exposure status. Approximately 80% of cases of MI were considered definite; the remainder were considered probable and excluded as endpoints in sensitivity analyses. Further details of the confirmation of endpoints are published elsewhere (17, 18). Other endpoints were total, cardiovascular, and alcohol-augmented mortality, according to Thun et al. including cirrhosis of the liver, alcohol-related cancers, accidents and other external causes, cancer of colon and rectum, pneumonia and hemorrhagic stroke (19).

Covariate information was derived from biennial questionnaires. Nutritional information was derived from food-frequency questionnaires. Participants self-reported cardiovascular risk factors (self report of physician-diagnosed diabetes and hypercholesterolemia; cigarette smoking; physical activity; and weight) every two years. Aspirin use included use of aspirin and aspirin-containing products at least twice weekly. Regular use of lipid-lowering therapy and several categories of anti-hypertensive medication (diuretics, beta-blockers, calcium-channel blockers, alpha-blockers, or other) were also recorded biennially. Nutritional

variables, anthropomorphic measures, aspirin use, and self-reported CVD risk factors have been validated (20-25).

We calculated person-years of follow-up from the date of return of the 1986 questionnaire (for men with hypertension at baseline) or date of diagnosis of hypertension (for incident cases of hypertension) to the date of first CVD event, the date of death, or January 31 2002. We used Cox proportional hazards models with time-varying covariates to estimate hazard ratios for categories of alcohol intake, relative to abstainers (26). We simultaneously controlled for age; smoking (never, former, 1-14 cigarettes/d, 15-24 cigarettes/d, ≥25 cigarettes/d); body mass index (in quintiles); diabetes; hypercholesterolemia; parental history of MI; aspirin use; lipid-lowering therapy; leisure-time physical activity (five categories); energy intake (in quintiles); and energy-adjusted intake of saturated fat, trans fatty acids, folate, vitamin E, magnesium, sodium, potassium, ω-3 fatty acids and dietary fiber (each in quintiles). Alcohol consumption and dietary variables were updated every four years; other potential confounders were updated every two years. Indicator variables were assigned where smoking, BMI or physical activity information was missing, but this only accounted for ~1% of cumulative person-time. To assess influence of missing information, a complete-case analysis was also performed. We stopped updating alcohol use upon the development of cancer (other than non-melanoma skin cancer) in all analyses. In secondary analyses for CHD outcomes, we censored men when they developed cancer. To examine possible confounding by socio-demographic status, we additionally adjusted for marital and employment status (full-time, part-time, retired), profession, and race in the multivariate model. We conducted tests of linear trend across increasing categories of alcohol consumption by treating the midpoints of consumption in categories as a continuous variable. The possibility of a non-linear relation between alcohol consumption and risk of MI was examined with restricted cubic splines (27) and tested with the likelihood ratio test, comparing models with only the linear term to those with linear and cubic spline terms. None of the non-linear models provided significantly better model-fit than linear models.

We used the number of anti-hypertensive medications that participants reported as a proxy for severity of hypertension to assess if severity of hypertension altered the association between alcohol consumption and MI. Indicator-variables for use of different hypertensive medications were examined in a similar manner. Interactions of alcohol

consumption with BMI, smoking, folate intake, and number of anti-hypertensive medication were investigated by comparing model likelihoods of models with main effects only to those additionally adjusted for interaction terms.

In primary analyses, we assessed the association of alcohol intake and risk of MI, stroke or mortality among both incident and prevalent hypertensive men. For analyses of beverage type and drinking frequency, we controlled for the standard covariates incorporated into other models. For beverage-specific analyses, we also included intake of each of the other beverage types in the model; sensitivity analyses that considered beverages individually were similar. We separated beer and liquor consumption into four categories (none, 0.1- 9.9 g/d, 10.0- 29.9 g/d, and ≥30 g/d), as they were consumed in larger quantities than other beverages, and white and red wine intake into three categories (none, 0.1-14.9 g/d, ≥15 g/d).

We also conducted secondary analyses restricted to men who developed hypertension during follow-up (i.e., 5237 incident hypertensive men from who we had valid information on alcohol use prior to and following diagnosis of hypertension) to assess if the association between alcohol consumption and risk of MI differed when we used pre-diagnosis or post-diagnosis alcohol intake, or when we included only those men whose alcohol consumption did not change by more than 7.5 g/day (~½ drink/day) after diagnosis. We assessed whether increasing or decreasing alcohol consumption by >7.5 g/day after diagnosis was associated with risk of MI compared with not changing by including indicator variables in a model with post-diagnosis alcohol consumption.

The Cox proportional hazards assumption was examined with interaction terms between time and alcohol consumption (28), with no violations detected. SAS statistical package, version 8.2 (SAS Institute, Cary, North Carolina), was used for all analyses.

RESULTS

Baseline characteristics

Table 1 shows the characteristics of the 11,982 men with hypertension, categorized by their alcohol consumption assessed on the first dietary questionnaire after diagnosis of hypertension. Approximately 50% of the study population used anti-hypertensive medication, which increased with increasing alcohol consumption. Heavier alcohol consumption was also associated with a greater prevalence of smoking and a lower prevalence of diabetes. Liquor and beer were the alcoholic beverages consumed in greatest quantity.

Alcohol consumption and Risk of MI

During 16 years of follow-up, we documented 683 cases of total MI, 293 fatal and 390 non-fatal. Table 2 shows the associations between alcohol consumption and risk of MI. There was little difference in risk between abstainers and men who consumed 0.1-4.9 g/day. We observed a roughly dose-dependent inverse association between alcohol consumption and risk of MI, both in age- and smoking-adjusted and multivariate models and for fatal and non-fatal endpoints. Analyses excluding dietary variables yielded similar results (hazard ratio for 15.0- 29.9 g/day: 0.74; 95%- CI: 0.56- 0.98). Adjusting for marital and employment status, profession and race in our models did not alter the results (0.75; 95%-CI: 0.56- 0.99), nor did adjusting for occurrence of renal failure or liver disease (0.75; 95%-CI: 0.57- 0.99). Restricting to confirmed cases of MI (0.80; 95%-CI: 0.57- 1.12) or excluding heavier drinkers of ≥50 g/day (0.76; 95%-CI: 0.57- 1.01) also showed similar results. A complete case analysis excluding those with missing information on covariates (~ 4%) also showed similar results (0.72; 95%-CI: 0.54- 0.97).

The number of anti-hypertensive medications that men reported using tended to be associated with an increased risk of MI (hazard ratio for each additional medication: 1.08; 95% confidence interval (CI): 0.96- 1.20) and total stroke (1.07: 95%-CI 0.87- 1.32), but inclusion of this factor in our analyses did not alter the observed associations for alcohol (0.76; 95%-CI: 0.57- 1.02), nor did adjustment for use of individual anti-hypertensive drug classes (0.75; 95%-CI: 0.56- 0.99). No interaction between alcohol consumption and number of anti-hypertensive medication ($p_{interaction}$= 0.60) was observed. This was also the case for interactions with smoking (p> 0.99), BMI (p= 0.68) and folate intake (p= 0.69). We had limited power to assess interactions with individual drug classes.

Table 1: Base-line characteristics[1] of 11,982 prevalent and incident hypertensive men from the Health Professionals Follow-up Study.

	Alcohol consumption (g/day)						
	0	0.1-4.9	5.0-9.9	10.0-14.9	15.0-29.9	30.0-49.9	>50.0
N	2587	2631	1639	1603	1767	1284	471
Age (years)	61 ±	61 ±	60 ±	61 ± 9	60 ± 9	61 ± 9	60 ±
BMI (kg/m2)	27.0	26.8	26.4	26.4	26.4	26.4	26.6
Physical activity (METs/week)[2]	27.0	26.5	30.1	28.6	31.5	27.3	24.8
Alcohol consumed (g/day)[3]							
Beer	0	0.7	2.1	3.6	5.4	10.3	24.6
Red wine	0	0.4	1.0	1.7	3.1	2.9	6.2
White wine	0	0.7	1.3	2.1	3.4	2.9	6.8
Liquor	0	0.6	2.1	4.4	7.5	21.4	31.7
Smoking (%)							
Never	54.2	44.8	41.3	34.7	31.4	22.2	21.5
Former	40.4	49.6	51.8	56.3	59.3	60.6	58.6
Current	4.4	4.7	6.0	6.4	7.8	15.4	18.0
Diabetes (%)	9.4	6.3	4.9	4.7	4.8	4.4	4.3
Hypercholesterolemia (%)	39.8	38.3	38.5	38.2	38.5	38.1	39.7
Family history of MI (%)	31.4	32.6	35.3	33.3	35.7	34.8	36.8
Aspirin use (%)	41.6	42.8	45.1	44.8	46.0	48.0	45.9
Lipid lowering therapy (%)	5.9	6.5	5.9	7.4	7.3	7.1	7.7
Anti-hypertensive medication							
0	57.2	53.6	50.9	53.5	51.4	47.7	48.8
1	34.7	37.7	38.1	36.7	38.0	39.0	39.7
2	7.4	8.0	9.6	8.8	9.5	11.8	10.8
>2	0.7	0.7	1.4	1.0	1.1	1.5	0.7
Energy intake (kcal/day)	1906	1909	1926	1910	2060	2122	2390
Saturated fatty acids (g)[4]	24.1	23.5	23.1	23.2	22.5	22.1	19.5
Trans fatty acids (g)[4]	3.4	3.4	3.2	3.1	3.0	2.9	2.4
Omega-3 fatty acids (g)[4]	0.28	0.34	0.35	0.33	0.35	0.29	0.28
Dietary fiber (g)[4]	23.0	22.6	22.3	21.9	21.1	18.5	16.8
Sodium (mg)[4]	2636	2599	2631	2659	2645	2575	2422
Potassium (mg)[4]	3464	3519	3531	3506	3475	3243	3052
Magnesium (mg)[4]	372	375	377	376	372	351	351
Folate (mcg)[4]	542	557	551	545	533	507	471
Vitamin E (mg)[4]	96	98	105	106	102	102	101

[1] All characteristics except age are age-adjusted
[2] MET= metabolic equivalent
[3] Absolute amount of alcohol (g/day) from each beverage type.
[4] Energy-adjusted

We also observed similar associations when abstainers who used any alcohol before diagnosis of hypertension were excluded. For example, the hazard ratio for risk of MI was 0.78 (95% CI, 0.58- 1.05) among men who consumed 15.0- 29.9 g alcohol/day, similar to the 0.76 (0.57- 1.02) in the main analyses. Similarly, excluding smokers from our analyses (0.76; 95%-CI: 0.55- 1.05) or censoring for development of cancer (0.73; 95%-CI: 0.54- 0.97) did not alter our results.

Table 2: Alcohol consumption and risk (95% confidence interval (CI)) of total myocardial infarction (MI), fatal coronary heart disease (CHD) and non-fatal MI among 11,982 hypertensive men from the Health Professionals Follow-up Study.

	Alcohol consumption (g/day)							P-value
	0	0.1-4.9	5.0- 9.9	10.0- 14.9	15.0- 29.9	30.0- 49.9	≥50.0	for trend
Total MI								
Cases, n	174	177	84	82	86	65	15	
Hazard ratio (95% CI)[1]	1.0	1.05	0.77	0.67	0.68	0.62	0.42	< 0.001
		(0.84- 1.32)	(0.59- 1.02)	(0.50- 0.88)	(0.52- 0.90)	(0.46- 0.85)	(0.23- 0.75)	
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	1.12	0.84	0.69	0.76	0.68	0.42	< 0.001
		(0.89- 1.41)	(0.63- 1.12)	(0.52- 0.92)	(0.57- 1.02)	(0.49- 0.95)	(0.23- 0.78)	
Fatal CHD								
Cases, n	82	67	40	28	39	29	8	
Hazard ratio (95% CI)[1]	1.0	0.87	0.77	0.47	0.67	0.56	0.41	0.003
		(0.62- 1.24)	(0.52- 1.16)	(0.30- 0.75)	(0.44- 1.01)	(0.35- 0.89)	(0.19- 0.93)	
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	0.95	0.88	0.47	0.84	0.62	0.42	0.021
		(0.65- 1.37)	(0.56- 1.35)	(0.29- 0.76)	(0.53- 1.31)	(0.37- 1.04)	(0.17- 1.03)	
Non-fatal MI								
Cases, n	92	110	44	54	47	36	7	
Hazard ratio (95% CI)[1]	1.0	1.21	0.77	0.84	0.70	0.68	0.41	< 0.001
		(0.90- 1.63)	(0.53- 1.13)	(0.59- 1.20)	(0.48- 1.01)	(0.45- 1.03)	(0.18- 0.97)	
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	1.27	0.84	0.87	0.72	0.73	0.41	0.002
		(0.94- 1.72)	(0.56- 1.24)	(0.60- 1.26)	(0.49- 1.06)	(0.47- 1.13)	(0.17- 1.00)	

[1]Age- and smoking adjusted.

[2]Adjusted for age smoking, BMI, physical activity, diabetes, hypercholesterolemia, family history of MI, aspirin use, lipid lowering therapy, energy intake and energy-adjusted quintiles of saturated fat, trans fatty acids, sodium, potassium, magnesium, folate, vitamin E, ω-3 fatty acids and dietary fiber.

Alcohol Consumption and Risks of Stroke and Death

The association between alcohol consumption and risks of stroke and mortality are shown in table 3. Although alcohol consumption tended to be associated with lower risk of cardiovascular mortality, no significant associations between alcohol consumption and total and cardiovascular mortality were observed. However, restricting to ischemic heart disease mortality showed a similar inverse association as for MI endpoints (hazard ratios 0.53 (95%-CI: 0.33-0.87) for 10.0-14.9 g/day, 0.77 (95%-CI: 0.48-1.23) for 15.0-29.9 g/day, and 0.64 (95-% CI: 0.38-1.09) for 30.0-49.9 g/day; ptrend= 0.031). Surprisingly, mortality from causes commonly associated with alcohol use was not associated with alcohol consumption in this population, but few cases (n= 142) were observed.

As seen in table 3, we also identified no significant associations or trends with risk of ischemic stroke, but the 95% confidence limits do not rule out an association of moderate strength. Although we only observed 38 cases of hemorrhagic stroke during follow-up, consumers of ≥30 g/day had a hazard ratio of 1.82 (95%-CI: 0.54- 6.07).

Beverage type, Drinking Frequency and Risk of MI

We explored the relationships of both beverage type and drinking frequency with risk of MI. For beverage type, liquor consumption (the alcoholic beverage most commonly used by these men) was most strongly associated with lower risk. For example, consumers of 10.0-29.9 g alcohol/day from liquor had a hazard ratio of 0.58 (95%-CI: 0.43- 0.79) for total MI (p_{trend}< 0.001). The corresponding hazard ratios were 1.00 (95%-CI: 0.61- 1.67; p_{trend}= 0.99) for red wine, 0.97 (95%-CI: 0.58- 1.62; p_{trend}= 0.70) for white wine and 1.04 (95%-CI: 0.71- 1.51; p_{trend}= 0.84) for beer.

Drinking frequency was inversely associated with risk of MI, with an adjusted hazard ratio of 0.68 (95%-CI: 0.54- 0.85) for intake ≥5 days/week, compared with intake <1 day/week (p_{trend} <0.001). When alcohol consumption was included in the model, the corresponding hazard ratio was 0.84 (95%-CI: 0.61- 1.16; p_{trend}= 0.175). However, we could not assess the relative importance of drinking frequency and quantity consumed per drinking day. Because nearly all men with hypertension consumed 1-2 drinks per drinking day, too little variation in drinks per drinking day existed to draw valid conclusions.

Table 3: Alcohol consumption and risks (95% confidence interval (CI)) of total and ischemic stroke and total, cardiovascular and alcohol-augmented mortality among 11,982 hypertensive men from the Health Professionals Follow-up Study.

Variable	Alcohol consumption (g/day)							P-value
	0	0.1-4.9	5.0- 9.9	10.0- 14.9	15.0- 29.9	30.0- 49.9	≥50.0	trend
Total mortality								
Cases, n	296	238	148	167	156	146	48	
Hazard ratio (95% CI)[1]	1.0	0.89 (0.74- 1.07)	0.86 (0.70- 1.06)	0.87 (0.71- 1.07)	0.77 (0.62- 0.94)	0.85(0.68- 1.06)	0.85 (0.61- 1.19)	0.177
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	0.98 (0.81- 1.18)	0.96 (0.77- 1.20)	0.95 (0.77- 1.18)	0.94 (0.75- 1.17)	1.00 (0.78- 1.27)	0.89 (0.61- 1.28)	0.65
Cardiovascular mortality								
Cases, n	104	92	46	51	53	47	17	
Hazard ratio (95% CI)[1]	1.0	0.98 (0.73- 1.33)	0.77 (0.53- 1.11)	0.74 (0.52- 1.07)	0.75 (0.52- 1.07)	0.74 (0.51- 1.07)	0.86 (0.48- 1.52)	0.175
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	1.08 (0.78- 1.48)	0.82 (0.56- 1.21)	0.79 (0.54- 1.15)	0.87 (0.59- 1.27)	0.81 (0.54- 1.22)	0.91 (0.48- 1.71)	0.36
Alcohol consumption (g/day)	0	0.1- 9.9		10.0- 29.9		≥50.0		
Alcohol-augmented mortality[3]								
Cases, n	39	36		39		28		
Hazard ratio (95% CI)[1]	1.0	0.62 (0.38- 1.01)		0.73 (0.45- 1.20)		0.81 (0.46- 1.43)		0.98
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	0.71 (0.42- 1.22)		0.84 (0.48- 1.48)		0.87 (0.43- 1.77)		0.94
Total stroke								
Cases, n	51	63		74		38		
Hazard ratio (95% CI)[1]	1.0	0.81 (0.55- 1.20)		1.22 (0.83- 1.80)		0.98 (0.61- 1.57)		0.53
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	0.83 (0.55- 1.24)		1.37 (0.91- 2.05)		0.94 (0.55- 1.60)		0.59
Ischaemic stroke								
Cases, n	31	34		45		23		
Hazard ratio (95% CI)[1]	1.0	0.71 (0.42- 1.19)		1.28 (0.78- 2.11)		0.99 (0.53- 1.83)		0.41
Multivariate-adjusted hazard ratio (95% CI)[2]	1.0	0.71 (0.41- 1.21)		1.50 (0.88- 2.55)		1.22 (0.60- 2.46)		0.142

[1]Age- and smoking adjusted.

[2]Adjusted for age smoking, BMI, physical activity, diabetes, hypercholesterolemia, family history of MI, aspirin use, lipid lowering therapy, energy intake and energy-adjusted quintiles of saturated fat, trans fatty acids, sodium, potassium, magnesium, folate, vitamin E, ω-3 fatty acids and dietary fiber.

[3]Including cirrhosis of the liver, alcohol-related cancers, accidents and other external causes, cancer of colon and rectum, pneumonia and hemorrhagic stroke.

Alcohol consumption before and after diagnosis of hypertension

Among incident hypertensive men of whom we had valid information on alcohol consumption both before and after diagnosis of hypertension, changes of alcohol consumption were assessed. Alcohol consumption slightly decreased by 0.4 g/day (inter-quartile range: -1.9 to 2.0 g/day) after diagnosis of hypertension. Approximately 78% of men did not alter their alcohol consumption by more than half a drink per day after diagnosis of hypertension. Alcohol consumption before and after diagnosis of hypertension were highly correlated (Spearman r=0.83; p< 0.001). About 12% of abstainers after diagnosis of hypertension reported any alcohol use before diagnosis, and ~2% reported use of >15 g/day before diagnosis.

As expected from the strong relationships between alcohol intake before diagnosis and after diagnosis in this population of men, the association between alcohol consumption with risk of MI was similar when we used pre-diagnosis or post-diagnosis (Table 2) intake levels. Using pre-diagnosis alcohol consumption, hazard ratios for MI were 1.23 (95%-CI: 0.86- 1.74) for 0.1-9.9 g/day, 0.95 (95%-CI: 0.64- 1.43) for 10.0- 29.9 g/day and 0.54 (95%-CI: 0.32- 0.94) for ≥30.0 g/day. Results were also quite similar when we restricted our analyses to men whose alcohol consumption did not change by more than 7.5 g/day following diagnosis. Compared to men who did not substantially change their alcohol consumption after diagnosis, hazard ratios for total MI were 0.89 (95%-CI: 0.56- 1.41) for men who increased and 1.45 (95%-CI: 0.85-2.49) for men who decreased alcohol consumption after diagnosis of hypertension (p_{trend}= 0.160).

DISCUSSION

In this prospective analysis of 11,982 hypertensive men, alcohol consumption was associated with a decreased risk of fatal and non-fatal MI, but not with risks of total and cardiovascular mortality. The associations of total and ischemic stroke with moderate alcohol consumption were indeterminate due to few cases of stroke.

An English-language Medline search to March 2006 revealed only four similar studies on this association that we are aware of (10-12, 29). Palmer et al. reported a reduced risk of ischemic heart disease mortality among hypertensive men consuming >21 units of alcohol per week (or approximately 24 g/day) (11). Malinski et al. found a strong, linear, inverse association of alcohol consumption with cardiovascular mortality among 14,125 male physicians with hypertension followed over 5.4 years (10). Neither of these studies, however, assessed whether alcohol consumption had changed after diagnosis of hypertension and, if so, whether this influenced the relation with CVD mortality. It seems likely that some men decrease their alcohol consumption after diagnosis of hypertension on the advice of their physician or because of therapeutic contraindications, although good evidence that this strategy decreases blood pressure is lacking (30). We did not find evidence of a substantial decrease of alcohol consumption after diagnosis in this group of men. As a consequence, the association between alcohol consumption and MI was similar using pre- or post-diagnosis intake levels.

We are unaware of previous studies of the association between alcohol consumption and non-fatal MI among hypertensive men. Some studies in the general population have suggested a more modest association of alcohol consumption with fatal than non-fatal events (31, 32), although other evidence suggests moderate alcohol intake is associated with a lower case-fatality rate of MI (33). In our analyses, the inverse association with alcohol consumption was similar for fatal and non-fatal events. In the only other study of non-fatal CVD, albeit limited to stroke, Kiyohara et al. observed a slightly lower risk of ischaemic stroke for consumers of <34 g/day and an increased risk for consumers of ≥34 g/day (29).

Despite the inverse associations between alcohol consumption and cardiovascular mortality in earlier studies (10, 11), we did not observe a significant association between alcohol consumption and total or cardiovascular mortality. However, cardiovascular

mortality only accounted for ~1/3 of total mortality, which may be due to a decline of case-fatality rate of MI and better secondary preventions strategies in the last few decades (34). Thus, cardiovascular mortality reflects several other factors in addition to MI incidence and, in this population, may particularly reflect other deaths from stroke and other CVD that are less strongly associated with alcohol consumption than MI (17), thereby attenuating the association. Indeed, our results cannot rule out a slightly increased risk of stroke with moderate to heavier intake of alcohol and restricting to ischemic heart disease mortality showed a similar inverse association as for MI endpoints.

One concern in any study of alcohol consumption and CVD is that any lower risk may be offset by higher of other, non-CVD related problems. We did, however, not observe significant associations between alcohol consumption and alcohol-augmented or cancer mortality, and censoring upon diagnosis of cancer in our analyses did not alter our results, although analyses of alcohol-augmented mortality were necessarily limited by the fortunate rarity of these causes of death. This makes it unlikely that the lower risk of MI for heavier drinkers observed in this study is caused by higher mortality due to other causes.

We could not determine the influences of drinking frequency and beverage type with precision in this study. We observed a significant inverse association between drinking frequency and risk of total MI, as is true for the full Health Professionals Follow-up Study cohort (35), but this could not be disentangled from average alcohol consumption due to the co-linearity of these two measures of alcohol intake among our subpopulation of hypertensive men.

Likewise, we observed the strongest inverse associations between alcohol consumption and risk of MI for liquor, the predominant beverage type in this population. However, we had limited power to explore the associations with other beverage types, making it difficult to draw firm conclusions on beverage type specific risk of MI. In a French study, Renaud et al. suggested that moderate alcohol consumption was associated with decreased mortality among hypertensives only for wine drinkers (12). Our findings and those of Renaud et al. (12) are consistent with the hypothesis that the beverage most widely consumed by a certain population is most likely to be inversely associated with the risk of CVD in that population (36). Therefore this inverse association is most likely due to ethanol itself. Statistical models suggest that about half of the association of alcohol consumption with CHD is mediated by an increase of HDL cholesterol (37, 38). A decrease

of inflammatory factors (39), improved insulin sensitivity (40) or change of thrombogenic markers (41) after moderate alcohol consumption may also be involved.

Certain limitations of this study need to be addressed. As in any observational study, our results could be influenced, at least in part, by differences in factors other than alcohol consumption. We simultaneously controlled for age, smoking, BMI, physical activity, diabetes, hypercholesterolemia, aspirin use, lipid-lowering therapy, diet and number and type of antihypertensive medication. Furthermore, our sample was homogeneous with respect to occupational class and gender, which should reduce residual confounding, but restricts our ability to generalize to women. Similar results were observed excluding abstainers at diagnosis of hypertension that reported having decreased their alcohol consumption (42).

In addition, we performed a sensitivity analysis using probabilistic methods to assess how large an unmeasured confounder would need to be to have produced our results (43, 44). We assumed a distribution of this confounder similar to that of smoking (40- 50% among consumers of 15.0- 29.9 g/day and 50- 60% among consumers of 0 g/day) and a relative risk for MI of this confounder ranging from two to six. The ranges of these assumptions were applied in 4000 simulation iterations, showing a median relative risk of moderate alcohol consumption with MI of 0.86 (95% simulation interval: 0.79- 0.97). Although such unmeasured confounding may attenuate the observed risk reduction, we believe it unlikely that a confounder with a magnitude and prevalence greater than this would remain undetected.

We relied on self-report of alcohol consumption, hypertension and other covariates in this study that may be subject to misclassification. The validity of alcohol consumption and other nutrients for the full cohort was confirmed against dietary records (15, 16). Despite this, we cannot exclude that misclassification and correlated errors are present in nutritional variables. Simulation studies have shown that highly correlated errors as can be present in nutritional variables may strongly affect observed estimates (45). The inclusion of nutrients in our models did not influence observed risk estimates, although our estimated confidence limits may nonetheless not fully reflect the variability induced by correlated error. We also performed a sensitivity analysis using probabilistic methods based on 3000 iterations (43, 44) for non-differential misclassification of alcohol intake assuming a sensitivity and specificity ranging from 0.8 to 1.0. This showed a lower median relative risk of 0.56, as

expected from correction of non-differential misclassification, but a wider 95% simulation interval from 0.08 to 0.75, reflecting decreased precision. Similar simulations for BMI and family history of MI suggested that measurement error would not change our estimates or risk or variability to a substantial degree. All of these issues should be taken into account when interpreting results of this and other studies using self-reported data.

In summary, in this population of male health professionals, moderate alcohol consumption is associated with a decreased risk of MI among hypertensive men to a similar degree as it is among non-hypertensive men. Despite this, alcohol consumption was not associated with total or cardiovascular mortality. Recommendations with regard to alcohol consumption depend on the individual characteristics of hypertensive patients, but, as in the general population, hypertensive men who drink moderately and safely may not need change their drinking habits.

Grant support

Supported by National Institute of Health Grants AA11181, HL35464 and CA55075 and a travel grant from the Dutch Heart Association and a research exchange award from European Research Advisory Board (JWB).

REFERENCES

1. Fields LE, Burt VL, Cutler JA, Hughes J, Roccella EJ, Sorlie P. The burden of adult hypertension in the United States 1999 to 2000: a rising tide. Hypertension. 2004; 44(4):398-404.
2. Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation. 1998; 97(18):1837-47.
3. Maclure M. Demonstration of deductive meta-analysis: ethanol intake and risk of myocardial infarction. Epidemiol Rev. 1993; 15(2):328-51.
4. Marmot M, Brunner E. Alcohol and cardiovascular disease: the status of the U shaped curve. BMJ. 1991; 303(6802):565-68.
5. Fuchs FD, Chambless LE, Whelton PK, Nieto FJ, Heiss G. Alcohol consumption and the incidence of hypertension: The Atherosclerosis Risk in Communities Study. Hypertension. 2001; 37(5):1242-50.
6. Thadhani R, Camargo CA, Jr., Stampfer MJ, Curhan GC, Willett WC, Rimm EB. Prospective study of moderate alcohol consumption and risk of hypertension in young women. Arch.Intern.Med. 2002; 162(5):569-74.
7. 2003 European Society of Hypertension-European Society of Cardiology guidelines for the management of arterial hypertension. J Hypertens. 2003; 21(6):1011-53.
8. Chobanian AV, Bakris GL, Black HR et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. JAMA. 2003; 289(19):2560-72.
9. Goldberg IJ, Mosca L, Piano MR, Fisher EA. AHA Science Advisory: Wine and your heart: a science advisory for healthcare professionals from the Nutrition Committee, Council on Epidemiology and Prevention, and Council on Cardiovascular Nursing of the American Heart Association. Circulation. 2001; 103(3):472-75.
10. Malinski MK, Sesso HD, Lopez-Jimenez F, Buring JE, Gaziano JM. Alcohol consumption and cardiovascular disease mortality in hypertensive men. Arch.Intern.Med. 2004; 164(6):623-28.
11. Palmer AJ, Fletcher AE, Bulpitt CJ et al. Alcohol intake and cardiovascular mortality in hypertensive patients: report from the Department of Health Hypertension Care Computing Project. J Hypertens. 1995; 13(9):957-64.
12. Renaud SC, Gueguen R, Conard P, Lanzmann-Petithory D, Orgogozo JM, Henry O. Moderate wine drinkers have lower hypertension-related mortality: a prospective cohort study in French men. Am.J.Clin.Nutr. 2004; 80(3):621-25.
13. USDA Nutrient Database for Standard Reference, Release 13. Washington DC; US Department of Agriculture Agricultural Research Service, editor. 1999. Accessed at http://www.nal.usda.gov/fnic/foodcomp/search/ on April 4, 2006.
14. Feskanich D, Rimm EB, Giovannucci EL et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J.Am.Diet.Assoc. 1993; 93(7):790-96.
15. Giovannucci E, Colditz G, Stampfer MJ et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am.J.Epidemiol. 1991; 133(8):810-17.
16. Rose GA, Blackburn H. Cardiovascular survey methods. WHO monograph series no. 58. 1982. Geneva, World Health Organization.
17. Mukamal KJ, Ascherio A, Mittleman MA et al. Alcohol and risk for ischemic stroke in men: the role of drinking patterns and usual beverage. Ann.Intern.Med. 2005; 142(1):11-19.
18. Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. N.Engl.J.Med. 1993; 328(20):1450-56.
19. Thun MJ, Peto R, Lopez AD et al. Alcohol consumption and mortality among middle-aged and elderly U.S.

adults. N.Engl.J.Med. 1997; 337(24):1705-1714.
20. Ascherio A, Rimm EB, Giovannucci EL et al. A prospective study of nutritional factors and hypertension among US men. Circulation. 1992; 86(5):1475-84.
21. Colditz GA, Giovannucci E, Rimm EB et al. Alcohol intake in relation to diet and obesity in women and men. Am.J.Clin.Nutr. 1991; 54(1):49-55.
22. Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Willett WC. Aspirin use and the risk for colorectal cancer and adenoma in male health professionals. Ann.Intern.Med. 1994; 121(4):241-46.
23. Rimm EB, Stampfer MJ, Colditz GA, Chute CG, Litin LB, Willett WC. Validity of self-reported waist and hip circumferences in men and women. Epidemiology. 1990; 1(6):466-73.
24. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am.J.Epidemiol. 1992; 135(10):1114-26.
25. Tanasescu M, Hu FB, Willett WC, Stampfer MJ, Rimm EB. Alcohol consumption and risk of coronary heart disease among men with type 2 diabetes mellitus. J.Am.Coll.Cardiol. 2001; 38(7):1836-42.
26. Therneau TM. Extending the Cox model. In: Lin DY, Fleming TR, eds. Proceedings of the First Seattle Symposium in Biostatistics: Survival Analysis. Springer Verlag; 1997:51-84.
27. Durrleman S, Simon R. Flexible regression models with cubic splines. Stat.Med. 1989; 8:551-561.
28. Ng'andu NH. An empirical comparison of statistical tests for assessing the proportional hazards assumption of Cox's model. Stat.Med. 1997; 16: 611-626.
29. Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Fujishima M. The impact of alcohol and hypertension on stroke incidence in a general Japanese population. Stroke. 1995;26:368-372.
30. Cushman WC, Cutler JA, Hanna E et al. Prevention and Treatment of Hypertension Study (PATHS): effects of an alcohol treatment program on blood pressure. Arch.Intern.Med. 1998; 158(11):1197-1207.
31. Corrao G, Rubbiati L, Bagnardi V, Zambon A, Poikolainen K. Alcohol and coronary heart disease: a meta-analysis. Addiction. 2000; 95(10):1505-23.
32. Rehm JT, Bondy SJ, Sempos CT, Vuong CV. Alcohol consumption and coronary heart disease morbidity and mortality. Am.J.Epidemiol. 1997; 146(6):495-501.
33. Wannamethee G, Whincup PH, Shaper AG, Walker M, Macfarlane PW. Factors determining case fatality in myocardial infarction "who dies in a heart attack"? Br.Heart J. 1995; 74(3):324-31.
34. Tunstall-Pedoe H, Kuulasmaa K, Mahonen M, Tolonen H, Ruokokoski E, Amouyel P. Contribution of trends in survival and coronary-event rates to changes in coronary heart disease mortality: 10-year results from 37 WHO MONICA project populations. Monitoring trends and determinants in cardiovascular disease. Lancet. 1999; 353: 1547-1557.
35. Mukamal KJ, Conigrave KM, Mittleman MA et al. Roles of drinking pattern and type of alcohol consumed in coronary heart disease in men. N.Engl.J.Med. 2003; 348(2):109-18.
36. Rimm EB. Alcohol consumption and coronary heart disease: good habits may be more important than just good wine. Am.J.Epidemiol. 1996; 143(11):1094-98.
37. Mukamal KJ, Jensen MK, Gronbaek M et al. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation. 2005; 112(10):1406-13.
38. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ. 1999; 319(7224):1523-1528.
39. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-

reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur.J.Clin.Nutr. 2002; 56(11):1130-36.

40. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA. 2002; 287(19):2559-62.
41. Hendriks HF, Veenstra J, Velthuis-te Wierik EJ, Schaafsma G, Kluft C. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. BMJ. 1994; 308(6935):1003-06.
42. Shaper AG, Wannamethee G, Walker M. Alcohol and mortality in British men: explaining the U-shaped curve. Lancet. 1988; 2(8623):1267-73.
43. Fox MP. Epidemiologic Method Research; Boston University Sensitivity Analysis Tools. Accessed at http://www.bu.edu/dbin/sph/departments/epidemiology/epidemiologic_methods_research.php on April 4 2006.
44. Fox MP, Lash TL, Greenland S. A method to automate probabilistic sensitivity analyses of misclassified binary variables. Int.J.Epidemiol. 2005; 34: 1370-1376.
45. Day NE, Wong MY, Bingham S et al. Correlated measurement error- implications for nutritional epidemiology. Int.J.Epidemiol. 2004; 33: 1373- 1381.

Chapter 3

Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1

Joline WJ Beulens

Aafje Sierksma

Arie van Tol

Natalie Fournier

Teus van Gent

Jean-Louis Paul

Henk FJ Hendriks

J Lipid Res (2004): 45(9):1716-23

ABSTRACT

Objective

Moderate alcohol consumption increases HDL cholesterol, which is involved in reverse cholesterol transport (RCT). The aim of this study was to investigate the effect of moderate alcohol consumption on cholesterol efflux, using J774 mouse macrophages and Fu5AH cells, and on other parameters in the RCT pathway.

Methods

Twenty-three healthy men (45-65 years) participated in a randomized, partially diet-controlled, cross-over trial. They consumed four glasses of whisky (40 g alcohol) or water daily during 17 days.

Results

After 17 days of whisky consumption serum capacity to induce ABCA1-dependent cholesterol efflux from J774 mouse macrophages was increased by 17.5% ($p= 0.027$) as compared with water. Plasma capacity to induce cholesterol efflux from Fu5AH cells increased by 4.6% ($p=0.002$). Pre β-HDL, ApoA-I and lipoprotein AI:AII also increased by respectively 31.6, 6.2 and 5.7% ($p<0.05$) after whisky consumption as compared with water consumption. Changes of cAMP-stimulated cholesterol efflux correlated ($r=0.65$; $p<0.05$) with changes of ApoA-I, but not with changes of pre-β HDL ($r=0.30$; $p=0.18$). Cholesterol efflux capacities from serum of lean men were higher than from overweight men.

Conclusion

This study shows that moderate alcohol consumption increases the capacity of serum to induce cholesterol efflux from J774 mouse macrophages, which may be mediated by ABCA1.

INTRODUCTION

Atherosclerosis is a condition of major arteries leading to cardiovascular disease (CVD), in which progressive occlusion of the arteries occurs by formation of atherosclerotic lesions. A major event in this process is the differentiation of monocytes to macrophages which accumulate lipoprotein-derived cholesterol to form 'foam' cells (1).

Epidemiologic studies have shown that moderate alcohol consumption is associated with a decreased risk of CVD (2). Several mechanisms, such as a reduction in blood coagulation and fibrinolysis, may be involved in the protective effect of moderate alcohol consumption on CVD (3, 4). However, more than 50% of the protective action of alcohol consumption is mediated by an increase of HDL cholesterol (HDL-C) (5, 6). In previous studies we have indeed shown that moderate alcohol consumption results in an increased blood HDL-C concentration (7- 9). The functional consequences of this increase in HDL-C concentration are not fully understood, but one of the protective actions may involve an increase of paraoxonase activity, an HDL-associated enzyme that may protect LDL against oxidation (8, 10, 11).

The role of HDL in reverse cholesterol transport (RCT) is another proposed mechanism for these protective effects. RCT consists of the following steps: efflux of free cholesterol (FC) from peripheral tissue, esterification of FC by plasma lecithin:cholesterol acyltransferase (LCAT) and incorporation of cholesteryl esters (CE) in the HDL particle. HDL-CE are cleared from plasma by the liver for catabolism via several pathways (12). The first step in RCT, cholesterol efflux, can be mediated by three mechanisms (13, 14). The first mechanism is aqueous diffusion, in which cholesterol molecules desorb from the plasma membrane to be captured by acceptors such as mature HDL particles. The second mechanism of efflux involves the scavenger receptor class B type I (SR-BI). SR-BI mediates cellular cholesterol efflux to mature HDL particles in addition to its role in selective lipid uptake (13, 14). In one of our previous studies we have shown that moderate alcohol consumption increased plasma capacity to induce cholesterol efflux from Fu5AH rat hepatoma cells, which have high levels of SR-BI (15).

The third mechanism of cholesterol efflux involves the release of FC to lipid-free or lipid-poor apolipoproteins, particularly apoA-1 and pre-β HDL, which is mediated by the ATP binding cassette transporter 1 (ABCA1). This lipid efflux is the first stage of HDL

biogenesis. Subsequently FC is esterified by LCAT and mature, spherical α-HDL particles are formed (13). Efficient cholesterol efflux from macrophages is critical for the prevention of foam cell formation and increased cellular ABCA1 levels may thus protect against atherosclerosis (16). To the best of our knowledge, the effect of moderate alcohol consumption on cholesterol efflux mediated by the ABCA1 receptor has never been investigated. We have therefore used the recently validated model of ABCA1-expressing J774 macrophages (17) to study the effect of moderate alcohol intake on serum cholesterol efflux capacity.

METHODS

Subjects

Twenty-four male subjects aged 45 to 65 years, all apparently healthy and non-smoking were recruited from the TNO BIBRA International (Carshalton, Surrey, UK) database of healthy human volunteers, by advertising in local newspapers and by leaflet drops to local residential areas. A questionnaire (self-report) was used for information on alcohol intake, medical history and family history of alcoholism. The questionnaire was checked by a medical investigator during an interview with the volunteer and subsequently a physical examination was performed. Subjects were considered healthy based on the values of the pre-study laboratory tests, their medical history and the physical examination. Subjects fulfilled the following inclusion criteria: consumption between 10 and 28 alcohol-containing beverages weekly, body mass index (BMI) between 20 and 35 kg/m2 and no family history of alcoholism. A wide range of BMIs was chosen to investigate whether the effect of moderate alcohol consumption on the outcome measures is modified by level of obesity. The study was conducted in accordance with the Declaration of Helsinki South Africa Revision 1996 and International Conference of Harmonisation Harmonized Tripartite Guideline for Good Clinical Practice. Approval to proceed with the study was given by an independent Medical Ethics Committee, and all subjects provided written informed consent before participation.

Study design

The subjects entered a randomized, partially diet-controlled, cross-over trial consisting of two periods of 17 days. A random sample of 12 men were allocated to the sequence of consuming whisky (Famous Grouse Scotch Whisky, 40 vol% alcohol) during dinner in the first period followed by drinking tap water (control beverage) during dinner in the second period. The other 12 men consumed water first, followed by whisky. The participants and staff administering the protocol were not blinded to the treatment sequence.

Four glasses (125 mL in total) of each beverage were consumed daily during dinner at TNO BIBRA. One glass was taken before dinner, two glasses during dinner and one glass after dinner. During the whisky period, alcohol intake equaled 40 gram per day. The daily dinner contained ~4,200 kJ and consisted of ~21% of energy from protein, ~38% of energy

from fat, and ~41% of energy from carbohydrate. The menu comprised of a four-day rotation. Dinner was prepared each day by a local caterer using the same source of ingredients throughout.

Each period was preceded by three alcohol-free days to prevent carry-over effects. Subjects were asked to continue their normal eating habits (except for the provided dinner) and carry on with their normal everyday activities. Compliance to the protocol was checked by a daily questionnaire. Body weight was measured on the first and last day of each treatment period with the subjects wearing indoor clothing, without shoes, wallet and keys. An alcohol breath test (Alcoholtest 7410, Dräger Nederland, Zoetermeer, The Netherlands) was performed during the whisky treatment to ensure that subjects left the institute safely. At the end of each treatment period, fasting blood samples were collected in the morning.

Blood was taken from an antecubital vein and collected in a tube containing lithium-heparin to obtain plasma and in a tube containing gel and clot activator to obtain serum (Vacutainer Systems; Becton Dickinson, Plymouth, UK). To obtain plasma, the blood was centrifuged for 20 minutes at 2,000 g and 4°C, between 15 and 30 minutes after collection. To obtain serum, the blood was centrifuged for 15 minutes at 2,000 g and 4°C, between 15 and 30 minutes after collection. The plasma and serum samples were stored at -80°C until analysis.

HDL was separated from the apoB fraction by precipitation of the latter with polyethylene glycol. Serum total and HDL-C (cholesterol oxidase/peroxidase-amidopyrine method) and triacylglycerols (glycerol phosphate oxidase/peroxidase-amidopyrine method), as well as unesterified cholesterol, triacylglycerol, and phospholipids (PLs) in HDL were analyzed enzymatically. The coefficients of variation within runs were 0.8% for total cholesterol (TC), 1.5% for triacylglycerol (TG), and 1.3% for HDL-C. CE were calculated as the difference between HDL-FC and total HDL-C. LDL cholesterol (LDL-C) was calculated by the equation of Friedewald, Levy, and Fredrickson (18). ApoA-I was analyzed in serum according to a commercial nephelometric assay on the Cobas Mira S (Roche, Switzerland). LipoproteinAI (LpA-I, containing apoA-I, but not apoA-II) concentrations were determined by double rocket immuno-electrophoresis using the assay kit provided by Sebia (Benelux N.V. Brussels, Belgium), according to the instructions of the manufacturer. The stained and dried gels were scanned and rocket heights were determined. A standard curve was made based on the linear relationship between rocket height and concentration. LpA-I

concentration was determined as a function of rocket height, whereas Lipoprotein AI:AII (LpA-I:A-II) was calculated by subtraction (total plasma apoA-I minus LpA-I).

Quantification of pre-β HDL by crossed immuno-electrophoresis

The crossed immunoelctrophoresis consisted of agarose electrophoresis in the first dimension for separation of lipoproteins with pre-β and α mobility. Antigen migration from the first agarose gel into the second agarose gel, containing goat anti-human apoA-I antiserum (3%, vol/vol), was used to quantitatively precipitate apoA-I. The antiserum was monospecific for human apoA-I. Lipoprotein electrophoresis was carried out in 1% (w/v) agarose gels in barbital buffer (50 mmol/l, pH 8.6). Plasma was applied at 3 µl/well and run in an LKB 2117 system (4 °C for 2 hours, 250 V for the first dimension). The track of the first agarose gel was excised and annealed with melted agarose to a gel containing 3% (vol/vol) goat anti-human apoA-I antiserum that was cast on GelBond film (Pharmacia). The plate was run in an LKB 2117 system (4 °C for 20 hours, 50 V) in barbital buffer. Unreacted antibody was removed by extensive washing in phosphate buffered saline. The gel was stained with Coomassie brilliant blue R250 and subsequently dried. Areas under the pre-β HDL and α-HDL peaks were scanned and calculated using Scion software. The pre-β HDL area can be expressed as the percentage of the sum of the pre-β HDL and the α-HDL areas. The pre-β HDL concentrations are given in absolute amounts (mg of apoA-I present in pre-β HDL per ml of plasma). These values were calculated from the percentage of apoA-I present in pre-β HDL and the total plasma apoA-I concentration.

Plasma cholesteryl ester transfer protein, LCAT and phospholipid transfer protein activity

Plasma cholesteryl ester transfer protein (CETP) activity was determined after removal of VLDL and LDL from each sample as described previously (19, 20). The isotope assay measures the transfer of [1-^{14}C-oleate]-CE from labeled LDL to an excess of unlabelled normal HDL. LCAT was inhibited with dithiobis-2-nitrobenzoic acid. CETP activity was calculated as the bi-directional transfer between labeled LDL and HDL. There is a strong correlation between the measured CETP activities and CETP mass (21). Plasma LCAT activity was measured using excess exogenous substrate as described (22). Plasma phospholipid transfer protein (PLTP) activity was also measured with exogenous substrates (20). Plasma samples were incubated with [^{3}H]-phosphatidylcholine labeled liposomes and

an excess of HDL. Thereafter, the liposomes were precipitated with a mixture of NaCl, $MgCl_2$ and heparin (final concentrations of 230 mmol/l, 92 mmol/l and 200 U/ml, respectively). This method is not affected by the PL transfer-stimulating action of CETP (20). The measured CETP, LCAT and PLTP activities are linearly related to the amount of plasma used in all incubations. The plasma CETP, LCAT and PLTP activities as obtained with these methods reflect the active amount of these proteins in plasma and are not influenced by the endogenous plasma lipoproteins.

For each assay, all plasma samples were analyzed in one run using the same batch of substrates. Plasma CETP, LCAT and PLTP activities were related to a reference pool of human plasma, obtained by mixing equal amounts of plasma, isolated at 4°C, from 250 healthy blood donors. All activities are expressed in arbitrary units (A.U.), corresponding to the percentages of the respective activities in the reference pool plasma; 100% is equivalent to the following activities: 216 nmol/ml/hr for CETP, 65 nmol/ml/hr for LCAT and 13.9 µmol/ml/h for PLTP. The within-assay coefficients of variation for the assays of plasma CETP, LCAT and PLTP activities amount to 2.7%, 4.5% and 3.5%, respectively.

Cholesterol efflux from J774 cells

Cholesterol efflux from J774 mouse macrophages cells was determined by use of a previously validated modification (17, 23, 24) of the general technique described by Sakr et al. (25). The current experiment is based on previous tests using normolipidemic human serum to obtain an optimal dilution for serum and carried out strictly according to the experimental conditions as described by Fournier et al. (17). Briefly, serum samples diluted to 1% were incubated at 37°C with the [^{3}H] cholesterol-labeled cells pretreated for 10–12 h with or without 0.3 mmol/l 8-(4-chlorophenylthio) cAMP (CPT-cAMP from Sigma). To prevent intracellular cholesterol esterification, the ACAT inhibitor GW 447C88 was added into the medium during the labeling period and all subsequent stages of the experiment. After four hours of incubation, the radioactivity released to the medium was expressed as the fraction of the total radioactive cholesterol present in the well. The percentage ABCA1-mediated FC efflux was calculated as the percentage FC efflux from cells upregulated with cAMP minus the percentage FC efflux from control J774 cells. This calculation controls for the contribution of FC efflux from aqueous diffusion mechanism, and yield data that are specific for the contribution of ABCA1 (ABCA1-dependent cholesterol efflux). All

determinations were made in triplicate. A standard pool of human serum was used to assess the daily variation in efflux experiments. The efflux values obtained with the standard pool of all experiments are averaged, and this serves as the 100% value. Each value of experiments with the standard pool is calculated as a percentage of the overall average, and this percentage is applied to correct all test values for that day.

Cholesterol efflux from Fu5AH cells

The capacity of plasma to induce cholesterol efflux from Fu5AH cells was measured as described by de la Llera Moya et al. (26). In short, Fu5AH cells were grown to confluency in the presence of [^{3}H]cholesterol. After removal of medium containing the labeled cholesterol, the cells were allowed to equilibrate for 24 h. Subsequently cholesterol efflux was measured in triplicate over four hours in the presence of 20-fold diluted plasma samples. Cholesterol efflux (radiolabel present in the culture medium after four hours), is expressed as a percentage of the radioactivity initially present in the cells (fractional efflux). Data were corrected for blanks, being the amount of label in the medium after four hours in the absence of plasma. The within-assay coefficient of variation of the cholesterol efflux assay is 5.5%. The fractional cholesterol efflux using Fu5AH cells is mainly attributed to SR-BI, since this cell line has a high expression of SR-BI (27).

Statistical analysis

Data analysis was performed using the SAS statistical software package (SAS/STAT Version 6.12, SAS Institute, Cary, NC, USA). Data were tested for normality using Shapiro-Wilk test and by visually inspecting normality plots. Treatment effects were evaluated by analysis of variance using the mixed model procedure. The factors treatment, moment, treatment order, BMI and the interaction term of treatment and BMI were included in the model. The effect of BMI on the outcome measures were obtained from the BMI factor in the ANOVA model. Correlation coefficients to assess association between (relative changes) in outcome measures were calculated according to Pearson or Spearman if variables were not distributed normally. Two-sided p-values were considered statistically significant at $p<0.05$.

RESULTS

Subjects

Of the 24 subjects included in the study, one subject withdrew from the study at day 21 due to a reason not related to treatment. Therefore only 23 subjects were included in data analysis of this study. Characteristics of these subjects are shown in table 1. Average body weight did not differ between the whisky and water treatment periods. The mean breath alcohol concentration at one hour after dinner with whisky was 0.43 g/l (range 0.26-0.68 g/l).

Table 1: Characteristics of the volunteers included in the data-analysis (n=23).

Characteristic[1]		
Age (y)	52 (5)	[45 - 65]
Body weight (kg)	81.4 (11.5)	[61.2 - 100.9]
BMI (kg/m^2)	26.7 (3.0)	[21.4 - 33.3]
Hemoglobin (mmol/L)	8.8 (0.6)	[7.5 - 10.0]
Triglycerides (mmol/L)	1.2 (0.7)	[0.3 - 3.0]
Total cholesterol (mmol/L)	5.6 (0.9)	[3.9 - 7.3]
HDL cholesterol (mmol/L)	1.4 (0.4)	[0.9 - 2.5]
LDL cholesterol (mmol/L)	3.6 (0.7)	[2.2 - 5.5]
Alkaline phosphatase (U/L)	56 (13)	[39 - 88]
Asparagine aminotransferase (U/L)	25 (5)	[18 - 41]
Alanine aminotransferase (U/L)	28 (10)	[14 - 51]
Gamma-glutamyltransferase (U/L)	26 (12)	[13 - 49]

[1] Data are expressed as mean (SD) and [range].

Cholesterol efflux capacity

Treatment effects on cholesterol efflux capacity of serum or plasma are shown in table 2. Cholesterol efflux capacity from Fu5AH cells was increased by 4.6% (p=0.002) after whisky consumption, and cholesterol efflux capacity from J774 cells without stimulation of cAMP also increased after whisky consumption by 3.3% (p=0.014). After stimulation with cAMP, cholesterol efflux capacity using J774 cells was elevated about twice as much (6.3%; p<0.001) after consumption of whisky as compared to water. This resulted in a 17.5% (p= 0.027) elevation after consumption of whisky as compared with water of the ABCA1-dependent cholesterol efflux in J774 cells. Individual serum ABCA1 dependent cholesterol

efflux capacities are shown in figure 1.

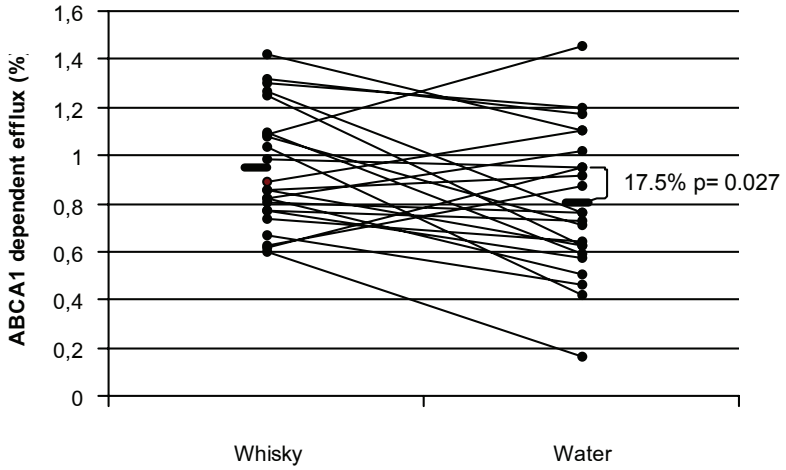


Figure 1: Individual serum ex vivo ABCA1 dependent cholesterol efflux capacities after consumption of whisky (40 g alcohol/day) or water for 3 weeks of 23 healthy male subjects.

Blood lipid and lipoprotein profile and related factors

Treatment effects on serum lipid profile and related factors are shown in table 3. A significant treatment effect on HDL-C was found, indicating a 7.4% increase of HDL-C after whisky consumption. The HDL-PL fraction increased by 9.3% ($p<0.0001$) after whisky consumption as compared with water consumption. No treatment effects on TC, LDL-C, VLDL-C and TG were found (table 3). Pre-β HDL increased by 31.6% ($p=0.026$), serum ApoA-I concentration by 6.2% ($p<0.001$) and concentration of LpA-I:A-II by 6.0% ($p=0.015$). A borderline significant ($p=0.069$) effect of moderate alcohol consumption was observed on

Table 2: Cholesterol efflux capacities (mean ±SEM) after consumption of whisky or water in 23 healthy middle-aged men

Cholesterol efflux capacity (%)	Water	Whisky	% change	p-value
Fu5AH cholesterol efflux	31.69 ± 0.53	33.15 ± 0.53	4.6	0.002
J774 cholesterol efflux (-cAMP)	3.37 ± 0.06	3.48 ± 0.06	3.3	0.014
J774 cholesterol efflux (+cAMP)	4.16 ± 0.07	4.42 ± 0.07	6.3	<0.001
ABCA1-dependent efflux	0.80 ± 0.06	0.94 ± 0.06	17.5	0.027

Table 3: Blood lipid profile and related factors (mean ±SEM) after consumption of water or whisky in 23 middle-aged men

Variable	Water	Whisky	% change	p-value
Total cholesterol (mmol/L)	6.01 ± 0.23	6.02 ± 0.23	0.2	0.94
LDL cholesterol (mmol/L)	4.28 ± 0.21	4.14 ± 0.21	-3.3	0.30
HDL cholesterol (mmol/L)	1.22 ± 0.05	1.31 ± 0.05	7.4	<0.001
VLDL cholesterol (mmol/L)	0.52 ± 0.06	0.57 ± 0.06	9.6	0.12
Triglycerides (mmol/L)	1.23 ± 0.15	1.36 ± 0.15	10.6	0.12
HDL phospholipids (mmol/L)	1.50 ± 0.05	1.64 ± 0.05	9.3	<0.0001
HDL triglycerides (mmol/L)	0.12 ± 0.01	0.13 ± 0.01	8.3	0.20
Pre-β HDL (mg/mL ApoA-I)	0.038 ± 0.010	0.050 ± 0.010	31.6	0.026
Total ApoA-I (mg/mL)	1.29 ± 0.03	1.37 ± 0.03	6.2	<0.001
LpA-I (g/L)	0.46 ± 0.03	0.49 ± 0.03	6.5	0.069
LpA-I:A-II (g/L)	0.84 ± 0.03	0.89 ± 0.03	6.0	0.015
LCAT activity (A.U.)[1]	88.95 ± 2.11	94.14 ± 2.08	5.8	0.007
PLTP activity (A.U.)[1]	76.49 ± 2.76	79.92 ± 2.73	0.5	0.16
CETP activity (A.U.)[1]	87.08 ± 4.91	85.94 ± 4.90	-1.4	0.51

[1]A.U.= arbitrary units, corresponding to the percentages of the respective activities in the reference pool plasma; 100 % is equivalent to the following activities: 216 nmol/ml/hr for CETP, 65 nmol/ml/hr for LCAT and 13.9 µmol/ml/h for PLTP.

LpA-I concentration, suggesting an increase of 6.5% after whisky consumption as compared with water consumption. LCAT activity was increased significantly by 5.8% after whisky consumption as compared to water. No treatment effects were found on CETP and PLTP activity.

Table 4: Cholesterol efflux capacities (mean ±SEM) in 12 lean and 11 overweight, middle-aged men

Cholesterol efflux capacity (%)	Lean	Overweight	% difference	p-value
Fu5AH cholesterol efflux	33.33 ± 0.67	31.53 ± 0.70	-5.7	0.076
J774 cholesterol efflux (-cAMP)	3.56 ± 0.08	3.29 ± 0.08	-8.2	0.028
J774 cholesterol efflux (+cAMP)	4.45 ± 0.08	4.13 ± 0.09	-7.7	0.018
ABCA1-dependent efflux	0.89 ± 0.07	0.85 ± 0.07	-4.7	0.68

BMI effect

The effect of BMI on cholesterol efflux, lipid and lipoprotein profile and related factors is shown in tables 4 and 5. Table 4 shows that cholesterol efflux capacities using J774 were higher in lean men (BMI<27) than overweight men (BMI≥27). Also, the cholesterol efflux capacity using Fu5AH cells tended to be lower in overweight men. However, ABCA1-dependent cholesterol efflux capacity was not different between lean and overweight men. Table 5 shows that HDL-C, HDL-PL, Apo-AI and Lp AI were higher in lean men than in overweight men, whereas LCAT activity was 9.2% higher in overweight men. VLDL-C and TG tended to be lower in lean than in overweight men. No significant interaction between BMI and alcohol consumption has been found for any of the efflux and lipoprotein variables.

Table 5: Blood lipid profile and related factors (mean ±SEM) in 12 lean and 11 overweight, middle-aged men

Variable	Lean	Overweight	% difference	p-value
Total cholesterol (mmol/L)	5.99 ± 0.29	6.04 ± 0.30	0.8	0.91
LDL cholesterol (mmol/L)	4.11 ± 0.27	4.30 ± 0.28	4.4	0.62
HDL cholesterol (mmol/L)	1.43 ± 0.07	1.09 ± 0.07	-31.2	0.003
VLDL cholesterol (mmol/L)	0.45 ± 0.08	0.64 ± 0.08	29.7	0.056
Triglycerides (mmol/L)	1.06 ± 0.18	1.53 ± 0.19	30.7	0.056
HDL phospholipids (mmol/L)	1.71 ± 0.06	1.43 ± 0.06	-19.6	0.004
HDL triglycerides (mmol/L)	0.12 ± 0.01	0.13 ± 0.01	7.7	0.49
Pre-β HDL (mg/mL ApoA-I)	0.043 ± 0.010	0.045 ± 0.010	4.4	0.65
Total ApoA-I (mg/mL)	1.44 ± 0.04	1.23 ± 0.05	-17.1	0.004
LpA-I (g/L)	0.55 ± 0.04	0.39 ± 0.04	-41.0	0.008
LpA-I:A-II (g/L)	0.88 ± 0.03	0.84 ± 0.04	-4.8	0.38
LCAT activity (A.U.)[1]	87.12 ± 2.65	95.97 ± 2.75	9.2	0.031
PLTP activity (A.U.)[1]	77.83 ± 3.06	78.58 ± 3.13	1.0	0.84
CETP activity (A.U.)[1]	85.91 ± 6.40	87.11 ± 6.64	3.8	0.89

[1]A.U.= arbitrary units, corresponding to the percentages of the respective activities in the reference pool plasma; 100 % is equivalent to the following activities: 216 nmol/ml/hr for CETP, 65 nmol/ml/hr for LCAT and 13.9 µmol/ml/h for PLTP.

Correlations

The changes of cAMP-stimulated cholesterol efflux capacity from J774 cells induced by whisky consumption correlated with changes of ApoA-I (r= 0.65, p=0.001), HDL-C (r=0.55, p=0.009), HDL-PL (r=0.56, p=0.008) and CETP activity (r=0.49; p=0.021). A borderline significant (p=0.054) correlation (r=0.42) between whisky-induced changes in cholesterol efflux capacity using cAMP-treated J774 cells and whisky-induced changes of cholesterol efflux capacity using Fu5AH cells was found. Changes of cholesterol efflux capacity from Fu5AH cells correlated (r=0.52, p=0.012) with changes of HDL-PL. Changes of cholesterol efflux capacity from cAMP-treated J774 cells did not correlate significantly with changes of pre-β HDL (r=0.30, p=0.18). However, whisky-induced changes of pre-β HDL did correlate with changes in PLTP activity (r=0.65, p=0.0014), ApoA-I (r=0.47, p=0.030) and of HDL-PL (r=0.54, p=0.010). Changes, induced by whisky consumption, in serum ApoA-I also correlated with changes in HDL-C (r=0.65, p=0.0015), LpA-I:A-II (r=0.61, p=0.0034) and HDL-PL (r=0.66, p=0.0012).

DISCUSSION

In the present study, we investigated the effect of moderate alcohol consumption on cholesterol efflux capacity of serum or plasma by using two different cell systems, providing information on both ABCA1- and SR-BI-mediated cholesterol efflux. We showed an increase of 6.3% in cAMP-stimulated cholesterol efflux capacity from J774 cells. Consequently the capacity of serum to induce ABCA1-dependent cholesterol efflux increased by 17.5%. The capacity of plasma to induce cholesterol efflux from Fu5AH cells showed a less pronounced increase of 4.6%. Effects on both cholesterol efflux capacities were associated with concomitant changes of other parameters in the RCT pathway.

The study was partially diet-controlled and performed according to a randomized cross-over design. No important deviations of the study protocol occurred and no significant changes of body weight were found during the study. It is therefore unlikely that the results of this study are confounded by carry-over effects or by changes in diet or body weight. In this study serum or plasma used for the cholesterol efflux experiments were diluted to 1% in order to approach the composition of the interstitial fluid that comes in direct contact with the cells. Cholesterol efflux capacities measured in this study were in the same order of magnitude as those described previously (15, 17, 28). Nevertheless, any extrapolation of the ex vivo cholesterol efflux capacity to the in vivo situation can only be done with caution. However, the consistency between our results of cholesterol efflux capacity and related parameters measured in vivo indicate that in this case this extrapolation is not unreasonable.

The findings of this study, however, do not agree with the study by Marmillot et al. (29), who showed a 21% decrease of cholesterol efflux from mouse J774 macrophages induced by plasma of male Wistar-Furth rats after chronic alcohol consumption. Apart from the species difference, the rats were fed a diet containing as much as 36 energy% of alcohol which is much higher than the moderate dose of alcohol (~10 energy%) in our study. This is another reason why the results of these studies cannot be directly compared. A similar inhibition of cholesterol efflux has been reported in a study with in-vitro fibroblasts by Avdulov et al. (30) using a high alcohol concentration.

In human intervention studies with moderate alcohol consumption the effect on cholesterol efflux has only been investigated using Fu5AH hepatoma cells. These studies

showed increases in cholesterol efflux of 5 to 7% (15, 31), which is in accordance with the findings of our current study using this cell system (4.6%). The impact of moderate alcohol consumption on cAMP stimulated (6.3%) and particularly on ABCA1-dependent cholesterol efflux capacity in J774 cells (17.5%) was greater than the effects observed using Fu5AH cells. Our results show that both mechanisms may play a role in the cardioprotective effect of moderate alcohol consumption but suggest that ABCA1 mediated cholesterol efflux may be a more relevant pathway in this respect.

Besides the increased cholesterol efflux capacity, other parameters involved in RCT were also increased by moderate whisky consumption (pre-β HDL, HDL-C, HDL-PL, ApoA-I, Lp AI, Lp AI:AII and LCAT activity). Moreover, total ApoA-I correlated significantly with cAMP-stimulated cholesterol efflux capacity, which is in agreement with the concept that cholesterol efflux from macrophages may be mediated by cholesterol-poor ApoA-I (32, 33). On the other hand, the increase of cholesterol efflux capacity did not correlate significantly with changes of pre-β HDL. This may simply be attributable to the relatively large variance in the cholesterol efflux capacity measure. However, because pre-β HDL was measured in vivo and cholesterol efflux ex vivo after four hours of incubation, serum proteins involved in the cycle of apoA-I between nascent and mature HDL species like LCAT, CETP or PLTP may influence the cholesterol efflux process (17, 34). LCAT, which increased significantly after whisky consumption in this study, may contribute to the consumption of the initial pre-β HDL concentration (17, 34). On the other hand additional formation of pre-β HDL through PLTP may also occur (35, 36), as we found a relatively strong correlation (r=0.65) between changes of pre-β HDL and PLTP activity in this study. Even using the highly diluted serum, these events could take place during the four hour incubation period and may lead to attenuation of the correlation between pre-β HDL and cholesterol efflux capacity. Finally, the chemical composition of pre-β HDL may also play a role. This composition is not entirely defined, but is likely to contain both precursors and products of ABCA1-mediated cholesterol efflux (34). Thus, because lipid content of pre-β HDL may influence structure and functional properties of ApoA-I (37), these variations may affect cholesterol efflux capacity and therefore attenuate its correlation with pre-β HDL concentration.

We also found significant positive correlations between cholesterol efflux capacity both from J774 and Fu5AH cells and HDL-PL. The latter correlation is in accordance with earlier studies that reported that SRBI-rich cells are highly sensitive to HDL-PL concentrations (38,

39). The correlation between cholesterol efflux capacity from cAMP-treated J774 cells and HDL-PL was also found in patients with primary hypertriglyceridemia by Brites et al. (40). Recent results of Yancey et al. (28) using plasma from human ApoA-I transgenic mice, however, are not entirely consistent with these data. This may be due to the relatively extreme changes in HDL composition due to overexpression in these transgenic mice or to a species difference.

Furthermore, this study shows that cholesterol efflux capacity of serum, HDL-C, HDL-PL, ApoA-I and LpA-I were significantly lower in obese than in lean subjects. VLDL-C and TG tended to be higher and LCAT activity was significantly higher in obese than in lean subjects. Although the differences in our study are less pronounced, our findings show the same pattern as the results of Sasahara et al. (41), who studied the pathway of cholesterol efflux from human fibroblasts in lean and obese subjects. They found a somewhat larger difference between lean and obese subjects, but this may be attributable to methodological differences such as another study population or the use of another cell system (fibroblasts). Syvänne et al. (42), using the Fu5AH cell system, observed a decreased cholesterol efflux capacity in patients with non-insulin dependent diabetes mellitus (7%) or coronary artery disease (7%) of the same magnitude as our findings in obese subjects.

To the best of our knowledge, this is the first study to show that moderate alcohol consumption increases ABCA1-dependent cholesterol efflux capacity of human serum measured with J774 macrophages. This increase of cholesterol efflux capacity using J774 cells was larger than the increase using Fu5AH cells, suggesting that the ABCA1 transporter is more relevant for the effect of moderate alcohol consumption on HDL-C than the SR-BI. At the same time, moderate alcohol consumption increased other parameters of the RCT pathway such as total ApoA-I, LpA-I:A-II, pre-β HDL, HDL-C and LCAT activity. The increase in serum cholesterol efflux capacity from J774 cells correlated with the increase of total ApoA-I, but the correlation with pre-β HDL did not reach statistical significance. Altogether, these findings suggest that moderate alcohol consumption may stimulate early steps in the RCT pathway mediated by ABCA1.

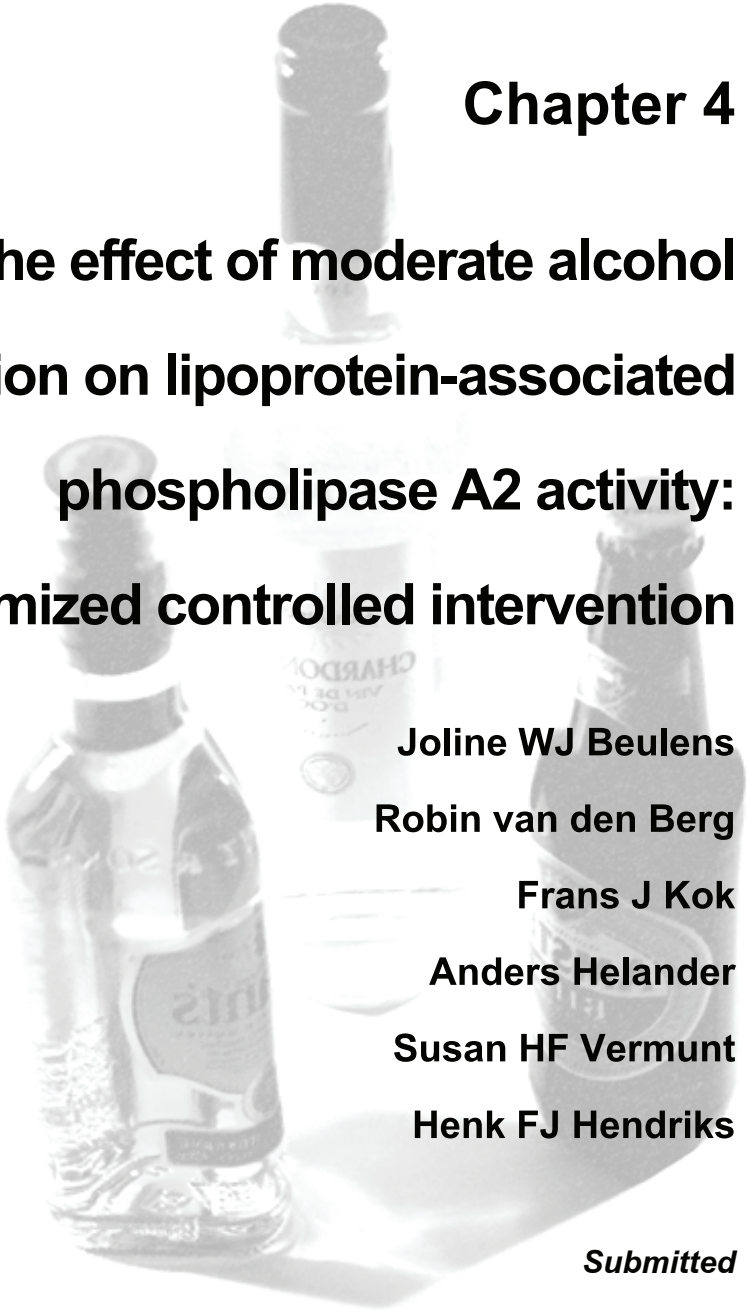
Acknowledgements

We want to thank the team that conducted this study and the volunteers for their enthusiastic participation. Dr. Pieter H.E. Groot and Leo M. Scheek are thanked for the preparation of the goat anti-human apoA-I antiserum, used in the quantitation of pre-β HDL. Leo M. Scheek also performed the PLTP and LCAT activity assays and Farah Sadeghi Niaraki measured CETP activities. Cora Groffen measured plasma capacities to induce cholesterol efflux from Fu5AH cells. Dr. Geesje Dallinga-Thie performed the assay of total plasma ApoA-I. The research described in this paper was funded by the Dutch Foundation for Alcohol Research.

REFERENCES

1. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801-809.
2. Grobbee DE, Rimm EB, Keil U, Renaud S. Alcohol and the Cardiovascular System. In Health Issues Related to Alcohol Consumption. 1999; I. MacDonald, editor. Blackwell Science Ltd, Oxford. 125-179.
3. Hendriks HF, Veenstra J, Velthuis-te Wierik EJ, Schaafsma G, Kluft C. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. BMJ 1994; 308: 1003-1006.
4. Pikaar NA, Wedel M, van der Beek EJ et al. Effects of moderate alcohol consumption on platelet aggregation, fibrinolysis, and blood lipids. Metabolism 1987; 36: 538-543.
5. Castelli WP, Doyle JT, Gordon T et al. Alcohol and blood lipids. The cooperative lipoprotein phenotyping study. Lancet 1977; 2: 153-155.
6. Criqui MH, Cowan LD, Tyroler HA. Lipoproteins as mediators for the effects of alcohol consumption and cigarette smoking on cardiovascular mortality: results form the Lipid Research Clinics Follow-up Study. Am. J. Epidemiol. 1987; 126: 629-637.
7. Hendriks HF, Veenstra J, van Tol A, Groener JE, Schaafsma G. Moderate doses of alcoholic beverages with dinner and postprandial high density lipoprotein composition. Alcohol Alcohol 1998; 33: 403-410.
8. van der Gaag MS, van Tol A, Scheek LM et al. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. Atherosclerosis 1999; 147: 405-410.
9. van Tol A, van der Gaag MS, Scheek LM, van Gent T, Hendriks HF. Changes in postprandial lipoproteins of low and high density caused by moderate alcohol consumption with dinner. Atherosclerosis 1998; 141 Suppl 1: S101-S103.
10. Mackness B, Mackness MI, Arrol S, Turkie W, Durrington PN. Effect of the human serum paraoxonase 55 and 192 genetic polymorphisms on the protection by high density lipoprotein against low density lipoprotein oxidative modification. FEBS Lett. 1998; 423: 57-60.
11. Mackness MI, Arrol S, Mackness B, Durrington PN. Alloenzymes of paraoxonase and effectiveness of high-density lipoproteins in protecting low-density lipoprotein against lipid peroxidation. Lancet 1997; 349: 851-852.
12. Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J. Lipid Res. 1995; 36: 211-228.
13. Owen JS, Mulcahy JV. ATP-binding cassette A1 protein and HDL homeostasis. Atherosclerosis. 2002; Suppl 3: 13-22.
14. Rothblat GH, Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC. Cell cholesterol efflux: integration of old and new observations provides new insights. J. Lipid Res. 1999; 40: 781-796.
15. van der Gaag MS, van Tol A, Vermunt SH, Scheek LM, Schaafsma G, Hendriks HF. Alcohol consumption stimulates early steps in reverse cholesterol transport. J. Lipid Res. 2001; 42: 2077-2083.
16. Singaraja RR, Fievet C, Castro G et al. Increased ABCA1 activity protects against atherosclerosis. J. Clin. Invest 2002; 110: 35-42.
17. Fournier N, Francone O, Rothblat G et al. Enhanced efflux of cholesterol from ABCA1-expressing macrophages to serum from type IV hypertriglyceridemic subjects. Atherosclerosis 2003; 171: 287-293.
18. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. 1972; 18: 499-502.
19. Groener JE, Pelton RW, Kostner GM. Improved estimation of cholesteryl ester transfer/exchange activity in serum or plasma. Clin. Chem. 1986; 32: 283-286.
20. Speijer H, Groener JE, van Ramshorst E, van Tol A. Different locations of cholesteryl ester transfer protein and phospholipid transfer protein activities in plasma. Atherosclerosis 1991; 90: 159-168.
21. van Venrooij FV, Stolk RP, Banga JD et al. Common cholesteryl ester transfer protein gene polymorphisms and the effect of atorvastatin therapy in type 2 diabetes. Diabetes Care 2003; 26: 1216-1223.
22. Joles JA, Willekes-Koolschijn N, Scheek LM, Koomans HA, Rabelink TJ, van Tol A. Lipoprotein phospholipid

composition and LCAT activity in nephrotic and analbuminemic rats. Kidney Int. 1994; 46: 97-104.
23. Fournier N, Atger V, Paul JL et al. Human ApoA-IV overexpression in transgenic mice induces cAMP-stimulated cholesterol efflux from J774 macrophages to whole serum. Arterioscler. Thromb. Vasc. Biol. 2000 ; 20: 1283-1292.
24. Fournier N, Cogny A, Atger V et al. Opposite effects of plasma from human apolipoprotein A-II transgenic mice on cholesterol efflux from J774 macrophages and Fu5AH hepatoma cells. Arterioscler. Thromb. Vasc. Biol. 2002; 22: 638-643.
25. Sakr SW, Williams DL, Stoudt GW, Phillips MC, Rothblat GH. Induction of cellular cholesterol efflux to lipid-free apolipoprotein A-I by cAMP. Biochim. Biophys. Acta 1999; 1438: 85-98.
26. de la Llera-Moya M, Atger V, Paul JL et al. A cell culture system for screening human serum for ability to promote cellular cholesterol efflux. Relations between serum components and efflux, esterification, and transfer. Arterioscler. Thromb. 1994; 14: 1056-1065.
27. Ji Y, Jian B, Wang N et al. Scavenger receptor BI promotes high density lipoprotein-mediated cellular cholesterol efflux. J. Biol. Chem. 1997; 272: 20982-20985.
28. Yancey PG, Kawashiri MA, Moore R et al. In vivo modulation of HDL phospholipid has opposing effects on SR-BI- and ABCA1-mediated cholesterol efflux. J. Lipid Res. 2004; 45: 337-346.
29. Marmillot P, Rao MN, Liu QH, Chirtel SJ, Lakshman MR. Effect of dietary omega-3 fatty acids and chronic ethanol consumption on reverse cholesterol transport in rats. Metabolism 2000; 49: 508-512.
30. Avdulov NA, Chochina SV, Igbavboa U, Wood WG. Cholesterol efflux to high-density lipoproteins and apolipoprotein A-I phosphatidylcholine complexes is inhibited by ethanol: role of apolipoprotein structure and cooperative interaction of phosphatidylcholine and cholesterol. Biochemistry 2000; 39: 10599-10606.
31. Senault C, Betoulle D, Luc G, Hauw P, Rigaud D, Fumeron F. Beneficial effects of a moderate consumption of red wine on cellular cholesterol efflux in young men. Nutr. Metab Cardiovasc. Dis. 2000; 10: 63-69.
32. Hara H, Yokoyama S. Interaction of free apolipoproteins with macrophages. Formation of high density lipoprotein-like lipoproteins and reduction of cellular cholesterol. J. Biol. Chem. 1991; 266: 3080-3086.
33. Yancey PG, Bielicki JK, Johnson WJ et al. Efflux of cellular cholesterol and phospholipid to lipid-free apolipoproteins and class A amphipathic peptides. Biochemistry 1995; 34: 7955-7965.
34. Barrans A, Jaspard B, Barbaras R, Chap H, Perret B, Collet X. Pre-beta HDL: structure and metabolism. Biochim. Biophys. Acta 1996; 1300: 73-85.
35. Lie J, de Crom R, Jauhiainen M et al. Evaluation of phospholipid transfer protein and cholesteryl ester transfer protein as contributors to the generation of pre beta-high-density lipoproteins. Biochem. J. 2001; 360: 379-385.
36. Pussinen PJ, Jauhiainen M, Ehnholm C. ApoA-II/apoA-I molar ratio in the HDL particle influences phospholipid transfer protein-mediated HDL interconversion. J. Lipid Res. 1997; 38: 12-21.
37. Sparks DL, Frank PG, Braschi S, Neville TA, Marcel YL. Effect of apolipoprotein A-I lipidation on the formation and function of pre-beta and alpha-migrating LpA-I particles. Biochemistry 1999; 38: 1727-1735.
38. Atger V, de la Llera-Moya M, Bamberger M et al. Cholesterol efflux potential of sera from mice expressing human cholesteryl ester transfer protein and/or human apolipoprotein AI. J. Clin. Invest 1995; 96: 2613-2622.
39. Fournier N, de la Llera-Moya M, Burkey BF et al. Role of HDL phospholipid in efflux of cell cholesterol to whole serum: studies with human apoA-I transgenic rats. J. Lipid Res. 1996; 37: 1704-1711.
40. Brites FD, Bonavita CD, De Geitere C et al. Alterations in the main steps of reverse cholesterol transport in male patients with primary hypertriglyceridemia and low HDL-cholesterol levels. Atherosclerosis 2000; 152: 181-192.
41. Sasahara T, Nestel P, Fidge N, Sviridov D. Cholesterol transport between cells and high density lipoprotein subfractions from obese and lean subjects. J. Lipid Res. 1998; 39: 544-554.
42. Syvanne M, Castro G, Dengremont C et al. Cholesterol efflux from Fu5AH hepatoma cells induced by plasma of subjects with or without coronary artery disease and non-insulin-dependent diabetes: importance of LpA-I:A-II particles and phospholipid transfer protein. Atherosclerosis 1996; 127: 245-253.



Chapter 4

The effect of moderate alcohol consumption on lipoprotein-associated phospholipase A2 activity: a randomized controlled intervention

Joline WJ Beulens

Robin van den Berg

Frans J Kok

Anders Helander

Susan HF Vermunt

Henk FJ Hendriks

Submitted

ABSTRACT

Objective

To investigate the effect of moderate alcohol consumption on lipoprotein associated phospholipase A2 (Lp-PLA2) activity, markers of inflammation and oxidative stress and whether these effects are modified by BMI.

Methods

Eleven lean (BMI: 18.5–25 kg/m^2) and nine overweight (BMI> 27 kg/m^2) men participated in a randomized controlled crossover trial. After consuming three cans of beer (40 g ethanol) or alcohol-free beer daily during three weeks, fasting blood samples were taken.

Results

HDL cholesterol increased by 18.2% ($p< 0.001$) after beer compared to alcohol-free beer, while LDL cholesterol decreased by 7.8% ($p= 0.008$). Lp-PLA2 activity was not different ($p= 0.23$) between beer (47.5 ±0.8) and alcohol-free beer (48.9 ±0.8). High sensitive C-reactive protein was unaffected, but urinary isoprostanes tended to increase ($p= 0.09$) after beer (114.0 ±6.9) compared to alcohol-free beer (96.9 ±6.5). An interaction between BMI and treatment ($p< 0.05$) on liver enzymes was observed, indicating an increase of liver enzymes after moderate alcohol consumption in overweight men only.

Conclusion

Despite profound effects on HDL and LDL cholesterol, moderate alcohol consumption did not affect Lp-PLA2 activity. Liver enzymes increased after alcohol consumption in overweight men only, suggesting a less favorable response to moderate alcohol consumption among overweight persons.

INTRODUCTION

Moderate alcohol consumption is associated with a decreased risk of cardiovascular disease (CVD) (1). About 50% of this association is explained by an increase of HDL cholesterol (1). Other factors such as a decrease of inflammatory (2) or fibrinolytic factors (3), or an increase of insulin sensitivity may also be involved (4;5). In addition, moderate alcohol consumption increases functional properties of HDL cholesterol such as paraoxonase activity (6) and cholesterol efflux (7). However, these effects have mainly been studied in middle-aged men or women (8) and a paucity of data exists for younger men.

Lipoprotein associated phospholipase A2 (Lp-PLA2) is an LDL- and HDL-associated enzyme (9). Similar to paraoxonase (10), Lp-PLA2 hydrolyses platelet activating factor, but it also effectively hydrolyses oxidized phospholipids (9). High plasma levels of Lp-PLA2 concentration (11) or activity (12) are independently associated with increased risk of CVD and Lp-PLA2 is suggested as an inflammatory marker (9). Whether moderate alcohol consumption also affects Lp-PLA2 has not been studied to date.

Serum liver enzymes are widely used as non-specific markers of chronic alcohol consumption (13), but elevated liver enzymes can also be present in individuals not consuming significant amounts of alcohol. This combination of histologic findings and liver enzyme elevation in absence of alcohol consumption is referred to as non-alcoholic steatohepatitis (NASH) (14). It is thought that inflammation and oxidative stress are involved in the development of NASH (14). Elevated liver enzymes are also independently associated with diabetes (15) and risk factors for CVD such as waist-to-hip ratio, hypertension and dyslipidemia (16;17). BMI is the strongest independent predictor of elevated liver enzymes (18) and may modify the association between alcohol consumption and γ-glutamyl transferase (GGT) (19). Whether BMI also modifies the effect of moderate alcohol consumption on other risk factors for CVD is unknown. This study investigated the effect of moderate alcohol consumption on Lp-PLA2 activity and markers of inflammation and oxidative stress and whether these effects are modified by BMI in young healthy men.

METHODS

Subjects & Design

Twenty healthy, lean (n=11; BMI: 18.5 -25 kg/m^2) or overweight (n=9; BMI> 27 kg/m^2) young men, aged 18 to 25 years, participated in a randomized, partially diet-controlled, crossover trial. According to self-report, the subjects were used to moderate alcohol consumption (10-28 units/week) and a habitual Western diet and lifestyle, had no family history of alcoholism, and did not smoke. Written informed consent was obtained from all participants after the study was carefully explained. The research protocol was approved of by the Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research (TNO) and the study was conducted according to the International Conference on Harmonisation Guideline for Good Clinical Practice.

The crossover trial consisted of two treatment periods of three weeks preceded by one week wash-out. The men were randomised based on BMI-group to receive the sequence beer (Amstel Bier, Amsterdam, The Netherlands; 5% vol ethanol) followed by alcohol-free beer (Amstel Malt Bier, Amsterdam, The Netherlands; <0.1 % vol ethanol) or the other way around. They consumed three cans of beer or alcohol-free beer daily with the evening meal, equaling 40 g ethanol/day during beer treatment. During the last ten days of each treatment period the diet was fully controlled. All food was supplied by TNO and subjects were not allowed to eat or drink anything except the foods supplied, tap water, tea or coffee. The energy content of the diet was adjusted to body weight and physical activity level of each participant. The composition of the diet was based on the Dutch Food Consumption Survey of 1998 (20) and consisted of 37 energy% fat, 15 energy% protein, and 48 energy% carbohydrates, excluding energy from alcohol.

Blood sampling and analysis

At the end of each treatment period (day 22) all subjects visited TNO after an overnight fast for blood sampling and blood pressure assessment. Blood was obtained from the antecubital vein and collected in tubes containing lithium-heparin or gel and clot activator (Becton Dickinson, Vacutainer Systems, Plymouth, UK). To obtain plasma or serum, blood was centrifuged for 15 minutes at 2000 g at 4 °C, between 15 and 30 minutes after

collection. Systolic and diastolic blood pressure was measured using a blood pressure monitor (Omron Healthcare Europe BV, The Netherlands) in triplo after a 15 minute rest.

Serum triacylglycerol and HDL cholesterol levels were determined enzymatically (Roche Diagnostics, Mannheim, Germany) and LDL cholesterol was calculated according to Friedewald (21). Activities of the liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), and alkaline phosphatase (ALP) were determined in serum using commercially available kits (Roche Diagnostics, Basel, Switzerland). High sensitive CRP (hs-CRP) was determined using a commercially available enzyme-linked immunosorbent assay (ELISA, Alpco Diagnostics). Plasma Lp-PLA2 activity was determined using a radiometric activity assay according to Oei et al. (12).

Urine sampling and analysis

Urine was collected during 24 hours on the last two days of the study and urine samples were stored at -80 °C until analysis. Ethyl glucuronide (EtG), a direct metabolite of ethanol, in 24-hour urine specimens was determined by LC-MS and was used as a marker of alcohol consumption during the previous days (22). Urinary 8-iso prostaglandin F2-alpha was determined using GC-MS according to a modified method of Morrow et al. (23). Data are expressed as pg 8-iso prostaglandin F2-alpha/mg creatinin.

Statistical analysis

Data were analyzed using the SAS statistical software package (SAS/STAT Version 8, SAS Institute, Cary, NC, USA). Treatment effects were assessed by analysis of variance using a mixed model with BMI, treatment order, period, treatment, and the interaction between BMI and treatment included in the model. Residuals were tested for normality and homogeneity of variance. These analyses were also performed among subgroups of lean and overweight subjects. Correlation coefficients were computed to assess associations between changes in outcome measures. In order to determine explanatory power of BMI, R^2 for these associations were obtained using regression analysis on those variables. Two-sided p-values below 0.05 were considered statistically significant.

RESULTS

Subjects

Table 1 shows the subject characteristics by BMI subgroup. Distinct differences between lean and overweight subjects were observed for serum cholesterol fractions and liver enzyme concentrations. Compliance to instructions for use of alcoholic beverages was good as judged by self-report and as indicated by all participants showing positive urinary EtG concentrations (mean ± sd: 6.6 ± 0.6 mg/l; range: 2.8- 14.0) after beer consumption, but no detectable EtG in all urine samples collected after drinking alcohol-free beer.

Table 1: Mean ± sd baseline characteristics among lean and overweight men

Variable	Lean	Overweight
N	11	9
Age (years)	19 ± 2	21 ± 2
BMI (kg/m^2)	20.1 ± 1.0[1]	31.3 ± 3.9
Haemoglobin (mmol/l)	9.4 ± 0.5	9.4 ± 0.5
Total cholesterol (mmol/l)	4.1 ± 0.6[1]	5.0 ± 0.8
HDL cholesterol (mmol/l)	1.6 ± 0.4[1]	1.2 ± 0.2
LDL cholesterol (mmol/l)	1.9 ± 0.5[1]	3.2 ± 0.9
Triacylglycerols (mmol/l)	1.2 ± 0.6	1.5 ± 0.4
Alkaline phosphatase (U/l)	100 ± 28	89 ± 27
Asparagine aminotransferase (U/l)	21 ± 4[1]	32 ± 21
Alanine aminotransferase (U/l)	15 ± 5[1]	60 ± 69
γ-glytamyl transferase (U/l)	18.0 ± 3.8[1]	43.3 ± 29.3

[1] $p<0.05$ lean compared to overweight group

Overall effect of moderate alcohol consumption

Table 2 shows Lp-PLA2 activity, blood lipid profile, hs-CRP, F2-isoprostanes and blood pressure after beer and alcohol-free beer consumption. Moderate alcohol consumption had profound effects on blood lipid profile in this group of young men. Fasting serum HDL cholesterol level showed a large increase of 18% ($p< 0.001$), while LDL cholesterol decreased by ~8% ($p= 0.008$) after beer compared with alcohol-free beer consumption. However, Lp-PLA2 activity was not different ($p= 0.23$) after beer (47.5 ± 0.8) compared with alcohol free beer consumption (48.9 ± 0.8). Hs-CRP was not affected either, but F2-

isoprostanes tended to increase (p= 0.09) after beer (114.0 ± 6.9) compared with alcohol-free beer consumption (96.9 ± 6.5). No effect of alcohol consumption on systolic blood pressure was found, while diastolic blood pressure tended to increase after beer compared with alcohol-free beer consumption (p= 0.10). Liver enzymes were slightly elevated after beer as compared with alcohol-free beer consumption, although only the effect on GGT and AST was statistically significant.

Table 2: Mean ±sem Lp-PLA2 activity, lipoprotein fractions and markers of inflammation and oxidative stress after 3 weeks consumption of beer and alcohol-free beer in the total study population (n=20).

	Alcohol-free beer	Beer	% change	p-value
Lp-PLA2 activity[1]	48.9 ± 0.8	47.5 ± 0.8	-2.9	0.23
Total cholesterol (mmol/l)	4.2 ± 0.1	4.3 ± 0.1	2.4	0.095
HDL cholesterol (mmol/l)	1.1 ± 0.03	1.3 ± 0.03	18.2	< 0.001
LDL cholesterol (mmol/l)	2.6 ± 0.04	2.4 ± 0.04	-7.8	0.008
Triacylglycerol (mmol/l)	1.1 ± 0.06	1.4 ± 0.06	27.3	0.003
Ratio total/HDL cholesterol	4.0 ± 0.1	3.6 ± 0.1	-10.0	< 0.001
High-sensitive CRP	1.6 ± 0.3	1.5 ± 0.3	-6.3	0.82
F2-isoprostanes[2]	96.9 ± 6.5	114.0 ± 6.9	17.6	0.085
Systolic blood pressure (mm Hg)	114 ± 1	116 ± 1	1.8	0.30
Diastolic blood pressure (mm Hg)	65 ± 1	68 ± 1	4.6	0.097
GGT (U/l)	22 ± 1	26 ± 1	18.2	0.011
AST (U/l)	23 ± 0.8	26 ± 0.8	13.0	0.033
ALT (U/l)	26 ± 2	28 ± 2	7.8	0.43
ALP (U/l)	89 ± 2	89 ± 2	0	0.84

[1] expressed as nmol/min/ml plasma

[2] expressed as pg 8-iso prostaglandin F2-alpha/mg creatinin

Interaction of treatment with BMI

Table 3 shows Lp-PLA2 activity, blood lipid profile, hs-CRP, F2-isoprostanes and blood pressure after beer and alcohol-free beer consumption among lean and overweight subjects. The interaction between BMI and treatment was (borderline) significant for both GGT (p= 0.046) and AST (p= 0.063). Subgroup analysis indicated that the increase of liver enzymes, particularly GGT and AST, was larger among overweight (26% and 20% respectively) than lean men (8% and 0%). No significant interactions between treatment and BMI were observed for other parameters. LDL cholesterol, however, decreased (p= 0.010) by 10% among lean subjects, while it did not change (p= 0.23) among overweight subjects.

Table 3: Mean ± sem Lp-PLA2 activity, lipoprotein fractions and markers of inflammation and oxidative stress after 3 weeks consumption of beer and alcohol-free beer in the lean subgroup (n=11) and the overweight subgroup (n=9).

	Lean group				Overweight group			
	Alcohol-free beer	Beer	% change	p-value	Alcohol-free beer	Beer	% change	p-value
Lp-PLA2 activity[1]	39.1 ± 1.2	36.8 ± 1.2	-5.9	0.21	58.5 ± 1.1	58.3 ± 1.1	-0.3	0.90
Total cholesterol (mmol/l)	3.7 ± 0.1	3.8 ± 0.1	2.7	0.046	4.7 ± 0.1	4.8 ± 0.1	2.1	0.61
HDL cholesterol (mmol/l)	1.3 ± 0.04	1.5 ± 0.04	15.4	0.003	1.0 ± 0.1	1.1 ± 0.04	10.0	0.030
LDL cholesterol (mmol/l)	2.0 ± 0.05	1.8 ± 0.05	-10.0	0.010	3.1 ± 0.09	3.0 ± 0.09	-3.2	0.23
Triacylglycerol (mmol/l)	0.8 ± 0.08	1.2 ± 0.08	50.0	0.017	1.4 ± 0.08	1.7 ± 0.08	21.4	0.097
Ratio total/HDL cholesterol	3.0 ± 0.08	2.7 ± 0.08	-10.0	0.023	5.0 ± 0.1	4.6 ± 0.1	-8.0	0.030
High-sensitive CRP	1.0 ± 0.2	0.8 ± 0.2	-20.0	0.59	2.2 ± 0.5	2.3 ± 0.5	4.5	0.87
F2-isoprostanes[2]	89.3 ± 11.6	109.6 ± 11.6	22.7	0.25	105.6 ± 4.0	117.7 ± 4.5	11.5	0.072
Systolic blood pressure[3]	109 ± 2	110 ± 2	0.9	0.65	119 ± 2	122 ± 2	2.5	0.27
Diastolic blood pressure[3]	62 ± 2	64 ± 2	3.2	0.43	67 ± 2	72 ± 2	7.5	0.19
GGT (U/l)	12 ± 0.3	13 ± 0.3	8.3	0.002	31 ± 2	39 ± 2	25.8	0.048
AST (U/l)	21 ± 0.8	21 ± 0.8	0	0.55	25 ± 2	30 ± 2	20.0	0.046
ALT (U/l)	12 ± 0.4	12 ± 0.4	0	0.59	40 ± 4	45 ± 4	12.5	0.37
ALP (U/l)	94 ± 2	92 ± 2	-2.1	0.46	82 ± 3	86 ± 3	4.9	0.43

[1] expressed as nmol/min/ml plasma

[2] expressed as pg 8-iso prostaglandin F2-alpha/mg creatinin

[3] expressed as mm Hg

BMI effects

Plasma Lp-PLA2 activity was higher ($p< 0.001$) among overweight (58.4 ± 0.9) than lean (38.0 ± 0.8) subjects. Hs-CRP was also higher ($p= 0.015$) among overweight (2.2 ± 0.3) than lean (0.9 ± 0.2) subjects. As expected diastolic blood pressure, total cholesterol, LDL cholesterol, triacylglycerol, ratio total to HDL cholesterol GGT and ALT were significantly higher and HDL cholesterol was significantly lower among overweight than lean subjects (data not shown).

Correlations

BMI correlated significantly ($r= -0.71$; $p< 0.001$) with changes of GGT during the study and explained these changes for about 50%, as indicated by an R^2 of 0.50. BMI also correlated significantly with changes of ALT ($r= -0.48$; $p= 0.031$) and AST ($r= -0.59$; $p=0.006$). Finally, initial HDL concentration correlated significantly with changes of HDL cholesterol ($r= 0.62$, $p= 0.004$) and explained these changes for about 40%, as indicated by an R^2 of 0.38.

Changes of Lp-PLA2 activity correlated modestly with changes of LDL cholesterol ($r= 0.40$; $p= 0.08$), HDL cholesterol ($r= -0.41$; $p= 0.08$) and ratio of total to HDL cholesterol ($r= 0.52$; $p= 0.02$). Changes of Lp-PLA2 activity correlated strongly with changes of GGT ($r= 0.72$; $p< 0.001$) and AST ($r= 0.58$; $p= 0.008$).

DISCUSSION

This study showed profound beneficial effects of moderate alcohol consumption on blood lipid profile in young healthy men. Despite this, Lp-PLA2 activity was not affected by moderate alcohol consumption. Hs-CRP was not affected either, but F2-isoprostanes tended to increase after moderate alcohol consumption. The effect of moderate alcohol consumption on blood liver enzymes was largely dependent on BMI and an elevation of liver enzymes within normal values after moderate alcohol consumption was only observed for overweight men.

The study was performed according to a randomized, controlled crossover design and the diet of the subjects was fully controlled during the last ten days of each treatment period. It therefore seems unlikely that our results are confounded by diet or lifestyle.

Using urinary EtG as a sensitive and specific marker for alcohol consumption we were perfectly able to discriminate between the alcohol and alcohol-free period of the study. This is in accordance with the study of Sarkola et al (24). Variation in our results was smaller, possibly because we assessed EtG in 24-hour urine, while spot morning urine was used by Sarkola et al. (24). Moreover, only negative results of EtG were found during the alcohol-free beer treatment, while Sarkola et al. found two positive samples during the placebo period suggesting incidental consumption of alcoholic beverages. Our results confirm both that urinary EtG may be a valuable marker of recent alcohol consumption and that compliance to study treatment was good.

The findings from our study are in line with the well-known effects of moderate alcohol consumption on blood lipid profile. The magnitude of effects observed in this population of young men is remarkable. We found an 18% increase of HDL cholesterol, which is almost twice the increase reported in previous studies (8). HDL cholesterol is considered as the only lipoprotein mediating the association of moderate alcohol consumption with CVD. However, in our study LDL cholesterol also decreased significantly by 8%. This has only been reported previously for women (25-27). Possibly a decrease of LDL cholesterol may be involved in the cardio-protective effect of moderate alcohol consumption in addition to HDL cholesterol, at least in specific populations such as females and young men.

Despite these large effects on HDL and LDL cholesterol, Lp-PLA2 activity did not change after moderate alcohol consumption. To our knowledge, this study is the first

investigating the effect of a nutritional intervention (moderate alcohol consumption) on Lp-PLA2 activity. Only in an observational study of Oei et al. (12) a small but significant correlation between alcohol consumption and Lp-PLA2 activity was observed. Probably effects of moderate alcohol consumption on Lp-PLA2 activity are relatively small and only apparent in observational studies using larger populations and reflecting longer time-periods. As expected from the well-known association of obesity with inflammation, Lp-PLA2 activity was much higher among overweight than lean subjects. This is in line with a positive correlation between Lp-PLA2 and BMI observed by Oei et al. (12). The correlations between changes of Lp-PLA2 activity and LDL and HDL cholesterol were more modest than those demonstrated by Schaefer et al. (28) investigating the effect of statins on Lp-PLA2 concentration and activity. This difference can be explained by the relatively small change of Lp-PLA2 activity after moderate alcohol consumption compared to the larger effect of statins (28).

In this study a significant interaction between BMI and alcohol consumption for liver enzymes was observed, indicating a larger increase of liver enzymes after moderate alcohol consumption among overweight than lean subjects. This is in line with the study of Poikolainen & Vartainen (1997) (19), showing that GGT increased with increasing alcohol intake in overweight subjects (BMI > 27 kg/m2), whereas in lean subjects an increase of GGT was only found at an intake of alcohol exceeding 300 g/week. Consistent with previous studies (18), we showed that BMI is an important predictor of elevation of liver enzymes.

Inflammation and oxidative stress are both involved in the development of NASH. We therefore hypothesized that BMI could modify effects on markers of inflammation and oxidative stress. Indeed, higher concentrations of inflammatory markers were observed in overweight than lean men, but in contrast to previous reports (2;29), we did not observe an effect of moderate alcohol consumption on hs-CRP. This may be due to the relatively large variation in these results. Some subjects had a mild form of common cold during the course of the study that may have confounded these results. However, moderate alcohol consumption tended to increase F2-isoprostanes which is in line with the study of Hartman et al. (30).

Unfortunately, we could not demonstrate significant interactions between BMI and treatment for markers inflammation and oxidative stress. However, the decrease of Lp-

PLA2 activity after moderate alcohol consumption was larger among lean than overweight subjects. Furthermore, changes of Lp-PLA2 activity correlated strongly with changes of GGT and AST indicating a larger decrease of Lp-PLA2 with a smaller increase of liver enzymes. Also, LDL cholesterol decreased stronger among lean than overweight subjects. Altogether, these findings suggest a less favorable effect of moderate alcohol consumption among overweight than lean subjects. This could be attributed to the presence of symptoms of liver damage due to obesity that may already be present at young age (31). Nevertheless, larger trials are needed to further investigate whether inflammatory markers are involved in these differences between lean and overweight men.

In this population of healthy, young men we demonstrated profound effects of moderate alcohol consumption on HDL and LDL cholesterol. Despite this, we did not observe that moderate alcohol consumption affected Lp-PLA2 activity in this study. The effect of moderate alcohol consumption on liver enzymes was largely dependent on BMI and an increase of liver enzymes after moderate alcohol consumption was only demonstrated in overweight men. This suggests that overweight or obese persons may respond less favorably to alcohol consumption than lean subjects.

Acknowledgements

We acknowledge all those involved in the conduct of the study and thank the volunteers for their enthusiastic participation. The research described in this article was funded by the Dutch Foundation for Alcohol Research.

REFERENCES

1. Grobbee DE, Rimm EB, Keil U, Renaud S. Alcohol and the Cardiovascular System. In: MacDonald I, ed. Health issues related to alcohol consumption. Bodmin: Blackwell Sciences Ltd 1999:125-79.
2. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur J Clin Nutr 2002;56:1130-6.
3. Hendriks HF, Veenstra J, Velthuis-te Wierik EJ, Schaafsma G, Kluft C. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. BMJ 1994;308:1003-6.
4. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
5. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
6. van der Gaag MS, van Tol A, Scheek LM et al. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. Atherosclerosis 1999;147:405-10.
7. Beulens JW, Sierksma A, van Tol A et al. Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1. J Lipid Res 2004;45:1716-23.
8. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523-8.
9. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 2005;25:923-31.
10. Rodrigo L, Mackness B, Durrington PN, Hernandez A, Mackness MI. Hydrolysis of platelet-activating factor by human serum paraoxonase. Biochem J 2001;354:1-7.
11. Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. Circulation 2004;110:1903-8.
12. Oei HH, van dM, I, Hofman A et al. Lipoprotein-associated phospholipase A2 activity is associated with risk of coronary heart disease and ischemic stroke: the Rotterdam Study. Circulation 2005;111:570-5.
13. Helander A. Biological markers in alcoholism. J Neural Transm Suppl 2003;15-32.
14. James OFW, Day CP. Non-alcoholic steatohepatitis (NASH): A disease of emerging identity and importance. J Hepatol 1999;29:495-501.
15. Perry IJ, Wannamethee SG, Shaper AG. Prospective study of serum gamma-glutamyltransferase and risk of NIDDM. Diabetes Care 1998;21:732-7.
16. van Barneveld T, Seidell JC, Traag N, Hautvast JG. Fat distribution and gamma-glutamyl transferase in relation to serum lipids and blood pressure in 38-year old Dutch males. Eur J Clin Nutr 1989;43:809-18.
17. Wannamethee G, Ebrahim S, Shaper AG. Gamma-glutamyltransferase: determinants and association with mortality from ischemic heart disease and all causes. Am J Epidemiol 1995;142:699-708.
18. Bruckert E, Giral P, Ratziu V et al. A constellation of cardiovascular risk factors is associated with hepatic enzyme elevation in hyperlipidemic patients. Metabolism 2002;51:1071-6.
19. Poikolainen K, Vartiainen E. Determinants of gamma-glutamyltransferase: positive interaction with alcohol and

body mass index, negative association with coffee. Am J Epidemiol 1997;146:1019-24.
20. Voedingscentrum. Zo eet Nederland 1998; resultaten van de Voedselconsumptiepeiling 1998. Den Haag: 2003.
21. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972;18:499-502.
22. Stephanson N, Dahl H, Helander A, Beck O. Direct quantification of ethyl glucuronide in clinical urine samples by liquid chromatography-mass spectrometry. Ther Drug Monit 2002;24:645-51.
23. Morrow JD, Zackert WE, Yang JP et al. Quantification of the major urinary metabolite of 15-F2t-isoprostane (8-iso-PGF2alpha) by a stable isotope dilution mass spectrometric assay. Anal Biochem 1999;269:326-31.
24. Sarkola T, Dahl H, Eriksson CJ, Helander A. Urinary ethyl glucuronide and 5-hydroxytryptophol levels during repeated ethanol ingestion in healthy human subjects. Alcohol Alcohol 2003;38:347-51.
25. Baer DJ, Judd JT, Clevidence BA et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr 2002;75:593-9.
26. Clevidence BA, Reichman ME, Judd JT et al. Effects of alcohol consumption on lipoproteins of premenopausal women. A controlled diet study. Arterioscler Thromb Vasc Biol 1995;15:179-84.
27. van der Gaag MS, Sierksma A, Schaafsma G et al. Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. J Womens Health Gend Based Med 2000;9:607-16.
28. Schaefer EJ, McNamara JR, Asztalos BF et al. Effects of atorvastatin versus other statins on fasting and postprandial C-reactive protein and lipoprotein-associated phospholipase A2 in patients with coronary heart disease versus control subjects. Am J Cardiol 2005;95:1025-32.
29. Imhof A, Woodward M, Doering A et al. Overall alcohol intake, beer, wine, and systemic markers of inflammation in western Europe: results from three MONICA samples (Augsburg, Glasgow, Lille). Eur Heart J 2004;25:2092-100.
30. Hartman TJ, Baer DJ, Graham LB et al. Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. Eur J Clin Nutr 2005;59:161-8.
31. Kichian K, McLean R, Gramlich LM, Bailey RJ, Bain VG. Nonalcoholic fatty liver disease in patients investigated for elevated liver enzymes. Can J Gastroenterol 2003;17:38-42.

Chapter 5

Alcohol consumption and risk of type 2 diabetes mellitus among older women

Joline WJ Beulens

Ronald P Stolk

Yvonne T van der Schouw

Diederick E Grobbee

Henk FJ Hendriks

Michiel L Bots

Diabetes Care (2005); 28(12); 2933-2938

ABSTRACT

Objective

This study aimed to investigate the relation between alcohol consumption and type 2 diabetes among older women.

Methods

Between 1993 and 1997 16,330 women aged 49-70 years and free from diabetes were enrolled in one of the Dutch Prospect-EPIC (European Prospective study Into Cancer and nutrition) cohorts and followed for 6.2 years (range 0.1 to 10.1). At enrollment, women filled in questionnaires and blood samples were collected.

Results

During follow-up, 760 cases of type 2 diabetes were documented. A linear, inverse association (p= 0.007) between alcohol consumption and type 2 diabetes risk was observed, adjusting for potential confounders. Compared to abstainers, the hazard ratio for type 2 diabetes was 0.86 (95%-CI: 0.66- 1.12) for women consuming 5-30 g alcohol per week, 0.66 (0.48- 0.91) for 30-70 g per week, 0.91 (0.67- 1.24) for 70-140 g per week, 0.64 (0.44- 0.93) for 140-210 g per week and 0.69 (0.47- 1.02) for >210 g alcohol per week. Beverage type did not influence this association. Lifetime alcohol consumption was associated with type 2 diabetes in a U-shaped fashion.

Conclusion

Our findings support the evidence of a decreased risk of type 2 diabetes with moderate alcohol consumption and expand this to a population of older women.

INTRODUCTION

Type 2 diabetes is a major disease burden in developed and developing countries and its prevalence is expected to double the next 20 years (1). Alcohol consumption is associated with type 2 diabetes in a U-shaped fashion, indicating a decreased risk of type 2 diabetes with moderate alcohol consumption compared to both abstaining and excessive drinking (2). This relation has mostly been investigated among male populations (2). Three studies included both men and women and two included younger women up to 55 years of age (3,4). Only one study of Lee et al. describing the association between dietary iron and type 2 diabetes reported on the association of alcohol consumption with type 2 diabetes among postmenopausal women (5).

Data on this relation among older women are therefore particularly scarce. Meanwhile, the prevalence of type 2 diabetes is increasing with age and the majority of type 2 diabetic patients are women (1,6). The cumulative influence of lifetime alcohol consumption also remains unexamined. Therefore we examined the relation between both current and lifetime alcohol consumption and risk of type 2 diabetes in a large population-based prospective cohort study, consisting of women aged > 50 years.

METHODS

Study Design

A follow-up study was performed among 17,357 women aged 49-70 who participated in the breast cancer screening PROSPECT-EPIC (European Prospective Investigation into Cancer and Nutrition) cohort between 1993 and 1997, one of two Dutch contributions to EPIC. The design, sampling strategies and examination techniques of the cohort have been described previously (7). All women signed informed consent before study inclusion. The study complies with the Declaration of Helsinki and was approved by the Institutional Review Board of the University Medical Center Utrecht. Of the total cohort of 17,357 women, we excluded 119 women with missing data on alcohol consumption, 367 with missing data on occurrence of type 2 diabetes and 17 with missing data on BMI. Furthermore, 524 women reported diabetes at baseline and were therefore excluded, leaving 16,330 women for the present study. From the original cohort of 17,357 women, a 10% random sample was drawn and the same exclusion criteria were applied, leaving 1385 women. Serum HDL cholesterol was assessed in this random sample.

Baseline measurements

At baseline, questionnaires were mailed to women who agreed to participate and these were returned when the women visited for breast cancer screening (7). A general questionnaire included questions on demographic characteristics and presence of and risk factors for chronic diseases. Diabetes mellitus was defined present based on a physician-diagnosed self-report. Waist and hip circumferences, height and weight were measured, and body mass index (BMI) was calculated. Systolic and diastolic blood pressure were measured twice at the right arm with an automated and calibrated blood pressure device with the subject in supine position, and the mean was calculated. Hypertension and hypercholesterolemia were defined present based on a physician-diagnosed self-report. Women were assumed to be post-menopausal when they reported not having menstrual periods for at least a year. Physical activity was assessed using a questionnaire validated in an elderly population (8). Daily energy intake was obtained from a food frequency questionnaire (FFQ) containing questions on the usual frequency of consumption of 77 main food items during the year preceding enrollment. This questionnaire allows the

estimation of the average daily consumption of 178 foods. The FFQ has been validated prior to the start of the study (9,10). At baseline all women donated a non-fasting blood sample. Serum HDL cholesterol was determined for the random sample using an automated enzymatic procedure on a Vitros 250 (Johnson & Johnson, Rochester, New York, USA).

Assessment of alcohol consumption

Alcohol consumption was assessed by the general questionnaire and FFQ. The validity of this alcohol intake assessment is good as confirmed by a Spearman correlation of 0.87 between the FFQ and 12 to 24 hour recalls (9). The general questionnaire contained four questions regarding previous alcohol consumption. Subjects were asked whether they had ever used alcohol. If so, they were asked how many units of beer, wine, port/sherry/vermouth and spirits they drank at ages 20 and 40 (none, less than one unit a week or to indicate the number of units a week). The FFQ contained one question regarding the number of units of alcohol-free beer, beer, white wine, red wine, port/sherry/vermouth, and spirits consumed during the year before enrollment. Subjects indicated their consumption frequency on a daily/weekly/monthly/yearly scale or as never consumed. Baseline alcohol intake was determined by multiplying the consumption of each beverage by its ethanol content (10 g for beer and fortified wine, 9.6 g for wine and 9.8 g for liquor) (11) and was categorized into seven categories; teetotalers, 0-4.9 g/ week, 5-29.9 g/week, 30-69.9 g/week, 70-139.9 g/week, 140-209.9 g/week and ≥210 g/week.

To assess the influence of alcohol consumption earlier in life, we calculated lifetime alcohol consumption based on reported alcohol consumption at age 20 and 40 and calculated the number of years drinking one standard alcoholic beverage of 10 g alcohol daily (alcohol-years). We determined the number of standard alcoholic beverages containing 10 g of alcohol consumed daily at age 20 and 40. The amount consumed at age 20 was assumed to remain unchanged until age 30 and the amount consumed at age 40 from 30 until inclusion in the study. Alcohol-years were calculated by multiplying the number of alcoholic beverages of 10 g daily with the total number of years this amount of alcohol was consumed: from 20 to 30 years and from 30 to inclusion. Alcohol-years from 20 to 30 years, 30 to inclusion were added up and categorized into seven groups; 0 (teetotalers), 0.1-4.9, 5-9.9, 10-19.9, 20-29.9, 30-49.9 and ≥50 years of drinking one standard alcoholic

beverage of 10 g alcohol daily. The validity of such retrospective assessment of components of the diet (including alcohol) has been recently investigated in a similar population and is shown to provide a reasonable record (12).

Assessment of type 2 diabetes

Two follow-up questionnaires regarding occurrence of disease were sent to the participants in three- to five-year intervals. The occurrence of type 2 diabetes was assessed by self-reported occurrence of diabetes in the follow-up questionnaires, and/or a urinary glucose strip test for detection of glucosuria and/or a Dutch register of hospital discharge diagnoses. Type 2 diabetes was defined present when either of these methods reported a positive response. Follow-up questionnaires contained seven questions addressing the occurrence of diabetes. Subjects were asked whether diabetes was diagnosed and if so; in what year, by whom and whether they were treated by a diet, oral blood glucose lowering drugs and/or insulin. With the first follow-up questionnaire subjects received a urinary glucose strip test and were asked whether the urine strip had turned purple after ten seconds, indicating glucosuria. Response rate of self-reported occurrence of type 2 diabetes was 80% from the first follow-up questionnaire, and 91% at the second follow-up questionnaire and 73% for returning the outcome of the urinary glucose strip test. Data on diagnosis of type 2 diabetes were obtained from the Dutch Centre for Health Care Information, which holds a standardized computerized register of hospital discharge diagnoses. Admission files have been filed continuously from all general and university hospitals in the Netherlands from 1990. Data on sex, date of birth, dates of admission and discharge were recorded whenever a patient is discharged from hospital. One mandatory principal diagnosis and up to nine optional additional diagnoses were reported. All diagnoses were coded according to the International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9; 250 for type 2 diabetes). Follow-up was complete until January 1, 2002. The database was linked to the cohort on the basis of birth date, gender, postal code, and general practitioner with a validated probabilistic method (13). Information on vital status was obtained through linkage with the municipal administration registries.

Statistical analysis

Data analysis was performed using SPSS for windows version 12.0 (SPSS Inc., Chicago, USA). The association between alcohol consumption and HDL cholesterol was determined using regression analysis. The duration of follow-up was calculated as the interval between date of study entry and the diagnosis of type 2 diabetes, death, loss to follow-up, or January 1, 2002. Hazard Ratios (HR) for each baseline alcohol intake category were calculated using Cox proportional hazards with teetotalers as a reference group. Age- and BMI-adjusted HRs and 95% confidence intervals were estimated. We used a multivariate model to adjust for other confounding factors: smoking status (non-, former, current smokers), physical activity level (five categories), menopausal status (pre-, postmenopausal, unknown), education (seven categories), systolic blood pressure (six categories), hypertension (present or not), hypercholesterolemia (present or not), family history of diabetes (none, one parent or both), daily energy intake (four categories), waist circumference (six categories) and hip circumference (five categories). Linear trends across alcohol consumption categories were computed by including alcohol intake categories in the model as linear covariate. The square of this term was used to assess the quadratic trend across alcohol consumption categories. The influence of alcohol consumption earlier in life independent from current alcohol consumption was assessed by estimating HRs based on categories of alcohol-years using the same models as for baseline alcohol consumption. We adjusted for baseline alcohol consumption by including it as a continuous variable in the model, because the categorical variables of baseline alcohol consumption and alcohol-years were correlated ($r= 0.73$; $p< 0.001$). We assessed the risk associated with individual beverage types controlling for the covariates included in the multivariate model and intake of each of the other beverage types. Teetotalers were excluded from this analysis. Wine consumption was separated in four categories, as it was consumed in larger quantities than the other beverages. Fortified wine and liquor were separated in three and beer in two categories. Population attributive risks were calculated according to Rothman & Greenland (1998) using teetotalers as a reference group (14). Two-sided p-values <0.05 were regarded significant.

RESULTS

Baseline characteristics

Baseline characteristics of the study population are shown in table 1. BMI and waist circumference were inversely associated with alcohol consumption, while smoking, energy intake, physical activity level and education showed positive associations with alcohol consumption. Systolic and diastolic blood pressure and prevalence of hypertension decreased with increasing alcohol consumption up to 140 g/week, but increased in the higher drinking categories.

To validate self-reported alcohol intake, we determined the relation between alcohol intake and HDL cholesterol in the random sample of 1385 women. This analysis showed a linear association ($p< 0.001$) between alcohol intake and HDL cholesterol concentrations (beta ±sem: 0.1 ±0.01 mmol/l per 10 gram alcohol/week) with levels ranging from 1.41 ±0.05 mmol/l among teetotallers to 1.84 ±0.04 mmol/l among alcohol consumers of ≥210 g/week, adjusted for all confounders from the multivariate model. Lifetime alcohol consumption was also positively associated ($p< 0.001$) with HDL cholesterol (0.03 ±0.001 per 10 gram alcohol/week). When adjusted for baseline alcohol consumption, this association lost significance ($p= 0.31$; 0.01 ±0.001 per 10 g alcohol/week).

Table 1: Baseline characteristics by alcohol consumption categories in 16,330 Dutch women

	Alcohol consumption (g/week)						
	Teetotaller	0-4.9	5-29.9	30-69.9	70-139.9	140-	≥ 210
Participants (n)	1513	3115	3787	2586	2384	1629	1316
Age (years)[1,2]	59 ±6	59 ±6	58 ±6	57 ±6	57 ±6	57 ±6	56 ±6
BMI (kg/m2)[2]	26.9	26.6	26.2	25.8	25.1	25.3	25.3
Waist circumference (cm)[2]	85.2	84.8	83.6	82.8	81.8	82.6	83.5
Beer (g/day)	0	0	0.1	0.4	0.8	1.0	2.6
Wine (g/day)	0	0.1	1.2	3.6	6.3	10.9	17.8
Fortified wine (g/day)	0	0.1	0.5	1.7	4.3	8.7	13.9
Spirits (g/day)	0	0	0.3	1.0	2.3	3.5	8.7
Systolic blood pressure (mm Hg)[2]	135.4	132.7	131.6	131.1	130.1	132.0	134.3
Diastolic blood pressure (mm Hg)[2]	79.5	78.7	78.2	78.2	77.5	78.9	80.2
Current smoker (%)[2]	18.2	21.3	17.2	18.6	23.9	33.0	43.6
Past smoker (%)[2]	14.8	30.6	31.7	39.7	43.2	41.3	38.8
Hypertension (%)[2]	23.4	22.4	19.5	17.8	15.4	17.0	18.6
Hypercholesterolemia (%)[2]	6.4	6.6	5.6	4.6	4.5	4.8	4.4
Mean daily energy intake (kcal)[2]	1737	1732	1765	1816	1834	1842	1939
Physical activity level[2,3]	5.5	5.9	6.8	7.6	7.5	7.2	6.8
Family history type 2 diabetes (%)[2]	23.2	22.9	21.6	21.4	19.9	19.4	16.3
Postmenopausal (%)[2]	83.9	85.9	84.0	81.8	81.6	81.7	82.6
Higher education (%)[2,4]	8.2	11.7	16.6	23.7	29.4	29.4	34.8

[1]All characteristics are age-adjusted except age.

[2]P-value ≤0.001 between alcohol intake categories

[3]Arbitrary units

[4]Higher education was defined as having finished higher secondary school, bachelor or higher

Baseline and lifetime alcohol consumption and type 2 diabetes

During 101,250 person-years of follow-up, 760 new cases of type 2 diabetes were documented. An inverse relation between alcohol intake and risk of type 2 diabetes was observed (Table 2), with a similar risk among teetotalers and women consuming very low amounts of alcohol (0-4.9 g/week). Adjusting for potential confounders, HRs for type 2 diabetes ranging from 0.86 (0.66- 1.12) for women consuming 5-29.9 g/week to 0.69 (0.47-1.02) for women consuming ≥210 g/week were observed. This equaled a population attributive risk for type 2 diabetes of 16% for never consuming alcohol. When lifetime

Table 2: Baseline and lifetime alcohol consumption and risk of type 2 diabetes among 16,330 women

	Alcohol consumption							P-value linear trend	P-value quadratic trend
Baseline alcohol consumption (g/week)	0 (teetotaller)	0-4.9	5.0- 29.9	30.0-69.9	70.0- 139.9	140.0- 209.9	≥ 210		
Cases, n	100	211	174	87	92	53	43		
Person-years	9297	19533	23755	16015	14643	10009	7998		
Age- & BMI-adjusted	1.0	1.05 (0.83- 1.33)	0.79 (0.61- 1.01)	0.65 (0.49- 0.87)	0.82 (0.62- 1.09)	0.68 (0.48- 0.95)	0.69 (0.48- 0.98)	0.007	0.06
Multivariate-adjusted[1]	1.0	1.04 (0.80- 1.34)	0.86 (0.66- 1.12)	0.66 (0.48- 0.91)	0.91 (0.67- 1.24)	0.64 (0.44- 0.93)	0.69 (0.47- 1.02)	0.007	0.25
Multivariate-adjusted[1]	1.0	1.02 (0.79- 1.32)	0.85 (0.65- 1.11)	0.64 (0.46- 0.89)	0.88 (0.63- 1.23)	0.61 (0.41- 0.92)	0.65 (0.42- 1.01)	0.014	0.25
Lifetime alcohol consumption (alcohol-years)[2]	0 (teetotaller)	0-4.9	5.0- 9.9	10.0- 19.9	20.0- 29.9	30.0- 49.9	≥ 50		
Cases, n	101	120	132	137	75	90	91		
Person-years	9492	11362	15594	22200	12471	14882	14104		
Age- & BMI-adjusted	1.0	1.06 (0.82- 1.39)	0.92 (0.71- 1.19)	0.70 (0.54- 0.91)	0.76 (0.56- 1.02)	0.76 (0.57- 1.02)	0.79 (0.60- 1.05)	0.003	0.10
Age-, BMI, alcohol-adjusted	1.0	1.06 (0.82- 1.39)	0.93 (0.72- 1.21)	0.73 (0.56- 0.95)	0.82 (0.60- 1.12)	0.89 (0.64- 1.22)	1.04 (0.71- 1.54)	0.006	0.02
Multivariate-adjusted[1]	1.0	1.14 (0.86- 1.50)	0.96 (0.72- 1.26)	0.75 (0.56- 0.98)	0.78 (0.56- 1.08)	0.87 (0.57- 1.06)	0.80 (0.59- 1.10)	0.007	0.26
Multivariate- & alcohol-adjusted[3]	1.0	1.14 (0.87- 1.51)	0.97 (0.74- 1.28)	0.78 (0.59- 1.03)	0.86 (0.61- 1.20)	0.91 (0.65- 1.28)	1.09 (0.73- 1.64)	0.024	0.05

[1]Adjusted for age, BMI, smoking status, education, systolic blood pressure, menopause, physical activity, family history of type 2 diabetes, daily energy intake and hypertension.

[2]Number of years consuming 1 standard alcoholic beverage of 10 g alcohol daily

[3]Adjusted for baseline alcohol consumption (continuous) and all covariates from footnote 1.

alcohol consumption was included in the model the association did not substantially change (table 2). Waist circumference was a stronger predictor of type 2 diabetes than BMI. When BMI was replaced by waist and hip circumference, the association between alcohol consumption and type 2 diabetes did not substantially alter (data not shown). We also restricted these analyses to cases of type 2 diabetes that reported use of medication or insulin or were confirmed by the medical register and a similar inverse association between alcohol consumption and type 2 diabetes (p= 0.004) with a HR of 0.50 (0.31- 0.80) for those consuming ≥140 g alcohol per week was observed.

The association between lifetime alcohol consumption, calculated as alcohol-years, and type 2 diabetes is shown in table 2. Lifetime alcohol consumption was inversely associated with type 2 diabetes, although more modestly than baseline alcohol consumption. When baseline alcohol consumption was included in the model, the association between lifetime alcohol consumption and type 2 diabetes changed to a U-shape. Women with 0-4.9 alcohol-years (1.14 [0.87- 1.51]) or with ≥50 alcohol-years (1.09 [0.73- 1.64]) had a slightly increased risk of type 2 diabetes, while women with 10 to 20 alcohol-years had a HR of 0.78 (0.59- 1.03). Altogether, this equaled a population attributive risk for type 2 diabetes of 3.5% for never consuming alcohol. Replacing BMI with waist and hip circumference in the model did not alter the observed associations (data not shown).

Beverage type and type 2 diabetes

Associations between individual alcoholic beverages and type 2 diabetes are shown in table 3. Only for consumption of wine, we observed an inverse association (p= 0.05) with type 2 diabetes with a HR of 0.66 (0.40- 1.10) for those consuming ≥140 g alcohol/week. Although for the other beverages light-to-moderate consumption (0.8- 69.9 g/week) was also associated with decreased HRs for type 2 diabetes, these associations were not statistically significant.

Table 3: Beverage type and risk of type 2 diabetes among 16,330 women

	Alcohol consumption (g/week)					P-value linear trend
	0- 0.7	0.8- 29.9	30.0- 69.9	70- 139.9	≥140	
Wine						
Cases, n	212	314	69	45	19	
Person-years	20236	44496	14760	8097	4324	
Multivariate-adjusted hazard ratio (95%-CI)[1]	1.0	0.95 (0.78- 1.15)	0.73 (0.54- 0.99)	0.92 (0.64- 1.32)	0.66 (0.40- 1.10)	0.05
Fortified wine						
Cases, n	359	202	31	67		
Person-years	43960	31163	5488	11302		
Multivariate-adjusted hazard ratio (95%-CI)[1]	1.0	0.96 (0.80- 1.16)	0.88 (0.59- 1.31)	0.88 (0.66- 1.16)		0.81
Beer						
Cases, n	531	111	17			
Person-years	70077	19014	2822			
Multivariate-adjusted hazard ratio (95%-CI)[1]	1.0	1.02 (0.81- 1.27)	0.86 (0.50- 1.48			0.72
Liquor						
Cases, n	453	144	25	39		
Person-years	57767	26664	2863	4619		
Multivariate-adjusted hazard ratio (95%-CI)[1]	1.0	0.80 (0.65- 0.98)	1.16 (0.75- 1.80)	1.07 (0.76- 1.51)		0.26

[1]Adjusted for age, BMI, smoking status, education, systolic blood pressure, menopause, physical activity, family history of type 2 diabetes, daily energy intake and hypertension.

DISCUSSION

In this cohort of older women, a linear inverse association between moderate alcohol consumption and risk of type 2 diabetes was observed. A similar linear inverse association was observed for lifetime alcohol consumption. However, when adjusted for current alcohol consumption, the association changed into a U-shape. Beverage type did not influence the association between alcohol consumption and type 2 diabetes.

Certain potential limitations of the study need to be addressed. First, the use of self-reported information on alcohol intake may have introduced misclassification in exposure. However, self-reported alcohol consumption was validated against HDL cholesterol in a random sample of 1385 women and was positively, linearly associated with HDL cholesterol after adjustment for potential confounders. The assessment of alcohol consumption with the FFQ was also validated against 12 to 24 hour recalls and both measures were highly correlated, showing that this assessment is valid to rank subjects according to their alcohol intake. Altogether, this makes substantial misclassification unlikely. Also, drinking frequency was not included in our questionnaires and we could thus not assess the influence of binge drinking. However, among moderately drinking older women, binge drinking does not occur frequently (15). It is therefore unlikely that this influenced our results to a large extent. Second, data on development of type 2 diabetes were partly based on self-reported values and a urine dipstick test. It seems likely that certain cases of type 2 diabetes remained unidentified, because response rates were not complete. However, assuming that the observed relation between alcohol consumption and type 2 diabetes is a 'true' association, such underreporting of type 2 diabetes would only have attenuated the association. Indeed, when restricting our analyses to clinically confirmed cases of diabetes, we observed a similar association with type 2 diabetes. Lastly, we cannot exclude that residual confounding from other co-morbidities such as cardiovascular disease may be present. However, when excluding cases with cardiovascular disease from the analysis similar results were obtained (data not shown).

This finding of a decreased risk of type 2 diabetes with moderate alcohol consumption is consistent with other reports from prospective studies (2) and expands this relation to older women and lifetime alcohol consumption. In two small studies among women, light drinking was modestly inversely associated with risk of type 2 diabetes, but the findings

were not significant (16,17). A larger study of Stampfer et al. (1988) also reported non-significant results (18). Lee et al. reported an inverse association between alcohol consumption and type 2 diabetes in the Iowa Women's Health Study (5). Two recent studies in a large population of younger women also show a decreased risk of type 2 diabetes with alcohol consumption up to levels of 29.9 g/day and 10 g/day (3,4). Beyond these drinking levels risk of type 2 diabetes increased compared to light to moderate drinking. Similarly, several studies in male populations also indicated heavy drinking as a risk factor for type 2 diabetes (16,17,19) and a recent meta-analyses concluded that alcohol consumption is associated with type 2 diabetes in a U-shaped fashion (2). In contrast, we did not find an increased HR for heavier drinkers using baseline alcohol consumption. Lifetime alcohol consumption, however, did show a U-shaped association with risk of type 2 diabetes. To the best of our knowledge, this the first study to report on lifetime alcohol consumption and risk of type 2 diabetes. When adjusted for current alcohol use, we observed that both heavier drinkers and very light drinkers (0.1- 4.9 alcohol-years) had a slightly increased risk of type 2 diabetes.

Few studies explored the influence of beverage type on risk of type 2 diabetes. Wannamethee et al. reported that the reduction in risk associated with light and moderate drinking was more apparent among beer and wine drinkers (4). The Atherosclerosis Risk in Communities Study also showed no benefit of moderate drinking for liquor drinkers (19). A report from the Health Professionals' Follow-up Study, in contrast, observed benefit of light to moderate alcohol consumption for all beverage types (20). Consistent with Wannamethee et al. (4), we observed a significant, inverse association only for consumption of wine. However, the other beverages showed similar HR's for type 2 diabetes. Because wine was the predominant beverage type in this population, these differences are probably due to limited power for the other beverages.

The observed relation between alcohol consumption and type 2 diabetes is compatible with a population attributive risk of 16% for not consuming alcohol. This figure is in accordance with a recent study of Knoops et al. that reported similar population attributive risks ranging from 13 to 20% for (combinations of) several lifestyle factors such as moderate alcohol consumption, physical activity or a Mediterranean diet (21).

This protective effect of moderate alcohol consumption for type 2 diabetes may be due to increased insulin sensitivity with moderate alcohol consumption (22,23). Davies et al.

showed that moderate alcohol consumption increased insulin sensitivity dose-dependently in postmenopausal women after eight weeks of consumption of 0, 15 or 30 g alcohol per day (22). Possibly anti-inflammatory effects (24) of moderate alcohol consumption may also be involved in this risk reduction. Moderate alcohol consumption was associated with decreased BMI and waist circumference in this study. Therefore BMI or waist circumference may also mediate the association with type 2 diabetes, but we could not find evidence for this in our study.

In conclusion, this study supports the view that moderate alcohol consumption decreases risk of type 2 diabetes in older women. Both current and lifetime alcohol consumption were associated with a decreased risk of type 2 diabetes. These data agree with previous observations and expand this evidence to older women and lifetime alcohol consumption.

REFERENCES

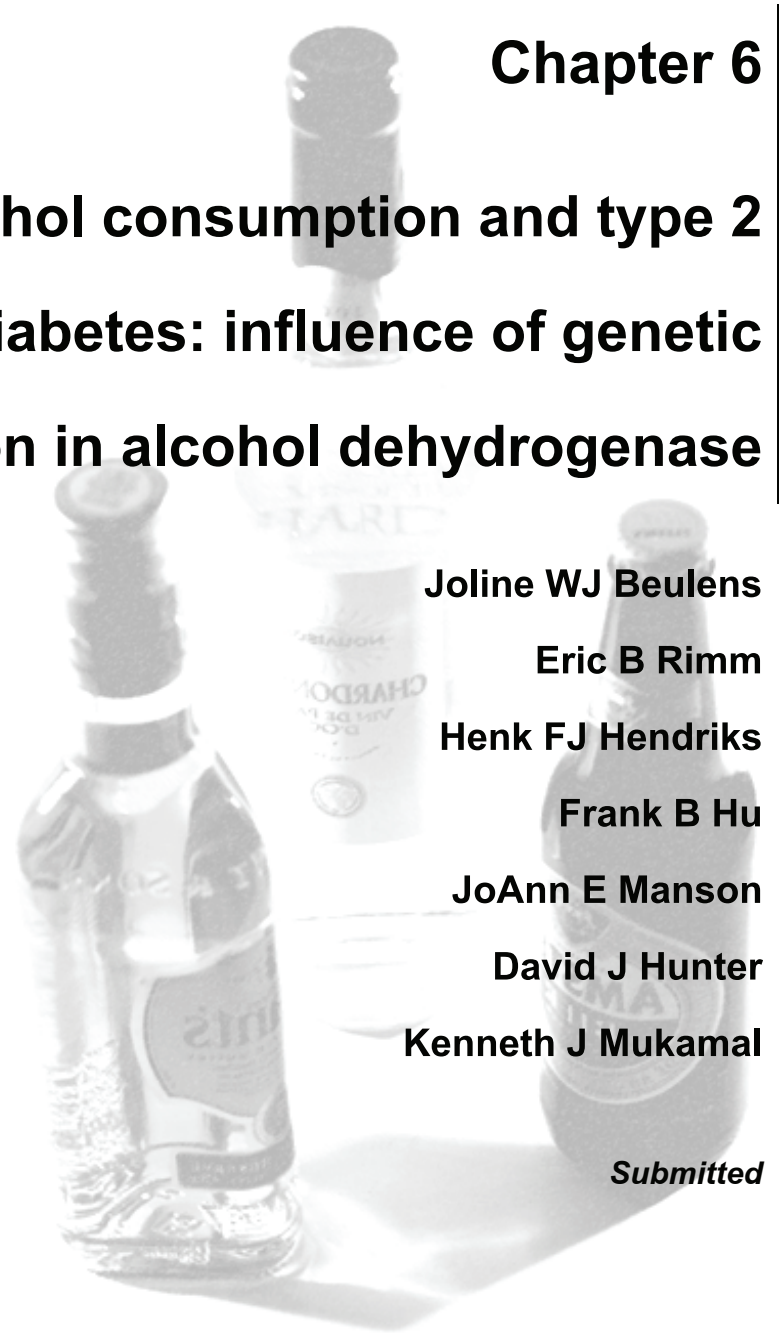
1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004; 27:1047-1053.
2. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005; 28:719-725.
3. Hu FB, Manson JE, Stampfer MJ et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N.Engl.J.Med. 2001; 345:790-797.
4. Wannamethee SG, Camargo CA, Jr., Manson JE, Willett WC, Rimm EB. Alcohol drinking patterns and risk of type 2 diabetes mellitus among younger women. Arch.Intern.Med. 2003; 163:1329-1336.
5. Lee DH, Folsom AR, Jacobs DR, Jr. Dietary iron intake and Type 2 diabetes incidence in postmenopausal women: the Iowa Women's Health Study. Diabetologia 2004; 47:185-194.
6. Stolk RP, Pols HA, Lamberts SW, de Jong PT, Hofman A, Grobbee DE. Diabetes mellitus, impaired glucose tolerance, and hyperinsulinemia in an elderly population. The Rotterdam Study. Am.J.Epidemiol. 1997; 145:24-32.
7. Boker LK, van Noord PA, van der Schouw YT et al. Prospect-EPIC Utrecht: study design and characteristics of the cohort population. European Prospective Investigation into Cancer and Nutrition. Eur.J.Epidemiol. 2001; 17:1047-1053.
8. Voorrips LE, Ravelli AC, Dongelmans PC, Deurenberg P, Van Staveren WA. A physical activity questionnaire for the elderly. Med.Sci.Sports Exerc. 1991; 23:974-979.
9. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int.J.Epidemiol. 1997; 26 Suppl 1:S37-S48.
10. Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int.J.Epidemiol. 1997; 26 Suppl 1:S49-S58.
11. Dutch Food Composition Table 2001. 2001. Den Haag.
12. Maruti SS, Feskanich D, Colditz GA et al. Adult recall of adolescent diet: reproducibility and comparison with maternal reporting. Am.J Epidemiol. 2005; 161:89-97.
13. Herings RM, Bakker A, Stricker BH, Nap G. Pharmaco-morbidity linkage: a feasibility study comparing morbidity in two pharmacy based exposure cohorts. J.Epidemiol.Community Health 1992; 46:136-140.
14. Rothman KJ, Greenland S: Modern Epidemiology. Philadelphia, USA, Lippincott-Raven, 1998.
15. Naimi TS, Brewer RD, Mokdad A, Denny C, Serdula MK, Marks JS. Binge drinking among US adults. JAMA 2003; 289:70-75.
16. Holbrook TL, Barrett-Connor E, Wingard DL. A prospective population-based study of alcohol use and non-insulin-dependent diabetes mellitus. Am.J.Epidemiol. 1990; 132:902-909.
17. Monterrosa AE, Haffner SM, Stern MP, Hazuda HP. Sex difference in lifestyle factors predictive of diabetes in Mexican-Americans. Diabetes Care 1995; 18:448-456.
18. Stampfer MJ, Colditz GA, Willett WC et al. A prospective study of moderate alcohol drinking and risk of diabetes in women. Am.J.Epidemiol. 1988; 128:549-558.
19. Kao WH, Puddey IB, Boland LL, Watson RL, Brancati FL. Alcohol consumption and the risk of type 2 diabetes mellitus: atherosclerosis risk in communities study. Am.J.Epidemiol. 2001; 154:748-757.
20. Conigrave KM, Hu BF, Camargo CA, Jr., Stampfer MJ, Willett WC, Rimm EB. A prospective study of drinking

patterns in relation to risk of type 2 diabetes among men. Diabetes 2001; 50:2390-2395.

21. Knoops KT, de Groot LC, Kromhout D et al. Mediterranean diet, lifestyle factors, and 10-year mortality in elderly European men and women: the HALE project. JAMA 2004; 292:1433-1439.
22. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002; 287:2559-2562.
23. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004; 27:184-189.
24. Sierksma A, van der Gaag MS, Kluft C, Hendriks HF. Moderate alcohol consumption reduces plasma C-reactive protein and fibrinogen levels; a randomized, diet-controlled intervention study. Eur.J.Clin.Nutr. 2002; 56:1130-1136.

Chapter 6

Alcohol consumption and type 2 diabetes: influence of genetic variation in alcohol dehydrogenase



Joline WJ Beulens

Eric B Rimm

Henk FJ Hendriks

Frank B Hu

JoAnn E Manson

David J Hunter

Kenneth J Mukamal

Submitted

ABSTRACT

Objective

Moderate alcohol consumption is inversely associated with risk of type 2 diabetes. A common polymorphism in the alcohol dehydrogenase gene (*ADH1C*) decreases the rate of ethanol oxidation and may modify the underlying biological effect of moderate alcohol consumption. This study aimed to investigate whether *ADH1C* genotype modifies the association between alcohol consumption and type 2 diabetes.

Methods

In nested, prospective case-control studies of 640 incident cases and 1000 controls from the Nurses' Health Study and 383 incident cases and 382 controls from the Health Professionals Follow-up Study, we determined the multivariate-adjusted associations between genotype of the ADH1C I350V polymorphism, self-reported alcohol consumption assessed with a validated food frequency questionnaire, and risk of type 2 diabetes.

Results

Alcohol consumption was associated ($p < 0.001$) with a decreased risk of type 2 diabetes among women, but not in the smaller study of men. *ADH1C* genotype modified the relation between alcohol consumption and diabetes for women ($p_{interaction} = 0.02$). The *ADH1C*2* allele, related to a slower rate of ethanol oxidation, was associated with an increased risk of diabetes among women consuming ≥5 g alcohol/day ($p = 0.002$). A qualitatively similar but non-significant trend was observed among men. Pooling both studies, we observed similar results ($p_{interaction} = 0.02$) with odds ratios for diabetes among moderate drinkers of 1.63 (1.02-2.61) for heterozygotes and 2.38 (1.08-5.22) for *ADH1C*2* homozygotes compared to *ADH1C*1* homozygotes ($p_{trend} = 0.02$).

Conclusion

ADH1C genotype modifies the association between alcohol consumption and type 2 diabetes. The *ADH1C*2* allele, related to a slower oxidation rate, is associated with an increased diabetes risk among moderate drinkers. This suggests that the association between alcohol consumption and type 2 diabetes may be causal but mediated by downstream metabolites such as acetate rather than ethanol itself.

INTRODUCTION

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes compared to abstention (1;2). Moreover, randomized controlled trials have shown that moderate drinking improves insulin sensitivity (3;4), suggesting that this relation may be causal and due to the effect of ethanol or its metabolites.

Ethanol, when consumed in moderation, is oxidized to acetaldehyde and acetate by alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) (5). Four genes of the ADH family, coding for class I (*ADH1A-C*) and class II (*ADH2*) enzymes, are involved in hepatic metabolism of ethanol (6). At the *ADH1B* locus, a functional polymorphism occurs exhibiting a 30-40-fold difference in Vmax for ethanol oxidation, but the variant allele is rare among Caucasians (< 5%) (7-9).

At the *ADH1C* locus, two linked variants occur encoding the γ1 and γ2 subunits, attributed to single amino acid substitutions of amino acids 271 and 349. This polymorphism shows a 2.5-fold difference of Vmax for ethanol oxidation, with the *ADH1C*2* variant conferring slower metabolism, and alleles are about equally distributed among Caucasians (7-9). Although the extent to which this polymorphism affects blood alcohol concentrations remains unclear (10), slower ethanol oxidation is thought to increase exposure to ethanol and therefore modify the association of alcohol consumption with disease risk. Indeed, *ADH1C* genotype is associated with alcohol-related diseases such as alcoholism (11) and head and neck cancer (12;13). It may furthermore modify the association of alcohol consumption with coronary heart disease and levels of HDL cholesterol (14).

Whether this *ADH1C* polymorphism also affects the association between alcohol consumption and type 2 diabetes has not been studied to date. The finding that *ADH1C* genotype modifies the association of alcohol intake with diabetes would support the hypothesis that the association is causal, because genotypes are distributed randomly and hence mimic the random assignment of alcohol exposure that would occur in a randomized trial (15). Therefore we investigated whether the *ADH1C* polymorphism modifies the relationship between alcohol consumption and type 2 diabetes in nested case-control studies from the Nurses' Health Study (NHS) and Health Professionals Follow-up Study (HPFS). Moderate drinking has previously been associated with lower risk of diabetes in the overall populations of both cohorts (16-19).

METHODS

Study Participants

The NHS began in 1976, when 121,700 female nurses aged 30 to 55 years responded to a questionnaire of health-related information. Questionnaires have been administered biennially to update health information and identify new cases of disease. During 1989-1990, 32,826 women free of diagnosed diabetes, coronary heart disease, stroke, or cancer provided blood samples. Women providing blood samples had higher prevalence of obesity and family history of diabetes and a lower prevalence of current smoking, but were otherwise similar to women not providing blood. By 2000, 678 of these women had a confirmed diagnosis of type 2 diabetes. Controls providing blood samples were matched to diabetes cases by year of birth, date of blood draw, race, and fasting status at blood draw. From 1990 until 1996, two controls were matched to each case based on the above factors. One of the two control subjects was also matched according to body-mass index (BMI) within 1 kg/m^2. After 1996, one control was matched to each case based on the same characteristics, and another control was matched on these characteristics and BMI to each of the cases in the top decile of the BMI distribution. Non-Caucasian women were excluded, leaving 640 cases and 1000 controls for analysis.

The HPFS began in 1986, when 51,529 male health professionals 40 to 75 years of age completed the initial questionnaire. Biennial follow-up has mirrored the NHS. During 1993-1994, 18,225 participants of the HPFS provided a blood sample. Characteristics of participants providing blood samples were similar to other HPFS participants. By 2002, 431 incident cases of type 2 diabetes, free from cardiovascular disease or cancer (except nonmelanoma skin cancer) at baseline, were confirmed. One control was matched per case by year of birth, month of blood collection, and fasting status at blood draw using the risk-set sampling strategy (20). We excluded non-Caucasian men, leaving 383 cases and 382 controls for analysis.

Subjects provided written informed consent, and the studies were approved by the institutional review board of Partners HealthCare System, Boston, Mass.

Ascertainment of Diabetes

Incident cases of type 2 diabetes were identified by self-report and confirmed by a validated supplementary questionnaire detailing symptoms, diagnostic laboratory test results, and diabetes treatment. The diagnosis was confirmed if participants reported at least one of the following on the questionnaire: treatment with either insulin or an oral hypoglycemic agent, at least one classic symptom of diabetes (for instance, polyuria, polydipsia, weight loss) plus elevated plasma glucose level, or an elevated plasma glucose level on at least on two occasions in the absence of symptoms. Elevated plasma glucose was defined as at least 140 mg/dL (≥7.8 mmol/L) fasting, or at least 200 mg/dL (≥11.1 mmol/L) nonfasting, or at least 200 mg/dL (≥1.1 mmol/L) at ≥2 hours after an oral glucose tolerance test for cases diagnosed before 1998; for cases diagnosed in 1998 and later, the fasting plasma glucose threshold was lowered to ≥126 mg/dL (≥7.0 mmol/L) (21). The validity of self-reported diabetes has been confirmed with medical record review in a sample (16;22).

Assessment of alcohol consumption

We assessed average alcohol consumption within a semi-quantitative food-frequency questionnaire (23) including separate items for beer, white wine, red wine and liquor. We specified standard portions as a glass, bottle, or can of beer; a four-ounce glass of wine; and a shot of liquor. For each beverage participants were asked to estimate their average consumption over the past year. We calculated ethanol intake by multiplying the frequency of consumption of each beverage by the alcohol content of the specified portion size (12.8 g for beer, 11.0 g for wine, and 14.0 g for liquor) and summing across beverages (24). We used alcohol consumption reported on the food frequency questionnaire in 1990 for women and 1994 for men and replaced information with data from 1986 onwards in case of missing data for alcohol consumption. In 1988, men and women also reported the number of days per week that they typically drank any form of alcohol.

We previously assessed the validity of alcohol consumption estimated with the food-frequency questionnaire against intake from two one-week dietary records collected approximately six months apart among 173 women and 136 men residing in eastern Massachusetts; the Spearman correlation coefficient between these two measures was 0.90 for women and 0.86 for men (25).

Assessment of lifestyle factors

Lifestyle factors were assessed using questionnaires, including smoking, body weight, physical activity, family history of diabetes, menopausal status, and use or nonuse of postmenopausal hormone therapy. Reported weights have been shown to correlate well with measured weights (r= 0.96) (26) and the assessment of physical activity was previously validated (27). We obtained energy intake, glycemic load, coffee consumption and energy-adjusted intakes of saturated fat, *trans* fatty acids, polyunsaturated fatty acids, and dietary fiber from the semi-quantitative food frequency questionnaire (28).

Laboratory procedures

All samples were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA), in 384-well format. The PCR amplifications were carried out on 5-20ng DNA using 1 X TaqMan® universal PCR master mix (No Amp-erase UNG). Amplification conditions on a AB 9700 dual plate thermal cycle (Applied Biosystems, Foster City, CA) were as follows: 1 cycle of 95°C for 10min, followed by 50 cycles of 92°C for 15s and 60°C for 1 min. TaqMan® primers and probes were designed using the Primer Express® Oligo Design software v2.0 (ABI PRISM) and are available on request.

Biomarkers related to diabetes were assessed only among women. Insulin levels were measured using a double antibody system with less than 0.2% cross-reactivity between insulin and its precursors (Linco Research, St Louis, Mo). Hemoglobin A_{1c} was measured by immunoassay (Roche Diagnostics, Indianapolis, Ind). The coefficients of variation were 3.5% to 11.7% for fasting insulin and 1.9% to 3.0% for hemoglobin A1c (HbA1c).

Statistical analysis

We used a chi-square test to determine whether *ADH1C* genotypes were in Hardy-Weinberg equilibrium. Logistic regression adjusted for matching factors was used to estimate odds ratios (and 95% confidence intervals) for type 2 diabetes based on alcohol consumption and *ADH1C* genotypes (*ADH1C*1/*1, ADH1C*1/*2, ADH1C*2/*2*). We simultaneously adjusted for BMI (quintiles) and for smoking (never-, former- or current smokers of 1-14, 15-34 or ≥35 cigarettes/day), family history of diabetes in a first degree relative (present or not), physical activity (five categories), postmenopausal hormone therapy (premenopausal, never-, past- or current user), energy intake, coffee consumption

and energy-adjusted intakes of saturated fat, *trans* fatty acids, polyunsaturated fatty acids, glycemic load and dietary fiber (each in quintiles for women and tertiles for men).

We first examined independent associations of alcohol consumption and *ADH1C* genotype with type 2 diabetes risk. Second, their interaction on type 2 diabetes risk was assessed and we examined the main effect of *ADH1C* genotype within strata of alcohol consumption (0, <5 and ≥ 5 g/day for women and 0, <10 and ≥10 g/day for men). Interaction terms of alcohol consumption (modeled on the log-scale to maximize model fit) multiplied by *ADH1C* genotype (modeled as the number of variant alleles) were tested. Finally, we examined the joint association of alcohol consumption and *ADH1C* genotype with risk of type 2 diabetes. To combine men and women, beta-coefficients of the interaction term were pooled using the DerSimonian and Laird random-effects model (29).

We also assessed whether *ADH1C* genotype modified the relation between alcohol consumption and fasting insulin and HbA1c among controls using analysis of variance adjusted for covariates described for logistic regression above. For these analyses, fasting insulin was log-transformed for analysis due to deviation from homogeneity of variance.

We performed sensitivity analyses using drinking frequency (0 days/week, 1-4 days/week and ≥5 days/week) rather than average quantity, using linear modeling and excluding women with HbA1c concentrations >6.5, with similar results. Analyses were performed using SAS statistical package, version 8.2 (SAS Institute, Cary, North Carolina) and Intercooled STATA 9.0 (STATA Corporation, College Station, Texas, USA).

RESULTS

Baseline characteristics and allele frequencies

Baseline characteristics of the cases and controls from NHS and HPFS are shown in table 1. Women who developed type 2 diabetes tended to have higher BMI, energy intake, fasting insulin, and HbA1c than controls. Alcohol consumption and physical activity were lower among women developing type 2 diabetes compared to matched controls. Men who developed type 2 diabetes had higher BMI and lower physical activity than controls. The frequencies of the *ADH1C*2* allele among controls were 41% among women and 40% among men. The distribution of *ADH1C* genotype was in Hardy-Weinberg equilibrium in the total population of NHS (p= 0.09) and HPFS (p= 0.72) and among controls from NHS (p= 0.41) and the HPFS (p= 0.38).

Table 1: Characteristics of incident cases of type 2 diabetes and matched controls.

	Women		**Men**	
	Controls	Cases	Controls	Cases
n	1000	640	382	383
Age (years)	57.0	57.0	60.4	60.6
BMI (kg/m^2)	27.3[1]	30.5	25.8[1]	29.0
Physical activity (Mets/week)	15.1[1]	12.4	36.6[1]	27.0
Alcohol consumption (g/day)	5.2[1]	2.9	11.5	10.7
Smoking (%)				
Never	46.7	42.2	49.2[1]	38.4
Past	42.0	44.2	46.1[1]	56.4
Current	10.8	13.6	3.7	4.7
Family history of diabetes (%)	21.2[1]	44.8	16.5[1]	26.6
Menopausal status (%)	79.5	82.0	NA	NA
Nutrients[2]:				
Energy (kcal)	1780	1828	2135	2130
Saturated fatty acids (g)	21.3[1]	22.5	22.3[1]	23.3
Trans fatty acids (g)	3.1	3.2	3.0	3.2
Polyunsaturated fatty acids (g)	11.9	12.2	12.2	12.5
Fiber (g)	19.9	20.2	22.3	21.4
Glycemic load	117.8	121.1	134.6[1]	130.6
Coffee consumption (cups/day)	2.2	2.0	2.1	1.9
Biomarkers:				
HbA1c (%)	5.6[1]	6.4	-	-
Insulin (μU/mL)	9.6[1]	13.5	-	-

[1] p< 0.05 cases versus controls.
[2] All nutrients are energy-adjusted except energy.

Main effects of alcohol consumption and *ADH1C* genotype

We first examined the main effects of alcohol consumption and *ADH1C* genotype. Alcohol consumption was inversely associated (p< 0.001) with risk of type 2 diabetes within this nested case-control population from the NHS, with a multivariate-adjusted odds ratio of 0.45 (0.33- 0.63) among women consuming ≥5 g alcohol/day compared to abstainers as previously reported for the entire NHS (17;18). Despite the previously reported inverse association between alcohol consumption and risk of type 2 diabetes in the entire HPFS cohort (16), alcohol consumption was not associated with risk of type 2 diabetes in this nested case-control study (odds ratio 1.08 (95%-CI: 0.67- 1.75) for intake of ≥10 g alcohol/day versus abstention).

ADH1C genotype per se was not associated with risk of type 2 diabetes among men or women (table 2). Adjusting for alcohol consumption did not alter the association.

Table 2: *ADH1C* polymorphism and risk of type 2 diabetes among 640 cases and 1000 controls from the Nurses Health Study and 383 cases and 382 controls from the Health Professionals Follow-up Study[1]

	ADH1C*1/*1	***ADH1C*1/*2***	***ADH1C*2/*2***	**P-value linear trend**
Cases	376	469	178	
Controls	479	665	238	
Matched, BMI-adjusted	1.0	0.93 (0.77- 1.12)	1.00 (0.78- 1.29)	0.83
Matched, multivariate	1.0	0.96 (0.79- 1.18)	1.05 (0.80- 1.36)	0.87
Matched, alcohol- & multivariate adjusted[2]	1.0	0.97 (0.79- 1.19)	1.11 (0.85- 1.45)	0.59

[1] Results obtained from pooling the beta-coefficient and standard error estimates for men and women using the DerSimonian and Laird random-effects model.

[2] Adjusted for BMI, physical activity, smoking, family history of diabetes, menopausal status, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption.

Interaction between alcohol consumption and *ADH1C* genotype

Second the main effect of *ADH1C* genotype within strata of alcohol consumption was assessed. *ADH1C* genotype significantly modified the association between alcohol consumption and type 2 diabetes among women ($p_{interaction}$= 0.02) (table 3). The *ADH1C*2* allele was positively associated with risk of type 2 diabetes in a dose-dependent manner

among consumers of ≥5 g alcohol/day, while *ADH1C* genotype was not associated with risk of type 2 diabetes among abstainers and light drinkers.

Among men, the overall interaction between alcohol consumption and *ADH1C* genotype was similar to women, albeit weaker (p=0.41). Among men consuming ≥10 g/day, we observed a graded but non-significant trend between number of *2 alleles and type 2 diabetes risk.

Because we found no heterogeneity in the interaction between alcohol consumption and genotype among women and men (p= 0.46), estimates for women and men were pooled. We observed a significant (p= 0.02) interaction between alcohol consumption and *ADH1C* genotype in the pooled analysis, with an approximate doubling of risk with homozygosity for the *ADH1C*2* allele specifically among moderate drinkers (table 3).

Table 3: Alcohol consumption, ADH1C polymorphism and risk of type 2 diabetes among 640 cases and 1000 controls from the Nurses Health Study and 383 cases and 382 controls from the Health Professionals Follow-up Study

	Women				**Men**				**Women and men[1]**			
	ADH1C			P-value	ADH1C			P-value	ADH1C			P-value
	*1/*1	*1/*2	*2/*2	for trend	*1/*1	*1/*2	*2/*2	for trend	*1/*1	*1/*2	*2/*2	for trend
Alcohol	**0 g/day**				**0 g/day**				**0 g/day**			
Cases/ Controls	139/ 32	144/ 192	40/ 53		34/ 32	46/ 48	19/ 18		173/ 164	190/ 240	59/ 71	
Matched, multivariate adjusted[2]	1.0	0.75 (0.52- 1.09)	0.81 (0.47- 1.41)	0.23	1.0	0.73 (0.33- 1.63)	1.04 (0.38- 2.84)	0.91	1.0	0.75 (0.54- 1.05)	0.86 (0.53- 1.40)	0.26
Alcohol	**0- 4.9 g/day**				**0- 9.9 g/day**				**0- 4.9 or 9.9 g/day3**			
Cases/ Controls	75/ 109	95/ 140	44/ 71		65/ 50	68/ 69	23/ 15		140/ 159	163/ 209	67/ 86	
Matched, multivariate adjusted[2]	1.0	1.32 (0.81- 2.15)	1.02 (0.56- 1.83)	0.79	1.0	0.77 (0.42-1.41)	1.11 (0.45- 2.75)	0.86	1.0	1.04 (0.61- 1.76)	1.04 (0.64- 1.71)	0.91
Alcohol	**≥5 g/day**				**≥10 g/day**				**≥5 or 10 g/day[3]**			
Cases/ Controls	24/ 106	49/ 141	30/ 56		39/ 50	67/ 75	22/ 25		63/ 156	116/ 216	52/ 81	
Matched, multivariate adjusted[2]	1.0	1.81 (0.93- 3.54)	3.48 (1.57- 7.68)	0.002	1.0	1.48 (0.77-2.84)	1.56 (0.65- 3.73)	0.26	1.0	1.63 (1.02- 2.61)	2.38 (1.08- 5.22)	0.02

[1]Results obtained from pooling the beta-coefficient and standard error estimates for men and women using the DerSimonian and Laird random-effects model.
[2]Adjusted for BMI, physical activity, smoking, family history of diabetes, menopausal status, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption.
[3]Cut-off for alcohol consumption of 5 g/day women and 10 g/day for men.

Finally, to demonstrate the joint effect of alcohol consumption and *ADH1C* genotype, abstainers homozygous for the *ADH1C*1* allele were used as a common reference category (Figure 1). Moderate alcohol consumption was associated with a decreased risk of type 2 diabetes. However, because the *2 allele tended to attenuate the lower risk associated with moderate drinking, the risk among moderate drinkers homozygous for the *2 allele was nearly identical to that among abstainers homozygous for the *1 allele.

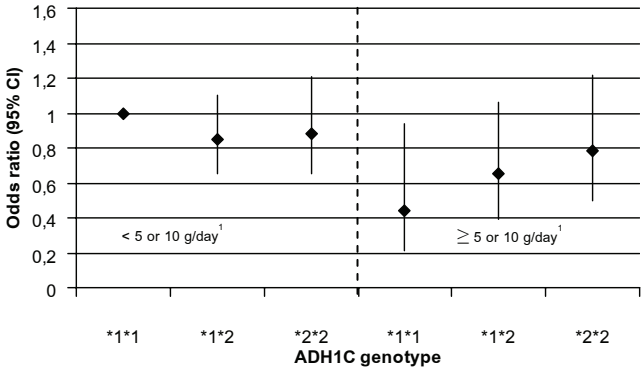


Figure 1: Alcohol consumption, ADH1C genotype and risk of type 2 diabetes among 640 cases and 1000 controls from the Nurses Health Study and 383 cases and 382 controls from the Health Professionals Follow-up Study. Odds ratio are adjusted for BMI, physical activity, smoking, family history of diabetes, menopausal status, energy intake and energy-adjusted intake of saturated fat, trans fatty acids, polyunsaturated fat, dietary fiber, glycemic load and coffee consumption and pooled for men and women using the DerSimonian and Laird random-effects model. [1] Cut-off for alcohol consumption of 5 g/day for women and 10 g/day for men.

Mediating biomarkers

We also assessed whether *ADH1C* genotype modified the relation between alcohol consumption and markers of type 2 diabetes among control participants. Alcohol consumption was inversely associated with fasting insulin (beta-coefficient ± se: -0.017 ± 0.010), with concentrations of 8.2 ±0.41, 8.0 ±0.41 and 6.9 ±0.39 among non-drinkers and consumers of 0.1- 4.9 and ≥5.0 g/day respectively. *ADH1C* genotype was not associated with fasting insulin among abstainers (p= 0.81) and light drinkers (p= 0.89). Among consumers of ≥5 g alcohol/day, homozygotes for *ADH1C*2/*2* (8.3 ± 1.4 μU/mL) tended to have higher concentrations of fasting insulin than homozygotes *ADH1C*1/*1* (7.0 ± 0.7 μU/mL) and heterozygotes (5.8 ± 0.6 μU/mL), but the interaction between alcohol consumption and *ADH1C* genotype was not significant (p= 0.86). No association between alcohol consumption and HbA1c was observed, nor was an interaction between alcohol consumption and *ADH1C* genotype (data not shown).

DISCUSSION

The primary function of *ADH1C* is ethanol oxidation. A common polymorphism of this gene is associated with a slower rate of oxidation and may increase exposure to ethanol. Therefore we hypothesized that *ADH1C* genotype would modify the association between alcohol consumption and type 2 diabetes risk. In line with this hypothesis, and in support of a causal relationship between alcohol consumption and type 2 diabetes, *ADH1C* genotype modified the association of alcohol consumption with type 2 diabetes among men and women in two pooled cohorts.

A key finding of this study was that the *ADH1C*2* allele, related to slower ethanol oxidation, was dose-dependently associated with an increased diabetes risk among moderate drinkers, while no associations between *ADH1C* genotype and diabetes were observed for those consuming less or no alcohol. These results are in the opposite direction from previous observations for cardiovascular disease (14), where the benefit from moderate alcohol consumption is mainly due to increased HDL cholesterol concentrations. Ethanol itself directly increases hepatic Apo A-1 production and HDL cholesterol in a dose-dependent manner (30). Thus slower gastric and hepatic metabolism of ethanol would lead to increased HDL cholesterol concentrations and reduced cardiovascular risk as observed (14). In contrast, we observed a lower risk of diabetes with moderate drinking in this study, but this was attenuated (rather than accentuated) by alleles conferring slower oxidation (Figure 1).

This disparity between our findings for type 2 diabetes and those for cardiovascular disease suggest different mechanisms underlying the associations with moderate drinking. Ethanol oxidation produces measurable downstream metabolites such as acetaldehyde and acetate (31), that could affect risk of type 2 diabetes themselves instead of ethanol. Slower ethanol oxidation would produce lower concentrations of these metabolites.

Perhaps the strongest data relating ethanol metabolites to type 2 diabetes risk involve acetate and its effects on peripheral tissue (32). Acetate, the end-product of ethanol oxidation, is oxidized to acetyl-CoA by acetyl-CoA syntethase (33;34) primarily in peripheral tissues such as muscle (35). This reaction of acetate to acetyl CoA generates AMP; a serving of 10 g of ethanol will produce about 200 mmoles of AMP (33;34). AMP generated by acetate metabolism may stimulate AMP-activated protein kinase, which improves insulin

sensitivity (36;37) by promoting muscular fatty acid oxidation and GLUT4 translocation (38-40). In addition, acetyl-CoA itself enters the Krebs cycle, stimulating oxidative phosphorylation (41). Several studies have shown that impaired mitochondrial oxidative phosphorylation is associated with insulin resistance (42) and type 2 diabetes (43;44). Moreover, in human studies, acetic acid and acetate acutely improve insulin sensitivity (45) and decrease free fatty acid concentrations (46).

Studies directly manipulating ADH activity confirm decreased acetate concentrations that may support our hypothesis. Sarkola et al. showed that 4-methylpyrazole, an ADH inhibitor, decreased production of acetate in men and women after ingestion of 0.4 g/kg of alcohol (31). Although the effect of genotype on blood alcohol and acetate concentrations remains uncertain, *ADH1C*2* genotype has been associated with decreased production of salivary acetaldehyde as expected (47).

Strengths of our study include its prospective design and detailed assessment of alcohol consumption, lifestyle factors and diet. Nonetheless, several limitations of this study need to be addressed. First, our study population consists of relatively light drinkers with limited variation in alcohol consumption. We could therefore not explore the effect of *ADH1C* genotype on the association between alcohol consumption and type 2 diabetes among heavier drinkers. However, it is likely that genetic variation in *ADH1C* would have less impact among heavier drinkers because most alcohol is metabolized through CYP2E detoxifying enzymes. Second, we could not replicate the inverse association between alcohol consumption and type 2 diabetes observed in the entire HPFS cohort in the smaller nested case-control study. This could be due to a weaker association of alcohol consumption with diabetes among men than women (2), but this does not completely explain our findings. Examining the entire cohort from 1994 (as opposed to 1986, the original baseline of the study) onward, we observed an inverse association between alcohol intake and risk of diabetes similar to our previous studies, suggesting that results in this smaller case-control study may be due to chance. The interaction, however, tended to be qualitatively similar among both men and women. Third, we cannot exclude the possibility that these results are based on chance and further studies are warranted to confirm our observations.

In conclusion, this study indicates that *ADH1C* genotype modifies the association between alcohol consumption and type 2 diabetes. Among moderate drinkers, the

*ADH1C*2* allele, related to a slower rate of ethanol oxidation, was dose-dependently associated with an increased risk of type 2 diabetes. This suggests that the association between alcohol consumption and type 2 diabetes may be causal but due to downstream metabolites such as acetate rather than ethanol itself.

Grant support

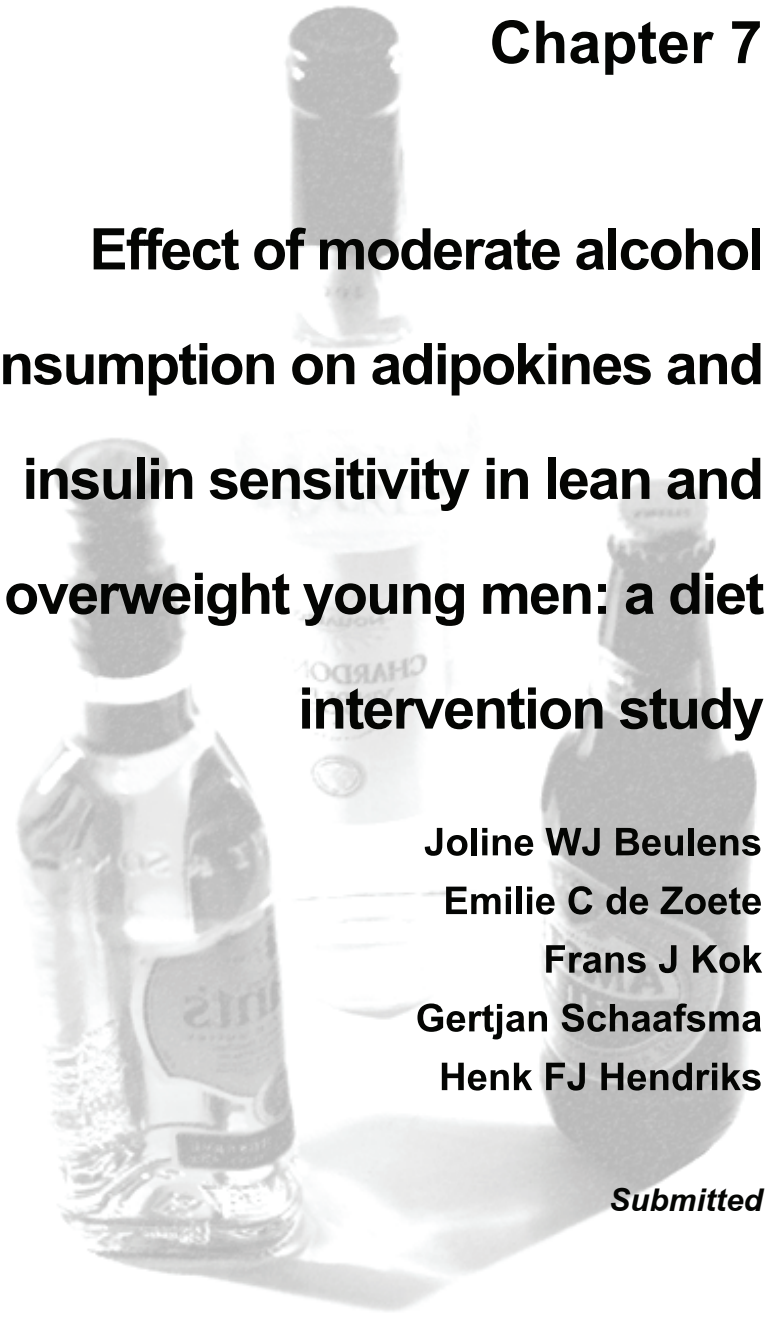
Supported by National Institutes of Health Grants AA11181, HL35464, HL60712, DK46519 and DK58845, and a travel-grant from the Dutch Heart Association and a research exchange award from European Research Advisory Board (JWB).

REFERENCES

1. Carlsson S, Hammar N, Grill V. Alcohol consumption and type 2 diabetes Meta-analysis of epidemiological studies indicates a U-shaped relationship. Diabetologia 2005;48:1051-4.
2. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
3. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
4. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
5. Edenberg HJ, Bosron WF. Alcohol dehydrogenase. New York: Elsevier Science Inc., 1997.
6. Han CL, Liao CS, Wu CW, Hwong CL, Lee AR, Yin SJ. Contribution to first-pass metabolism of ethanol and inhibition by ethanol for retinol oxidation in human alcohol dehydrogenase family--implications for etiology of fetal alcohol syndrome and alcohol-related diseases. Eur J Biochem 1998;254:25-31.
7. Agarwal DP, Goedde HW. Pharmacogenetics of alcohol metabolism and alcoholism. Pharmacogenetics 1992;2:48-62.
8. Bosron WF, Lumeng L, Li TK. Genetic polymorphism of enzymes of alcohol metabolism and susceptibility to alcoholic liver disease. Mol Aspects Med 1988;10:147-58.
9. Goedde HW, Agarwal DP, Fritze G et al. Distribution of ADH2 and ALDH2 genotypes in different populations. Hum Genet 1992;88:344-6.
10. Neumark YD, Friedlander Y, Durst R et al. Alcohol dehydrogenase polymorphisms influence alcohol-elimination rates in a male Jewish population. Alcohol Clin Exp Res 2004;28:10-4.
11. Chao YC, Liou SR, Chung YY et al. Polymorphism of alcohol and aldehyde dehydrogenase genes and alcoholic cirrhosis in Chinese patients. Hepatology 1994;19:360-6.
12. Harty LC, Caporaso NE, Hayes RB et al. Alcohol dehydrogenase 3 genotype and risk of oral cavity and pharyngeal cancers. J Natl Cancer Inst 1997;89:1698-705.
13. Peters ES, McClean MD, Liu M, Eisen EA, Mueller N, Kelsey KT. The ADH1C polymorphism modifies the risk of squamous cell carcinoma of the head and neck associated with alcohol and tobacco use. Cancer Epidemiol Biomarkers Prev 2005;14:476-82.
14. Hines LM, Stampfer MJ, Ma J et al. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N Engl J Med 2001;344:549-55.
15. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet 2001;358:1356-60.
16. Conigrave KM, Hu BF, Camargo CA, Jr., Stampfer MJ, Willett WC, Rimm EB. A prospective study of drinking patterns in relation to risk of type 2 diabetes among men. Diabetes 2001;50:2390-5.
17. Hu FB, Manson JE, Stampfer MJ et al. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. N Engl J Med 2001;345:790-7.
18. Stampfer MJ, Colditz GA, Willett WC et al. A prospective study of moderate alcohol drinking and risk of diabetes in women. Am J Epidemiol 1988;128:549-58.
19. Wannamethee SG, Camargo CA, Jr., Manson JE, Willett WC, Rimm EB. Alcohol drinking patterns and risk of type 2 diabetes mellitus among younger women. Arch Intern Med 2003;163:1329-36.

20. Prentice R, Breslow N. Retrospective studies and failure time models. Biometrika 1978;65:153-8.
21. Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 1997;20:1183-97.
22. Manson JE, Rimm EB, Stampfer MJ et al. Physical activity and incidence of non-insulin-dependent diabetes mellitus in women. Lancet 1991;338:774-8.
23. Willett WC. Nutritional Epidemiology. In: Rothman KJ, Greenland S, eds. Modern Epidemiology. Philadelphia, PA: Lippincott-Raven Publishers 1998:623-42.
24. USDA Nutrient Database for Standard Reference, Release 13. US Department of Agriculture Agricultural Research Service. 1999.
25. Giovannucci E, Colditz G, Stampfer MJ et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol 1991;133:810-7.
26. Willett W, Stampfer MJ, Bain C et al. Cigarette smoking, relative weight, and menopause. Am J Epidemiol 1983;117:651-8.
27. Hu FB, Sigal RJ, Rich-Edwards JW et al. Walking compared with vigorous physical activity and risk of type 2 diabetes in women: a prospective study. JAMA 1999;282:1433-9.
28. Feskanich D, Rimm EB, Giovannucci EL et al. Reproducibility and validity of food intake measurements from a semiquantitative food frequency questionnaire. J Am Diet Assoc 1993; 93: 790-6.
29. Rothman KJ, Greenland S. Modern Epidemiology. Philadelphia, USA: Lippincott-Raven, 1998.
30. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523-8.
31. Sarkola T, Iles MR, Kohlenberg-Mueller K, Eriksson CJ. Ethanol, acetaldehyde, acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. Alcohol Clin Exp Res 2002;26:239-45.
32. McCarty MF. Does regular ethanol consumption promote insulin sensitivity and leanness by stimulating AMP-activated protein kinase? Med Hypotheses 2001;57:405-7.
33. Fujino T, Kondo J, Ishikawa M, Morikawa K, Yamamoto TT. Acetyl-CoA synthetase 2, a mitochondrial matrix enzyme involved in the oxidation of acetate. J Biol Chem 2001;276:11420-6.
34. Ishikawa M, Fujino T, Sakashita H, Morikawa K, Yamamoto T. Kinetic properties and structural characterization of highly purified acetyl-CoA synthetase from bovine heart and tissue distribution of the enzyme in rat tissues. Tohoku J Exp Med 1995;175:55-67.
35. Yamashita H, Kaneyuki T, Tagawa K. Production of acetate in the liver and its utilization in peripheral tissues. Biochim Biophys Acta 2001;1532:79-87.
36. Pold R, Jensen LS, Jessen N et al. Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 2005;54:928-34.
37. Smith JL, Patil PB, Fisher JS. AICAR and hyperosmotic stress increase insulin-stimulated glucose transport. J Appl Physiol 2005;99:877-83.
38. Buhl ES, Jessen N, Schmitz O et al. Chronic treatment with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. Diabetes 2001;50:12-7.
39. Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR simultaneously increases fatty acid and glucose oxidation in resting rat soleus muscle. J Physiol 2005;565:537-46.
40. Smith AC, Bruce CR, Dyck DJ. AMP kinase activation with AICAR further increases fatty acid oxidation and blunts triacylglycerol hydrolysis in contracting rat soleus muscle. J Physiol 2005;565:547-53.

41. Antoshechkin AG. Physiological model of the stimulative effects of alcohol in low-to-moderate doses. Ann N Y Acad Sci 2002;957:288-91.
42. Pitteloud N, Mootha VK, Dwyer AA et al. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care 2005;28:1636-42.
43. Mootha VK, Lindgren CM, Eriksson KF et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat Genet 2003;34:267-73.
44. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med 2004;350:664-71.
45. Johnston CS, Kim CM, Buller AJ. Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type 2 diabetes. Diabetes Care 2004;27:281-2.
46. Laurent C, Simoneau C, Marks L et al. Effect of acetate and propionate on fasting hepatic glucose production in humans. Eur J Clin Nutr 1995;49:484-91.
47. Visapaa JP, Gotte K, Benesova M et al. Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. Gut 2004;53:871-6.



Chapter 7

Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight young men: a diet intervention study

Joline WJ Beulens
Emilie C de Zoete
Frans J Kok
Gertjan Schaafsma
Henk FJ Hendriks

Submitted

ABSTRACT

Objective

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes. This study investigates the effect of moderate alcohol consumption on adipokines and insulin sensitivity.

Methods

Twenty healthy, lean (BMI: 18.5 – 25 kg/m^2; n=11) or overweight (BMI > 27 kg/m^2; n=9) men (18- 25 years) consumed three cans of beer (40 g alcohol) or alcohol-free beer daily during three weeks in a randomized, partially diet-controlled, cross-over trial. After each treatment, adiponectin, leptin, acylation-stimulating protein (ASP), resistin, ghrelin and insulin sensitivity index (ISI) by an oral glucose tolerance test (OGTT), were assessed.

Results

Adiponectin and ghrelin concentrations increased ($p<0.01$) by 11% and 8%, while ASP concentrations decreased by 12% ($p=0.04$) after moderate alcohol consumption. Concentrations of leptin and resistin remained unchanged. ISI was not affected by moderate alcohol consumption, but 2-hour glucose concentrations were lower ($p=0.01$) after beer (4.5 ±0.1 mmol/l) than alcohol-free beer (4.9 ±0.1 mmol/l). Both free fatty acids and glucagon concentrations showed a stronger increase ($p\leq0.01$) after 90 minutes during OGTT after beer than alcohol-free beer. Changes of adiponectin were positively correlated ($r=0.69$, $p<0.001$) and changes of leptin ($r=-0.53$, $p=0.016$) and ASP ($r=-0.43$, $p=0.067$) were negatively correlated with changes of ISI.

Conclusion

Moderate alcohol consumption increased adiponectin and ghrelin, while it decreased ASP concentrations. These changes are in line with the hypothesized improvement of insulin sensitivity, but did not lead to an increase of insulin sensitivity after three weeks of moderate alcohol consumption. Adiponectin concentrations may particularly predict changes of insulin sensitivity.

INTRODUCTION

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes (1). This risk reduction could be explained by an increase of insulin sensitivity after moderate alcohol consumption (2;3). A positive association between alcohol consumption and insulin sensitivity is consistently reported in cross-sectional studies (4;5), but randomized controlled studies report contradictory results ranging from no change (6-9) to a dose-dependent improvement of insulin sensitivity (2).

Sierksma et al. suggested that an increase of adiponectin could precede changes of insulin sensitivity with moderate alcohol consumption (3). Adiponectin is thought to improve insulin sensitivity by suppression of hepatic glucose production, or increased glucose uptake and fatty acid oxidation in muscle tissue (10). Adipose tissue secretes several other proteins that may also be related to insulin sensitivity (11;12). The adipokines leptin and resistin are inversely associated with insulin sensitivity (11;12). Acylation-stimulating protein (ASP) stimulates storage of free fatty acids (FFA) as triglycerides in adipose tissue, but also increases glucose uptake (11). ASP-deficient mice indeed have decreased clearance of FFA, but improved insulin sensitivity (13). Similarly, plasma ASP concentrations are inversely correlated to insulin sensitivity in humans (14). Ghrelin, a peptide produced in the fundus of the stomach, is known as a regulator of food intake (12) and ghrelin concentrations are positively associated with insulin sensitivity (15;16).

Some studies suggest that decreased FFA concentrations may mediate (17-19) an increase of insulin sensitivity with moderate alcohol consumption, but the mechanism still is not entirely clear. Possibly, early changes of adipokines could play a role. Therefore we studied the effect of moderate alcohol consumption on adipokines and insulin sensitivity. We hypothesized that changes of adiponectin and ghrelin are positively, and changes of leptin, resistin and ASP are negatively associated with changes of insulin sensitivity.

METHODS

Subjects

We recruited twenty healthy, non-smoking, young men, aged 18 to 25 years from the pool of volunteers of TNO Quality of Life (Zeist, the Netherlands) and by advertisements in local newspapers. Subjects reported their alcohol intake, physical activity, diet, medical history and family history of alcoholism and were considered healthy based on pre-study laboratory tests, their medical history and a physical examination. Subjects were eligible when complying with the following criteria: alcohol consumption between 10 and 28 units/week, BMI between 18.5 and 25 kg/m^2 or BMI >27 kg/m^2, normal Dutch eating habits, less than eight hours/week of intense exercise and no family history of alcoholism. The subjects were divided in a lean subgroup (BMI 18.5-25 kg/m^2, n=11) and an overweight subgroup (BMI >27 kg/m^2, n=9) to explore whether BMI modifies the effect of moderate alcohol consumption on insulin sensitivity. Subjects gave written informed consent after the study was carefully explained. TNO Medical Ethics Committee approved the research protocol and we conducted the study according to the Declaration of Helsinki (2000) and the International Conference on Harmonisation Guideline for Good Clinical Practice.

Study design

The study was performed according to a randomized, partially diet-controlled, crossover trial, consisting of two three-week periods preceded by one week wash-out. Subjects were randomized based on BMI-group to the sequence beer (Amstel Bier, Amsterdam, The Netherlands; 5% vol alcohol) followed by alcohol-free beer (Amstel Malt Bier, Amsterdam, The Netherlands; <0.1 % vol alcohol) or the other way around. They consumed three cans (990 ml) of beer or alcohol-free beer daily with the evening meal, equaling 40 g alcohol/day during beer treatment. Their diet was fully controlled during the last ten days of each treatment period when all food was supplied by TNO and the subjects were not allowed to eat or drink anything but the foods supplied, except tap water, tea or coffee. We adjusted energy content of the diet to body weight and physical activity level of each participant. The composition of the diet was based on the Dutch Food Consumption Survey of 1998 (20) and consisted of 37 energy% fat, 15 energy% protein, and 48 energy% carbohydrates, excluding energy from alcohol. Differences in energy or carbohydrate content between beer

and alcohol-free beer were compensated for by the diet. During the entire study period (including wash-out periods) subjects were not allowed to drink any alcoholic beverages, besides those supplied by TNO Quality of Life. They were asked to maintain their habitual food consumption, body weight and physical activity pattern (except for habitual food consumption in diet-controlled period). Body weight was measured every visit wearing indoor clothing, without shoes, wallet and keys. Compliance was checked by daily questionnaires, return of empty cans and measuring ethyl glucuronide, a direct metabolite of alcohol consumption in the urine (21), and HDL cholesterol.

OGTT, blood and urine sampling and analysis

Subjects visited TNO at the end of each treatment period after an overnight fast for an OGTT and blood sampling. For the OGTT subjects drank 75 g glucose load dissolved in 300 ml water. Blood samples were taken before (fasting) and 30, 60, 90, 120 and 150 minutes after the glucose-load to assess glucose, insulin, FFA and glucagon concentrations. Fasting samples were used for determination of adipokines. Blood was collected from the antecubital vein in tubes containing clot activator (for serum) and in ice-chilled tubes containing citrate thiophylline, adenosine and dipyridamole (CTAD) for plasma (Vacutainer Systems, Becton Dickinson, Plymouth, UK). The blood was centrifuged for 15 minutes at 2.000 g at 4°C, within 15-30 minutes after collection and serum and plasma samples were stored at -80°C. Urine was collected during 24 hours on the last two days of the study and samples were stored at -80 °C.

Serum glucose was determined by hexokinase with a commercially available kit (Roche Reagens, Mannheim, Germany) on a Hitachi 911 (Hitachi Corporation, Japan). Serum insulin was determined by immunoenzymomatric assay (Tosho, Japan) on an AIA-600 (Tosho, Japan). FFA levels were determined enzymatically (Randox Laboratories, Antrim, U.K) and glucagon by EIA (Linco Research, Inc., St. Charles, Missouri, USA). Adiponectin, leptin and ghrelin concentrations were determined by radioimmunoassay method (Linco Research, Inc., St. Charles, Missouri, USA). ASP was determined by radioimmunoassay (Amersham Biosciences AB, Uppsala, Sweden) and resistin levels were determined by ELISA (BioVendor, Brno, Chech Republic). HDL was measured by homogenous enzymatic colorimetric (Roche reagens, Mannheim, Germany) on a Hitachi 911 (Hitachi Corporation, Japan). Ethyl glucuronide in 24 hour urine was determined according to Sarkola et al (21).

Power calculation

Sample size of this study was based on a power calculation according to Schouten (22) for the insulin sensitivity index (ISI) according to Cederholm (23). An estimate of treatment effects was obtained from studies of Sierksma et al. (3) and Davies et al. (2). A sample size of 20 is sufficient to detect a 12% difference in ISI based on a mean of 71 and standard deviation of 16 obtained from Cederholm (23) with a power of 80% and accepting a two-sided α of 0.05. The subgroups of 10 subjects would enable us to detect a 15% difference in ISI.

Statistical analysis

Data were analyzed using the SAS statistical software package (SAS/STAT Version 8, SAS Institute, Cary, NC, USA). ISI's were calculated based on the OGTT according to Cederholm and Gutt (23;24). Treatment effects were assessed by analysis of variance using a mixed model with BMI, treatment order, period, treatment, and the interaction between BMI and treatment included in the model. Differences between lean and overweight men were obtained from the main BMI effect in this model. Body weight was included in the model as a random factor to adjust for changes of body weight. Treatment order and the interaction between treatment and BMI were removed from the model if not significant. Treatment effects during the OGTT (curves over time) were analyzed by analysis of variance for repeated measurements (time*treatment interaction) using the same models as described for analysis of variance. Residuals were tested for normality and homogeneity of variance. Subgroup analysis based on BMI was performed in case of an interaction between BMI and treatment with a p-value below 0.1. Changes were calculated as difference between alcohol-free beer minus beer. Correlation coefficients were computed according to Spearman to assess associations between changes in outcome measures. Two-sided p-values below 0.05 were considered statistically significant.

RESULTS

Table 1 shows the characteristics of our study population. BMI of lean and obese subgroups differed profoundly, which is reflected in significant differences of total, HDL, LDL cholesterol and triglycerides and liver enzymes (AST, ALT and GGT) between the two groups. Compliance to guidelines for alcohol consumption and controlled diet was good as judged by self-report questionnaires and in general no important deviations from the study protocol occurred. Another indication for good compliance was the 18% increase ($p< 0.001$) of fasting serum HDL cholesterol after beer as compared to alcohol-free beer consumption. Moreover, urinary ethyl glucuronide concentration after consumption of beer was 6.6 ±0.6 mg/l, while no ethyl glucuronide was detected (0 ±0 mg/l) after consumption of alcohol-free beer. On average, subjects lost weight during the study, but this decrease was slightly higher ($p= 0.02$) during the alcohol-free beer (-1.3 ±0.2) than the beer drinking period (-0.6 ±0.2). Results were adjusted for this difference in body weight, but did not essentially change. The unadjusted results are therefore presented here.

Table 1: Baseline characteristics (mean ± sd) among lean and overweight men.

Variable	Lean	Overweight
N	11	9
Age (years)	19 ± 2	21 ± 2
BMI (kg/m^2)	20.1 ± 1.0[1]	31.3 ± 3.9
Body weight (kg)	69.0 ± 6.7[1]	106.0 ± 13.0
Height (m)	1.85 ± 0.07	1.84 ± 0.07
Glucose (mmol/l)	5.4 ± 0.3	5.4 ± 0.3
Haemoglobin (mmol/l)	9.4 ± 0.5	9.4 ± 0.5
Total cholesterol (mmol/l)	4.1 ± 0.6[1]	5.0 ± 0.8
HDL cholesterol (mmol/l)	1.6 ± 0.4[1]	1.2 ± 0.2
LDL cholesterol (mmol/l)	1.9 ± 0.5[1]	3.2 ± 0.9
Triacylglycerols (mmol/l)	1.2 ± 0.6	1.5 ± 0.4
Alkaline phosphatase (U/l)	100 ± 28	89 ± 27
Asparagine aminotransferase (U/l)	21 ± 4[1]	32 ± 21
Alanine aminotransferase (U/l)	15 ± 5[1]	60 ± 69
γ-glutamyl transferase (U/l)	18 ± 4[1]	43 ± 29

[1] $p<0.05$ lean compared to overweight group

Adipokines

Plasma adiponectin concentration increased by 11% and plasma ghrelin concentrations increased by 8%, while plasma ASP decreased by 12% after consumption of beer compared to alcohol-free beer (Table 2). Serum leptin and resistin concentrations remained unchanged (Table 2). A borderline significant (p< 0.10) BMI*treatment interaction was observed for serum resistin. After beer consumption, serum resistin increased by 9% (p= 0.04) among lean subjects, but did not change among overweight subjects (p= 0.49).

Table 2: Mean ±SEM ISI[1] and adiponectin, ghrelin, leptin and ASP and resistin concentrations after three weeks consumption of beer or alcohol-free beer

	Alcohol-free beer	Beer	P-value
Adipokines			
Adiponectin (mg/l)	4.4 ± 0.08	4.9 ± 0.08	< 0.001
Leptin (ng/ml)	6.0 ± 0.2	6.0 ± 0.2	0.86
Ghrelin (ng/ml)	734.6 ± 13.1	791.9 ± 13.1	0.006
ASP (ng/ml)	115.7 ±4.2	102.5 ± 4.2	0.043
Resistin total group (ng/ml)	4.5 ± 0.1	4.5 ± 0.1	0.68
Insulin sensitivity index			
Cederholm	32.8 ± 0.8	32.1 ± 0.8	0.55
Gutt	50.4 ± 2.6	57.4 ± 2.6	0.068

[1] Insulin sensitivity index; unit: $mg \cdot l^2/mmol \cdot mU \cdot min$

Oral glucose tolerance test

Figure 1 shows the results of serum glucose, insulin, FFA and glucagon concentrations during the OGTT after beer and alcohol-free beer consuming periods. Two-hour glucose concentrations were lower (p= 0.01) after beer (4.5 ± 0.1 mmol/l) than alcohol-free beer (4.9 ± 0.1 mmol/l) consumption (Figure 1A). Serum insulin concentrations were not different after beer than alcohol-free beer consumption (Figure 1B). FFA concentrations showed a larger increase during the last hour of the OGTT after beer than alcohol-free beer consumption (Figure 1C). Similar results were observed for glucagon concentrations (Figure 1D). $ISI_{Cederholm}$ was not affected by beer compared with alcohol-free beer consumption, but ISI_{Gutt} tended to increase after beer consumption (Table 2).

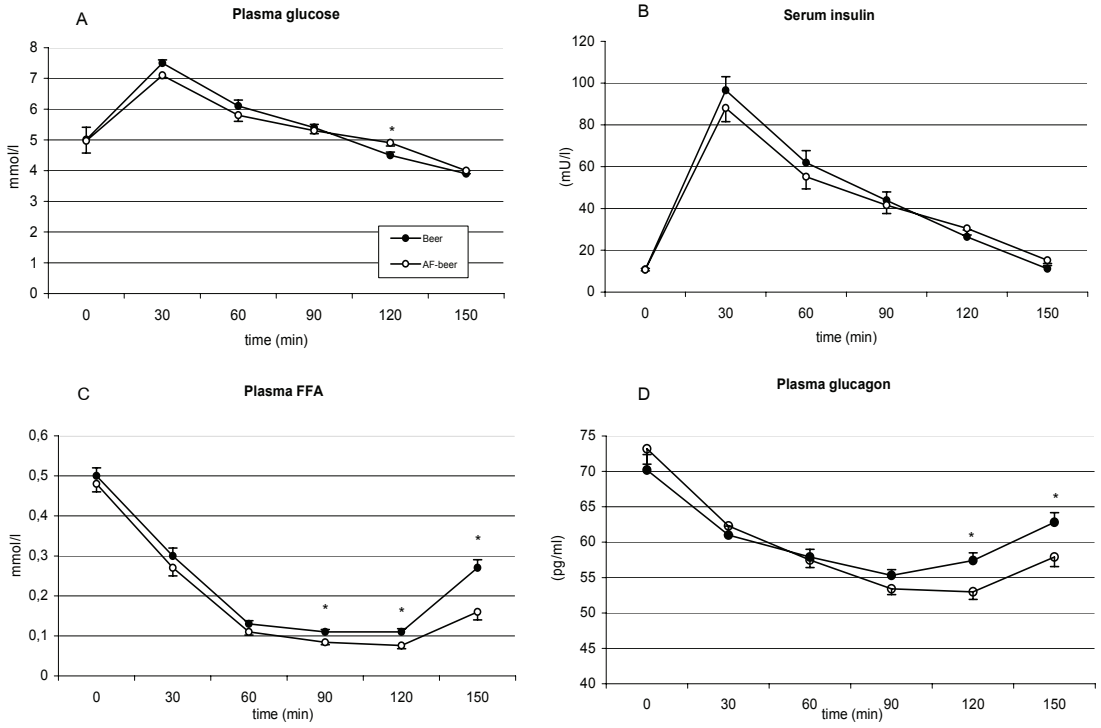


Figure 1: Mean (± SEM) plasma glucose (A), serum insulin (B), plasma free fatty acids (FFA: C) and glucagon (D) concentrations during an oral glucose tolerance test before and after 3 week consumption of beer and alcohol-free beer (AF-beer) among 20 healthy, lean and overweight men. Time*treatment interactions during the course of the oral glucose tolerance test were significant for glucose (p=0.02), free fatty acids (p=0.01) and glucagon (p<0.0001). * p<0.05 between beer and alcohol-free beer consumption at time-point during the oral glucose tolerance test.

BMI effects

Plasma adiponectin and resistin were not different between overweight and lean subjects, but plasma leptin and ASP were higher in overweight subjects, while plasma ghrelin tended to be lower in overweight than lean subjects (Table 3). Figure 2 shows serum glucose, insulin, FFA and glucagon concentrations during the OGTT for lean and overweight subjects. Fasting glucose levels were higher (p= 0.03) among overweight than lean subjects, but changes over time were not different (Figure 2A). Serum insulin was higher (p< 0.03) among overweight than lean subjects (Figure 2B). Changes of FFA during OGTT were different between lean and overweight men (Figure 2C). Plasma glucagon was higher (p< 0.07) in overweight than lean subjects throughout the curve (Figure 2D).

Table 3: Mean ±SEM ISI[1] and adiponectin, ghrelin, leptin, ASP and resistin concentrations in lean and overweight subjects

	Lean group	Overweight group	P-value
Adipokines			
Adiponectin (mg/l)	4.9 ± 0.07	4.3 ± 0.08	0.58
Leptin (ng/ml)	0.6 ± 0.2	11.4 ± 0.2	< 0.001
Ghrelin (ng/ml)	817.0 ± 12.4	709.0 ± 13.7	0.067
ASP (ng/ml)	91.9 ± 4.0	126.3 ± 4.5	0.010
Resistin (ng/ml)	4.4 ± 0.1	4.6 ± 0.1	0.80
Insulin sensitivity index			
Cederholm	37.0 ± 0.7	27.8 ± 0.8	< 0.001
Gutt	63.4 ± 2.5	44.4 ± 2.7	< 0.001

[1]Insulin sensitivity index; unit: $mg \cdot l^2/mmol \cdot mU \cdot min$

Correlations

Changes of adiponectin were positively correlated with changes of ISI ($r= 0.69$, $p< 0.001$), while changes of leptin ($r= -0.53$, $p= 0.016$) and ASP ($r= -0.43$, $p= 0.067$) were inversely correlated with changes of ISI. Changes of ghrelin and resistin were not correlated with changes of ISI. Adjusting correlation coefficients for BMI augmented the correlation between changes of ISI and adiponectin ($r= 0.73$, $p< 0.001$), because this correlation was mostly attributable to lean people ($r= 0.92$, $p< 0.001$). The difference of two-hour glucose concentrations between alcohol-free beer and beer was negatively correlated with the difference between alcohol-free beer and beer of changes of FFA concentrations during the last hour of the curve ($r= -0.75$, $p< 0.001$). This was not present for changes of glucagon during the last hour ($r= -0.21$, $p= 0.38$).

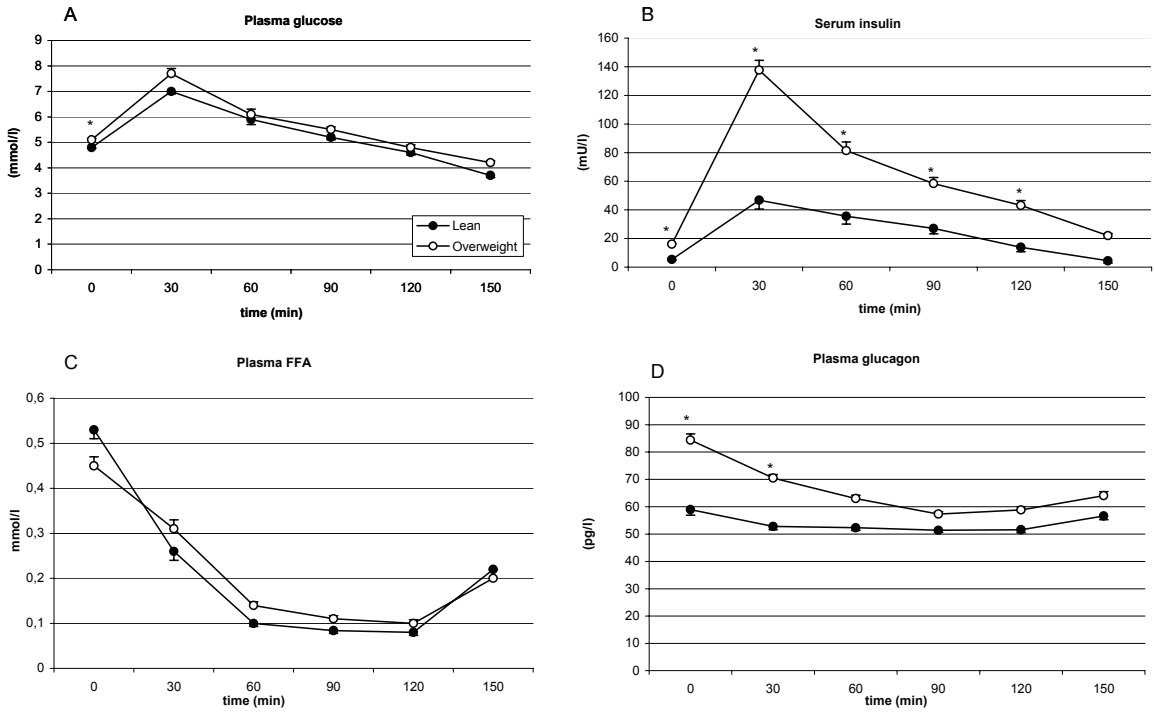


Figure 2: Mean (± SEM) plasma glucose (A), serum insulin (B), plasma free fatty acids (FFA: C) and glucagon (D) concentrations during the oral glucose tolerance test in lean and overweight subjects. Time*BMI interactions during the course of the oral glucose tolerance test were significant for insulin ($p<0.001$), free fatty acids ($p<0.001$) and glucagon ($p<0.001$). * $p<0.05$ between lean and overweight subjects at time-point.

DISCUSSION

The present study showed that moderate alcohol consumption increased adiponectin and ghrelin and decreased ASP concentrations. These changes are in line with the hypothesized improvement of insulin sensitivity, but did not lead to an increase of insulin sensitivity after three weeks of moderate alcohol consumption. Changes in adiponectin were positively associated with changes in insulin sensitivity, especially among lean subjects, while changes of leptin and ASP were modestly, negatively correlated with changes in insulin sensitivity.

This study was performed according to a randomized, controlled cross-over design and the diet was fully controlled during the last ten days of each treatment period. Changes of body weight were slightly different between interventions, but adjustment for these changes did not essentially change the results. It therefore seems unlikely that our results are distorted by changes in body weight, diet or lifestyle. Furthermore, HDL cholesterol increased by 18% after beer consumption and urinary ethyl glucuronide was not detected during the alcohol-free period, indicating good compliance to study treatments.

Changes of plasma adiponectin, ghrelin and ASP concentrations observed in this study after moderate alcohol consumption are in line with the hypothesized increase of insulin sensitivity. In addition, changes of adiponectin, ASP and leptin were correlated with changes of insulin sensitivity. These results are consistent with previous studies. Sierksma et al. showed an alcohol-induced increase of adiponectin that was positively associated with insulin sensitivity (3). A study of Roth et al. showed increased leptin concentrations after moderate alcohol consumption that were negatively associated with insulin sensitivity (25). Ours is the first study to show that moderate alcohol consumption decreases ASP concentrations. The inverse association with insulin sensitivity is consistent with the finding of increased insulin sensitivity in ASP-deficient mice and the inverse correlation of ASP with insulin sensitivity observed by Koistinen et al. (13;14). The magnitude of associations with insulin sensitivity suggests that adiponectin could particularly predict changes of insulin sensitivity with moderate alcohol consumption. The mechanism, however, by which moderate alcohol consumption increases adiponectin has not been investigated to date, but effects on gene expression may be involved. Low doses of alcohol have been shown to alter gene expression of both tissue plasminogen activator and plasmingen activator

inhibitor-1 (26). Like adiponectin, these markers are affected by moderate alcohol consumption (27;28) and activated by peroxisome proliferator-activated receptor gamma (29).

Ghrelin and resistin were not associated with insulin sensitivity. Ghrelin may therefore be indirectly related to insulin sensitivity via effects on energy metabolism and body weight (12). Resistin, on the other hand, seems to be associated with inflammation rather than insulin sensitivity in humans (30;31). We indeed observed in this study that changes of resistin correlated with changes of high-sensitive CRP ($r= 0.47$; $p= 0.034$; unpublished data).

Despite changes of adipokines, moderate alcohol consumption did not affect insulin sensitivity in this study. Our relatively small sample size could preclude detection of small effects on insulin sensitivity. However, based on our results, this sample size of 20 subjects is sufficient to detect a difference as small as 6%. As we observed a difference in ISI of only 2.5%, our non-significant results are probably not due to insufficient power.

Our results are contradictory to a randomized controlled trial of Davies et al. (2). This disparity may be due to the different methods used for evaluating insulin sensitivity that reflect different aspects of insulin sensitivity. The OGTT and ISI used in our study are suggested to represent peripheral insulin sensitivity (23) and were chosen because moderate alcohol consumption has been proposed to specifically affect peripheral insulin sensitivity (32). However, one of our previous studies applying the hyperinsulinemic, euglycemic clamp technique, representing whole-body insulin sensitivity, showed similar results as reported here (6). Furthermore, using ISI according to Matsuda that is highly correlated with the clamp (33) did not affect our results (data not shown). It is therefore unlikely that our results are explained by the method used. Davies et al. relied on fasting levels of glucose, insulin and triglycerides, suggested to represent hepatic insulin sensitivity (2). Triglycerides decreased significantly in their study, while most studies show an elevation or no change of triglycerides after moderate alcohol consumption (34). This could be due to the population of postmenopausal women, that show a different lipoprotein changes after moderate alcohol consumption with decreased concentrations of LDL cholesterol and triglycerides (35;36). Therefore, this measure used in a population of postmenopausal women may explain the disparity with our results. Other experimental conditions such as the three week treatment period of our study may also play a role.

Possibly this period may have been too short compared to the eight week intervention of Davies et al. (2).

We indeed observed some subtle changes during the OGTT such as a decreased two-hour glucose concentration that could precede changes of insulin sensitivity. FFA and glucagon concentrations were also increased during the last hour of the curve after beer consumption. Some studies report that increased two-hour FFA or glucagon concentrations are related to impaired glucose tolerance (37;38). However, such impaired glucose tolerance particularly leads to a decreased suppression of FFA and glucagon in the first 30 minutes of the curve in response to insulin secretion (37;38). Because we only observed increased FFA and glucagon concentrations during the last hour, this could indicate that FFA and glucagon are released earlier as energy fuel and to counteract the lower two-hour glucose concentration. This is confirmed by a negative correlation between the changes in FFA over time with two-hour glucose concentrations ($r=-0.75$, $p<0.001$). Longer interventions may be needed to observe changes of insulin sensitivity with moderate alcohol consumption.

In conclusion, moderate alcohol consumption increased adiponectin and ghrelin, while it decreased ASP concentrations. These changes are in line with the hypothesized improvement of insulin sensitivity, but did not lead to an increase of insulin sensitivity after moderate alcohol consumption. Adiponectin may particularly predict changes of insulin sensitivity, but longer interventions may be needed to detect changes of insulin sensitivity with moderate alcohol consumption.

Acknowledgements

We acknowledge all those involved in the conduct of the study and thank the volunteers for their enthusiastic participation. The research described in this article was funded by the Dutch Foundation for Alcohol Research.

REFERENCES

1. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
2. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
3. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
4. Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB, Jr., Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care 2000;23:1630-6.
5. Kiechl S, Willeit J, Poewe W et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). BMJ 1996;313:1040-4.
6. Beulens JW, van Beers RM, Stolk RP, Schaafsma G, Hendriks HF. The effect of moderate alcohol consumption on fat distribution and adipocytokines. Obesity (Silver Spring) 2006;14:60-6.
7. Cordain L, Bryan ED, Melby CL, Smith MJ. Influence of moderate daily wine consumption on body weight regulation and metabolism in healthy free-living males. J Am Coll Nutr 1997;16:134-9.
8. Cordain L, Melby CL, Hamamoto AE et al. Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. Metabolism 2000;49:1473-8.
9. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. Diabetes Care 2003;26:608-12.
10. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-6.
11. Havel PJ. Update on adipocyte hormones: regulation of energy balance and carbohydrate/lipid metabolism. Diabetes 2004;53 Suppl 1:S143-S151.
12. Meier U, Gressner AM. Endocrine regulation of energy metabolism: review of pathobiochemical and clinical chemical aspects of leptin, ghrelin, adiponectin, and resistin. Clin Chem 2004;50:1511-25.
13. Murray I, Sniderman AD, Havel PJ, Cianflone K. Acylation stimulating protein (ASP) deficiency alters postprandial and adipose tissue metabolism in male mice. J Biol Chem 1999;274:36219-25.
14. Koistinen HA, Vidal H, Karonen SL et al. Plasma acylation stimulating protein concentration and subcutaneous adipose tissue C3 mRNA expression in nondiabetic and type 2 diabetic men. Arterioscler Thromb Vasc Biol 2001;21:1034-9.
15. Poykko SM, Kellokoski E, Horkko S, Kauma H, Kesaniemi YA, Ukkola O. Low plasma ghrelin is associated with insulin resistance, hypertension, and the prevalence of type 2 diabetes. Diabetes 2003;52:2546-53.
16. Purnell JQ, Weigle DS, Breen P, Cummings DE. Ghrelin levels correlate with insulin levels, insulin resistance, and high-density lipoprotein cholesterol, but not with gender, menopausal status, or cortisol levels in humans. J Clin Endocrinol Metab 2003;88:5747-52.
17. Avogaro A, Watanabe RM, Gottardo L, de Kreutzenberg S, Tiengo A, Pacini G. Glucose tolerance during moderate alcohol intake: insights on insulin action from glucose/lactate dynamics. J Clin Endocrinol Metab 2002;87:1233-8.
18. Avogaro A, Watanabe RM, Dall'Arche A, de Kreutzenberg SV, Tiengo A, Pacini G. Acute alcohol consumption improves insulin action without affecting insulin secretion in type 2 diabetic subjects. Diabetes Care 2004;27:1369-74.

19. Furuya D.T., Binsack R, Onishi ME, Monteiro SP, Fabres MU. Low ethanol consumption induces enhancement of insulin sensitivity in liver of normal rats. Life Sci 2005;77:1813-24.
20. Voedingscentrum. Zo eet Nederland 1998; resultaten van de Voedselconsumptiepeiling 1998. Den Haag: 2003.
21. Sarkola T, Dahl H, Eriksson CJ, Helander A. Urinary ethyl glucuronide and 5-hydroxytryptophol levels during repeated ethanol ingestion in healthy human subjects. Alcohol Alcohol 2003;38:347-51.
22. Schouten HJA. Aantal mensen of proefdieren in twee groepen, II. Klinische Statistiek: Een praktische inleiding in methodologie en analyse. Houten: Bohn Stafleu Van Loghum 1995:199-206.
23. Cederholm J, Wibell L. Insulin release and peripheral sensitivity at the oral glucose tolerance test. Diabetes Res Clin Pract 1990;10:167-75.
24. Gutt M, Davis CL, Spitzer SB et al. Validation of the insulin sensitivity index (ISI(0,120)): comparison with other measures. Diabetes Res Clin Pract 2000;47:177-84.
25. Roth MJ, Baer DJ, Albert PS et al. Relationship between serum leptin levels and alcohol consumption in a controlled feeding and alcohol ingestion study. J Natl Cancer Inst 2003;95:1722-5.
26. Booyse FM, Aikens ML, Grenett HE. Endothelial cell fibrinolysis: transcriptional regulation of fibrinolytic protein gene expression (t-PA, u-PA, and PAI-1) by low alcohol. Alcohol Clin Exp Res 1999;23:1119-24.
27. Hendriks HF, Veenstra J, Velthuis-te Wierik EJ, Schaafsma G, Kluft C. Effect of moderate dose of alcohol with evening meal on fibrinolytic factors. BMJ 1994;308:1003-6.
28. Sierksma A, van der Gaag MS, Kluft C, Bakker M, Hendriks HF. Moderate alcohol consumption and fibrinolytic factors of pre- and postmenopausal women. Nutr Res 2001;21:171-81.
29. Marx N, Bourcier T, Sukhova GK, Libby P, Plutzky J. PPARgamma activation in human endothelial cells increases plasminogen activator inhibitor type-1 expression: PPARgamma as a potential mediator in vascular disease. Arterioscler Thromb Vasc Biol 1999;19:546-51.
30. Gomez-Ambrosi J, Fruhbeck G. Do resistin and resistin-like molecules also link obesity to inflammatory diseases? Ann Intern Med 2001;135:306-7.
31. Lee JH, Chan JL, Yiannakouris N et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J Clin Endocrinol Metab 2003;88:4848-56.
32. McCarty MF. Does regular ethanol consumption promote insulin sensitivity and leanness by stimulating AMP-activated protein kinase? Med Hypotheses 2001;57:405-7.
33. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-70.
34. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523-8.
35. Baer DJ, Judd JT, Clevidence BA et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr 2002;75:593-9.
36. van der Gaag MS, Sierksma A, Schaafsma G et al. Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. J Womens Health Gend Based Med 2000;9:607-16.
37. Ahren B, Larsson H. Impaired glucose tolerance (IGT) is associated with reduced insulin-induced suppression of glucagon concentrations. Diabetologia 2001;44:1998-2003.
38. Laws A, Hoen HM, Selby JV, Saad MF, Haffner SM, Howard BV. Differences in insulin suppression of free fatty acid levels by gender and glucose tolerance status. Relation to plasma triglyceride and apolipoprotein B concentrations. Insulin Resistance Atherosclerosis Study (IRAS) Investigators. Arterioscler Thromb Vasc Biol 1997;17:64-71.

Chapter 8

The effect of moderate alcohol consumption on fat distribution and adipocytokines

Joline WJ Beulens

Roderik M van Beers

Ronald P Stolk

Gertjan Schaafsma

Henk FJ Hendriks

Obesity (2006); 14(1): 60-66.

ABSTRACT

Objective

To investigate the effect of moderate alcohol consumption on fat distribution, adipose tissue secreted proteins (adiponectin and resistin) and insulin sensitivity in healthy middle-aged men with abdominal obesity.

Methods

Thirty-four healthy men with increased waist circumference (≥94cm), aged between 35 and 70 years participated in a randomized, controlled crossover design. They used 450 mL of red wine (40 grams of alcohol) or 450mL de-alcoholized red wine daily during four weeks. At the end of each treatment period fat distribution, adipose tissue proteins and insulin sensitivity index (ISI) were measured.

Results

Subcutaneous and abdominal fat contents and body weight did not change after four weeks of moderate alcohol consumption. Liver fat (quip-index) was slightly higher after consumption of red wine (6.8 ±0.1) as compared to de-alcoholized red wine (6.5 ±0.1), but not significantly different ($p= 0.09$). Plasma adiponectin concentration increased ($p<0.01$) to 6.0 ±0.1 µg/ml after 28 days of moderate alcohol consumption compared with de-alcoholized red wine (5.5 ±0.1 µg/ml). Serum resistin concentrations and ISI were not affected by alcohol consumption. Percent changes of serum resistin correlated significantly with changes of ISI ($r=-0.69$, $p<0.01$), whereas this correlation was not present between changes of plasma adiponectin and ISI ($r=0.31$, $p=0.22$).

Conclusion

Moderate alcohol consumption for four weeks is not associated with differences of subcutaneous and abdominal fat contents or body weight. The 10% increase of adiponectin was thus not associated with a change in fat distribution or body weight change.

INTRODUCTION

The metabolic syndrome (MS) is characterized by insulin resistance combined with a clustering of cardiovascular risk factors including abdominal obesity, low serum HDL-cholesterol, increased serum triglycerides, hypertension and elevated plasma glucose (1). The prevalence of this syndrome is estimated to be 15% in non-diabetic European adults (2) and the presence of MS is associated with an increased risk of cardiovascular disease (CVD) and type 2 diabetes mellitus (DM2) (2;3).

Moderate alcohol consumption is associated with a decreased risk of both CVD and DM2 (4;5) due to effects on several components of MS, such as an increase of HDL cholesterol and enhanced insulin sensitivity (6-8). Recently it has been shown that moderate alcohol consumption is also associated with a decreased risk of developing MS (9). Both (central) adiposity and insulin resistance are key features of MS (1;10). The effect of moderate alcohol consumption on these features is, however, contradictory. A positive association between alcohol consumption and body weight has not been consistently observed in cross-sectional studies (11). For insulin sensitivity, on the other hand, positive associations with alcohol consumption are consistently shown in cross-sectional studies (12;13). Randomized controlled trials, however, have reported contradictory results, showing increased insulin sensitivity after moderate alcohol consumption (7), no effect (14;15) or only in an insulin-resistant sub-group (8).

In addition to storing energy in the form of triglycerides, adipose tissue secretes several factors, such as adiponectin and resistin, that are involved in energy and glucose homeostasis (16). Consequently both adiponectin and resistin are suggested as possible markers for insulin sensitivity (16). Therefore we investigated the effect of moderate alcohol consumption on fat distribution, adiponectin, resistin and insulin sensitivity index (ISI) in middle-aged men with abdominal obesity.

METHODS

Subjects

The study was conducted at TNO Quality of Life (Zeist, The Netherlands). The study was performed according to the International Conference on Harmonisation Guideline for Good Clinical Practice, complied with the Declaration of Helsinki and was approved by the independent Medical Ethics Committee of TNO. Thirty-six subjects, all non-smoking, were recruited from the pool of volunteers of TNO Quality of Life and through an advertisement in a local newspaper. The volunteers received complete information about the study by verbal briefing and in writing and subsequently signed an informed consent. A questionnaire (self-report) was used for information on habitual alcohol intake, medical history and (family) history of alcoholism. Subjects fulfilled the following inclusion criteria: men, between 35 and 70 years old, habitual consumption between 10 and 28 alcohol-containing beverages weekly, waist circumference ≥94 cm and no (family) history of alcoholism, DM2 or CVD. Sample size of the study was based on a previous study of Sierksma et al. (8) showing that a sample size of 23 is sufficient to detect a 10% difference on adiponectin concentrations. The sample size of the subgroup for the ISI measurement was also based on this study. Sierksma et al. observed a 20% increase of ISI in an insulin-resistant subgroup that is comparable to our study population. Based on these data, a sample size of 18 subjects is sufficient to detect a 20% difference of ISI in a crossover design with a standard deviation of the difference of 1.0, a power of 80% and accepting a two-sided alpha of 5%.

Study design

Subjects entered a randomized crossover trial consisting of two periods of four weeks in which they consumed red wine or de-alcoholized red wine. The red wine and de-alcoholized red wine were specially bottled for this study (Carl Jung GmbH, Rüdesheim am Rhein, Germany). Red wine had an alcohol content of 11.5 vol%. De-alcoholized wine (alcohol content of 0.13 vol%) was made from exactly the same base wine, however it was sweetened with sugar (4%). Alcohol was extracted from the wine by vacuum distillation at low temperature (<30°C) to maintain the taste and characteristics of the wine.

One half of the subjects were randomly allocated to the sequence red wine followed by

de-alcoholized red wine. The other half of the subjects consumed de-alcoholized red wine first followed by red wine. Subjects were instructed to drink 450 mL (four glasses) of red wine (40 grams of alcohol) or de-alcoholized red wine (control) with dinner. Beverages were provided in bottles at the start and halfway each treatment period together with a measuring cup marked at 450 mL. Subjects were asked to maintain their normal dietary habits and exercise patterns and not to consume any (additional) alcoholic beverages throughout the study. Each day subjects completed a questionnaire detailing beverage intake, dietary habits, exercise performed, medications taken and illnesses experienced. The medical investigator routinely reviewed the questionnaires and all problems identified were discussed with the subjects during the next visit or by telephone call. In addition, compliance was checked by questionnaire and counting the number of bottles returned and measuring the leftovers. Body weight was determined halfway and at the end of each treatment period, with subjects wearing indoor clothing, without shoes, wallet and keys. At the end of each treatment period (day 29 and 57) fasting venous blood samples were collected. Blood was obtained from the antecubital vein and collected in a tube containing lithium-heparin and in a tube containing gel and clot activator (Becton Dickinson, Vacutainer Systems, Plymouth, UK). To obtain plasma or serum, the blood was centrifuged for 15 minutes at 2,000g and 4°C, between 15 and 30 minutes after collection. All samples were stored at -80 °C until analysis.

Ultrasound

The ultrasound measurement of liver, abdominal and subcutaneous fat depots was performed on day 28 and 56 of the study. The ultrasound abdominal fat imaging-protocol includes two distance measurements: from the peritoneum to the body of the corpus of the vertebra (abdominal measurement) and from the skin to the linea alba (subcutaneous measurement). A strict protocol was used. Each distance was measured at three positions, and was performed in threefold, without distortion (by compression) of the abdominal cavity. Details and validation of this protocol have been published elsewhere (17). The ultrasound measurements of liver fat were based on the quip program developed for veterinary sciences (18). Measurements were performed separately in the left and right liver lobes at the position of the portal vein. The mean of the two measurements was used in the analyses. All ultrasonography was performed by one radiologist using an HDI 3000

(Philips medical Systems, Eindhoven, The Netherlands) with a 2 to 4 MHZ transducer. The subcutaneous fat measurement of two persons was missing, and therefore the data of 32 subjects were analyzed.

Biological sample collection and analysis

Serum HDL-cholesterol levels were determined enzymatically (Roche Diagnostics, Mannheim, Germany). A validated sandwich enzyme-linked immunosorbent assay was used (BioVendor, Brno, Czech Republic) for determination of serum resistin. Plasma adiponectin was determined using a validated sandwich enzyme-linked immunosorbent assay employing an adiponectin-specific antibody (ANOC9108). The intra- and inter-assay coefficients of variation were 3.3% and 7.4% respectively. More detailed information on this assay and the antibody used are described by Arita et al. (1999) (19). Levels of serum glucose and insulin during the last hour of the clamp were determined using commercially available kits (Roche Diagnostics; Tosoh Corporation, Tokyo Japan and Euro-Diagnostica, Malmö, Sweden, respectively). All samples were analyzed in one run after the finish of the study. Total antioxidant capacity and flavonoid content of the red wine and de-alcoholized red wine were determined at the end of the study. Total antioxidant capacity was measured using the Trolox equivalent antioxidant assay as described by Van den Berg et al. (20). Flavonoid analysis was performed using high-performance liquid chromatography equipped with photodiode array detection according to method described by Hertog et al. (21).

Hyperinsulinemic isoglycemic glucose clamp

In a random subgroup of 24 subjects, at the end of each treatment period (day 29 and 57) after an overnight fast of al least ten hours, the sensitivity to insulin-mediated glucose uptake was assessed by the hyperinsulinemic, isoglycemic glucose clamp technique according to the principles described by DeFronzo et al. (22). Insulin and glucose solutions were administered through a cannulated vein. Arterialized (by warming at ~55°C) venous blood was sampled through a cannulated antecubital vein from the other arm. Insulin was constantly infused (1mU/kg body weight/min). Blood glucose concentration was maintained at the basal concentration throughout the clamp by monitoring the glucose concentration at 5 minutes intervals, using a glucometer (Accutrend, Boehringer Mannheim, East Sussex,

UK), and adjusting the infusion rate of a 20% w/v glucose solution. A steady state serum glucose level was reached between 60 and 110 minutes. Blood was collected at 0, 30 and 60 minutes for serum insulin and c-peptide measurements. The serum glucose levels were used for the calculation of insulin sensitivity.

Because the rate of glucose utilization is related to the blood glucose concentration, the metabolic clearance rate was calculated by dividing the average glucose infusion during the last hour of the clamp by the average serum glucose concentration and multiplied by five. To correct for differences in the steady state plasma insulin achieved during the clamp, the metabolic clearence rate was divided by the steady state plasma insulin, to obtain the insulin sensitivity index (ISI). The ISI ([(glucose infusion rate/steady state serum glucose)*5]/steady state plasma insulin) (mg glucose/kg body weight.min/μU insulin/mL), was taken as the measure of insulin sensitivity (22).

Of the 24 subjects that underwent the clamp, one subject had a variation in serum glucose levels >15% during the last hour of the clamp and therefore that measurement was excluded from the data analysis. Six subjects dropped out of this subgroup, due to a treatment unrelated reason. Finally 17 subjects were included in the analyses. The stability of the serum glucose level in the last hour of the clamp, indicated by the coefficient of variation, was during red wine 4.4% ± 2.0 and during de-alcoholized red wine 4.1% ±1.6.

Statistical analysis

Statistical analyses were performed with SAS statistical software package (SAS/STAT Version 8.02, SAS Institute, Cary, NC, USA). Treatment effects were assessed by the mixed model procedure that included fixed terms for treatment (red wine and de-alcoholized red wine) and period. Treatment order was added to the model to correct for possible carry-over effects. Body weight was included in the model as a random factor to adjust treatment effects for changes in body weight. Model terms were considered significant at $p \leq 0.05$. Correlation coefficients (Pearson) were computed to assess associations between the percentage changes of several outcome measures. Data are presented as means and SEM.

RESULTS

Subjects' characteristics are shown in table 1. From the 36 subjects who enrolled in this study, two subjects dropped out due to treatment unrelated reasons. From the subgroup for ISI measurements of 24 subjects only 17 subjects successfully completed the measurements twice due to treatment unrelated reasons. Compliance with the beverage intake was good, as judged from the daily questionnaire and the return of empty bottles. Another indication that the subjects were compliant with the beverage intake was their fasting serum HDL-cholesterol level, which increased with ~6% after four weeks red wine consumption compared with the de-alcoholized red wine consumption (1.12 mmol/l and 1.06 mmol/l, respectively; $P<0.01$). No carry-over effects in outcome measures were observed. Average body weight did not differ between red wine and de-alcoholized red wine in the treatment period (table 3). Nevertheless, treatment effects on all parameters were adjusted for changes in body weight and this did not substantially change the results. Therefore, results without adjustment for changes in body weight are presented here.

Table 1 Characteristics of the volunteers included in the data analysis (n=34).

Variable	Mean	SD[1]
Age (years)	53.2	± 9.2
Alcohol consumption (drinks/week)	15	± 6
Body weight (kg)	96.8	± 15.9
BMI (kg/m^2)	29.1	± 4.2
Waist circumference (cm)	109.1	± 10.9
Waist/hip ratio	1.02	± 0.05
Gamma-glutamyl transpeptidase (U/L)	37.2	± 17.5
Alanine aminotransferase (U/L)	32.7	± 14.5
Aspartate aminotransferase (U/L)	23.9	± 6.8
Total Cholesterol (mmol/L)	5.93	± 0.85
HDL cholesterol (mmol/L)	1.25	± 0.18
LDL Cholesterol (mmol/L)[2]	3.84	± 0.79
Triglycerides (mmol/L)	1.91	± 0.84
Glucose (mmol/L)	5.7	± 0.4
Insulin (mU/L)	10.7	± 5.6

[1]SD: standard deviation

[2]Measured in n=33

Total antioxidant capacity and flavonoids concentrations of red wine and de-alcoholized red wine are shown in table 2. Both antioxidant capacity and concentrations of several major free and glycosylated flavonoids were similar for red wine and de-alcoholized red wine.

Table 2: Total antioxidant capacity and flavonoid content of de-alcoholized red wine and red wine.

	De-alcoholized red wine	Red wine
TEAC[1] (mmol Trolox equivalents/L)	24.3	24.5
Free flavonoids (mg/L)		
Myricitin	2.8	3.0
Quercetin	3.1	3.2
Isoramnetin	0.2	0.4
Glycosylated flavonoids (mg/L)		
Myricitin	6.2	5.1
Quercetin	4.5	4.6
Isoramnetin	1.6	1.3

[1]Trolox equivalent antioxidant capacity

Ultrasound quantification of abdominal and liver fat

The results of the ultrasound measurements are shown in table 3. The ultrasound measurements of abdominal fat depots did not differ (p=0.68) between red wine (9.8 cm ±0.2) and de-alcoholized red wine (9.9 cm ±0.2). The subcutaneous fat depots did not differ (p=0.15) between red wine (2.7 cm ±0.1) and de-alcoholized red wine (2.8 cm± 0.1) treatment. The amount of fat in the liver was slightly higher after four weeks red wine consumption (6.8 ±0.1) compared with the de-alcoholized red wine consumption (6.5 ±0.1), but the difference was not significant (p=0.09).

Table 3: Mean (± SEM) body weight, fat mass, adipocytokines and ISI after de-alcoholized red wine and red wine in 34 healthy middle-aged men with increased waist circumference.

	De-alcoholized red wine	Red wine	p-value
Bodyweight (kg)	97.2 ± 0.2	97.6 ± 0.2	0.10
Subcutaneous fat (cm)	2.8 ± 0.1	2.7 ± 0.1	0.15
Abdominal fat (cm)	9.8 ± 0.2	9.9 ± 0.2	0.68
Liver-fat (quip-index)	6.6 ± 0.1	6.8 ± 0.1	0.09
Adiponectin (mg/mL)	5.5 ± 0.1	6.0 ± 0.1	<0.01
Resistin (ng/mL)	3.0 ± 0.1	3.0 ± 0.1	0.67
ISI[1,2]	7.1 ± 0.5	6.6 ± 0.6	0.24

[1]ISI is expressed as mg glucose/kg body weight.min/ μU insulin/ml.

[2]Measured in a subgroup of 17 subjects.

Plasma adiponectin, serum resistin and ISI

Plasma adiponectin concentration increased by 10% after 28 days of consumption of red wine as compared with de-alcoholized red wine consumption ($p<0.01$, table 3). Serum resistin concentration did not differ between the two treatment groups ($p=0.67$, table 3). ISI was not significantly different ($p=0.24$) between consumption of de-alcoholized red wine (7.1 ± 0.5) and consumption of red wine (6.6 ± 0.6, table 3).

Correlations

BMI correlated positively with subcutaneous ($r= 0.74$; $p< 0.01$) and abdominal ($r= 0.50$; $p< 0.01$) fat mass. Percent change of serum resistin correlated significantly with changes of ISI ($r=-0.69$, $p<0.01$), whereas this correlation was not present between plasma adiponectin and ISI ($r=0.31$, $p=0.22$). Changes of plasma adiponectin or serum resistin did not correlate with percent changes of BMI, subcutaneous, abdominal or liver fat.

DISCUSSION

The present study showed that four weeks moderate alcohol consumption increased plasma adiponectin concentration in middle-aged men with abdominal overweight, whereas fat distribution, serum resistin and ISI were unaffected. Changes of adiponectin were not associated with changes of body weight, fat distribution or ISI, while changes of resistin correlated significantly with changes of ISI.

This study was performed according to a randomized, controlled, crossover design. Total antioxidant capacity and concentrations of several major free and glycosylated flavonoids were similar in red wine and de-alcoholized red wine. Thus, it is unlikely that these differences affected our results to a large extent. Moreover, two previous studies of Davies et al. and Sierksma et al. showed differences of ISI after moderate alcohol consumption that can only be attributed to ethanol content (7;8). A recent meta-analysis of Koppes et al. also concluded that the association of alcohol consumption and DM2 was independent from beverage type (5). Therefore the effects of moderate alcohol consumption on ISI and DM2 are most likely to be due to ethanol itself.

Subjects did not change diet and lifestyle throughout the study. Only a slight increase of body weight was observed after consumption of red wine as compared to de-alcoholized red wine. Adjustment for changes of body weight did, however, not substantially change our results. It therefore is unlikely that our results are disrupted by body weight, diet or lifestyle. ISI was measured with the clamp technique, which is regarded as the reference method for quantifying insulin sensitivity. The small number of persons in this subgroup could preclude the detection of small effects on insulin sensitivity. Based on the data of our study, however, the subgroup of 17 subjects would have been sufficient to detect a 13% difference of insulin sensitivity. Therefore this sample size was sufficient to detect the 20% difference observed by Sierksma et al. (8). However, the difference observed in this study was only 8%. Therefore, our non-significant results are probably not due to insufficient power. Still, larger trials are needed to confirm these results.

It is generally expected that alcohol consumption, because of its high energy content could result in obesity. In our study, a 28-day intervention of red wine did not significantly change body weight or subcutaneous and abdominal fat depots. The results in our study are in agreement with cross-sectional studies that observed no clear correlation between

alcohol consumption and body weight (23). The relation between alcohol consumption and waist circumference, on the other hand, is not consistent. Some studies showed positive associations (24;25), whereas others observed a negative association between alcohol and waist circumference or no relation at all (26). A large prospective study among 16,587 men observed no association of alcohol consumption with waist gain after 9 years follow-up (27). Results from our study are consistent with this study, but this may also be due to our relatively short intervention period. The effect of moderate alcohol consumption on fat distribution and body weight, therefore, needs to be studied over a longer study period.

Plasma adiponectin concentration increased by 10% after 28 days moderate consumption of red wine compared with de-alcoholized red wine. This increase of adiponectin is consistent with the 11% increase observed by Sierskma et al. (8) and two observational studies (28;29). Although weight loss is associated with increased levels of adiponectin (30;31), the increase of plasma adiponectin was independent of changes in body weight or fat distribution. This suggests that other mechanisms mediate the effect of moderate alcohol consumption on adiponectin concentrations. This mechanism has not been studied to date. It seems probable though that effects on gene expression may be involved, because low doses of alcohol have been shown to alter gene expression of both tissue plasminogen activator and plasminogen activator inhibitor-1 as well (32). Animal models have shown that infusion of adiponectin increases insulin sensitivity, lowers plasma glucose and free fatty acid concentrations and increases fatty acid oxidation in skeletal muscle (33). Therefore, we hypothesized that the increase of adiponectin after moderate alcohol consumption would correlate with an increase in ISI. However, we did not observe an increased ISI after moderate alcohol consumption. This may be explained by the short intervention period. Another possibility is that the 40 gram alcohol daily used in this study is too high to observe an effect on ISI, since the association between alcohol consumption and incidence of DM2 is described in a J-shaped fashion (5). A third explanation for the absence of an effect on ISI could be that our subjects were rather overweight. Arky et al. suggested that overweight subjects may be less responsive to alcohol intake, as they observed a significant drop of plasma glucose after infusion of alcohol (0.24 g/min) in normal-weight subjects, while obese subjects were resistant to the hypoglycemic effect of alcohol (34).

Animal studies suggested that resistin influences glucose homeostasis (35). In human studies, however, no direct relationship between circulating levels of resistin and insulin action in peripheral tissue was observed (36;37). Lee et al. (37) proposed that given the incomplete homology between human and mouse resistin and the differences in energy metabolism between mice and humans, the physiology of resistin in mice differs from that in humans. Our 28 days alcohol intervention did not result in a significant change of resistin. On the other hand, changes of resistin correlated quite strongly ($r= -0.69$; $p< 0.01$) with changes of ISI. This was also observed in the study of Azuma et al. showing that changes of serum resistin correlated with changes of ISI after weight loss intervention, while serum resistin did not correlate with ISI cross-sectionally (37;38). Possibly intra-individual changes of resistin are associated with intra-individual changes of ISI, while this relation is not present cross-sectionally. Inconsistencies in correlations between ISI and resistin may also be due to the population under study. Our study and that of Azuma et al. consisted of obese subjects. In an unpublished study from our group we observed a significant interaction of BMI (lean versus obese subjects) with the effect of moderate alcohol consumption on serum resistin concentrations.

In conclusion, this study showed that moderate alcohol consumption did not result in fat accumulation in subcutaneous and abdominal fat depots. Therefore, increased fat mass does not explain the increased plasma adiponectin concentration observed in this study. Moderate alcohol consumption did not affect serum resistin concentrations and ISI, but changes of resistin concentrations were associated with changes of ISI. This was not the case for changes of adiponectin. Whether and under what circumstances adiponectin and resistin may serve as markers for ISI therefore needs to be further explored.

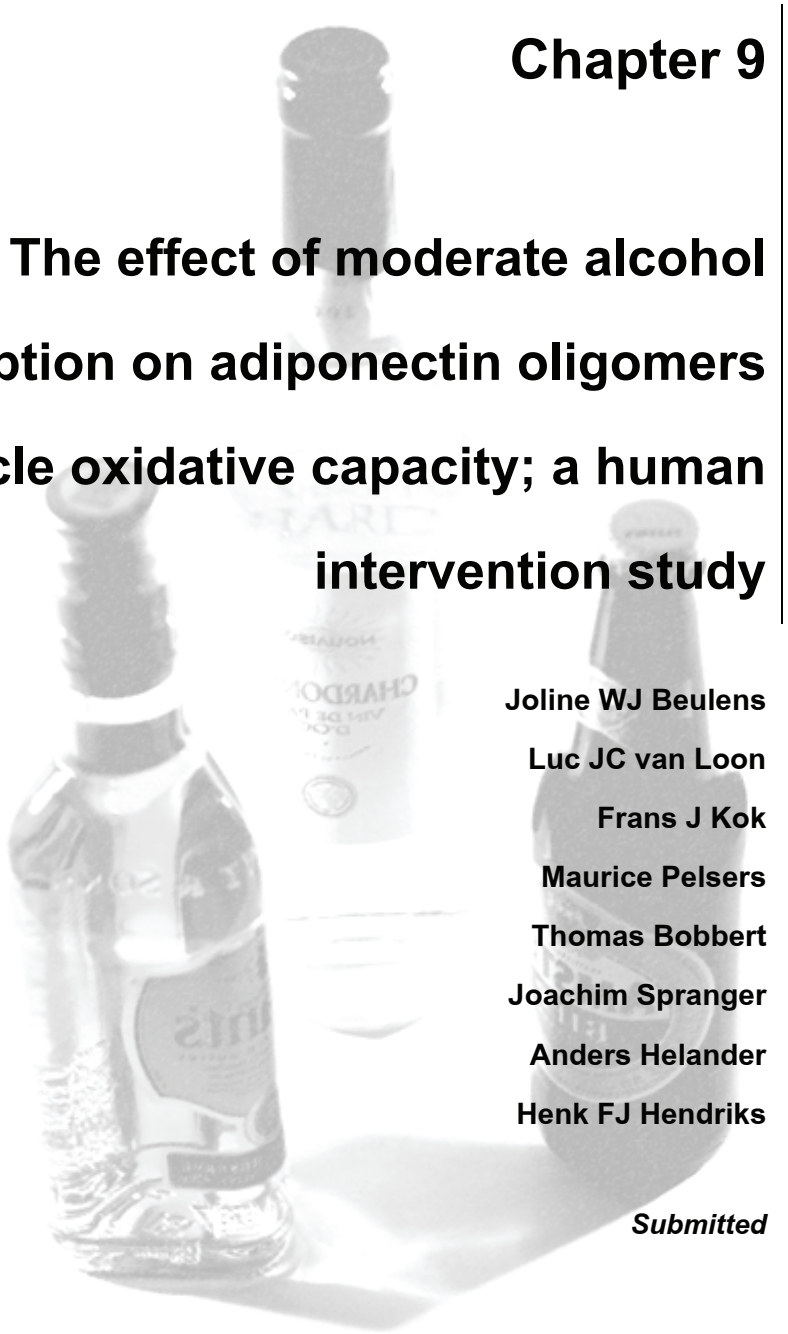
Acknowledgements

The authors thank the volunteers for their enthusiastic participation. This research was funded by the Dutch Foundation for Alcohol Research.

REFERENCES

1. Grundy SM, Brewer HB, Jr., Cleeman JI, Smith SC, Jr., Lenfant C. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433-8.
2. Hu G, Qiao Q, Tuomilehto J, Balkau B, Borch-Johnsen K, Pyorala K. Prevalence of the metabolic syndrome and its relation to all-cause and cardiovascular mortality in nondiabetic European men and women. Arch.Intern.Med. 2004;164:1066-76.
3. Lorenzo C, Okoloise M, Williams K, Stern MP, Haffner SM. The metabolic syndrome as predictor of type 2 diabetes: the San Antonio heart study. Diabetes Care 2003;26:3153-9.
4. Grobbee DE, Rimm EB, Keil U, Renaud S. Alcohol and the Cardiovascular System. In: MacDonald I, ed. Health issues related to alcohol consumption. Bodmin: Blackwell Sciences Ltd 1999:125-79.
5. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
6. Beulens JW, Sierksma A, van Tol A et al. Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1. J.Lipid Res. 2004;45:1716-23.
7. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
8. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
9. Djousse L, Arnett DK, Eckfeldt JH, Province MA, Singer MR, Ellison RC. Alcohol consumption and metabolic syndrome: Does the type of beverage matter? Obes Res. 2004;12:1375-85.
10. Carr DB, Utzschneider KM, Hull RL et al. Intra-abdominal fat is a major determinant of the National Cholesterol Education Program Adult Treatment Panel III criteria for the metabolic syndrome. Diabetes 2004;53:2087-94.
11. Macdonald I, Derby G, and Westerterp K. Alcohol and overweight. Heatlth issues related to alcohol consumption. 263-279. 1993. Brussels, ILSI Europe.
12. Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB, Jr., Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care 2000;23:1630-6.
13. Kiechl S, Willeit J, Poewe W et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). BMJ 1996;313:1040-4.
14. Cordain L, Bryan ED, Melby CL, Smith MJ. Influence of moderate daily wine consumption on body weight regulation and metabolism in healthy free-living males. J.Am.Coll.Nutr. 1997;16:134-9.
15. Cordain L, Melby CL, Hamamoto AE et al. Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. Metabolism 2000;49:1473-8.
16. Beltowski J. Adiponectin and resistin--new hormones of white adipose tissue. Med.Sci.Monit. 2003;9:RA55-RA61.
17. Stolk RP, Wink O, Zelissen PM, Meijer R, van Gils AP, Grobbee DE. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. Int.J.Obes.Relat Metab Disord. 2001;25:1346-51.
18. Whittaker AD, Park B, Thane BR, Miller RK, Savell JW. Principles of ultrasound and measurement of intramuscular fat. J.Anim Sci. 1992;70:942-52.
19. Arita Y, Kihara S, Ouchi N et al. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity.

Biochem.Biophys.Res.Commun. 1999;257:79-83.
20. van den Berg R, Haenen GRMM, Van den Berg H, Bast A. Applicability of an improved Trolox equivalent antioxidant capacity (TEAC) assay for evaluation of antioxidant capacity measurements of mixtures. Food Chemistry 1999;66:511-7.
21. Hertog MG, Hollman PC, Venema DP. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetable and fruits. J Agric Food Chem 1992;40:1591-8.
22. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am.J.Physiol 1979;237:E214-E223.
23. Liu S, Serdula MK, Williamson DF, Mokdad AH, Byers T. A prospective study of alcohol intake and change in body weight among US adults. Am.J.Epidemiol. 1994;140:912-20.
24. Dallongeville J, Marecaux N, Ducimetiere P et al. Influence of alcohol consumption and various beverages on waist girth and waist-to-hip ratio in a sample of French men and women. Int.J.Obes.Relat Metab Disord. 1998;22:1178-83.
25. Han TS, Bijnen FC, Lean ME, Seidell JC. Separate associations of waist and hip circumference with lifestyle factors. Int.J.Epidemiol. 1998;27:422-30.
26. Keenan NL, Strogatz DS, James SA, Ammerman AS, Rice BL. Distribution and correlates of waist-to-hip ratio in black adults: the Pitt County Study. Am.J.Epidemiol. 1992;135:678-84.
27. Koh-Banerjee P, Chu NF, Spiegelman D et al. Prospective study of the association of changes in dietary intake, physical activity, alcohol consumption, and smoking with 9-y gain in waist circumference among 16 587 US men. Am.J.Clin.Nutr. 2003;78:719-27.
28. Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am.J Clin.Nutr. 2005;81:780-6.
29. Shai I, Rimm EB, Schulze MB, Rifai N, Stampfer MJ, Hu FB. Moderate alcohol intake and markers of inflammation and endothelial dysfunction among diabetic men. Diabetologia 2004;47:1760-7.
30. Baratta R, Amato S, Degano C et al. Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. J.Clin.Endocrinol.Metab 2004;89:2665-71.
31. Yang WS, Lee WJ, Funahashi T et al. Weight reduction increases plasma levels of an adipose-derived anti-inflammatory protein, adiponectin. J.Clin.Endocrinol.Metab 2001;86:3815-9.
32. Booyse FM, Aikens ML, Grenett HE. Endothelial cell fibrinolysis: transcriptional regulation of fibrinolytic protein gene expression (t-PA, u-PA, and PAI-1) by low alcohol. Alcohol Clin.Exp.Res. 1999;23:1119-24.
33. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat.Med. 2001;7:941-6.
34. Arky RA, Abramson EA, Freinkel N. Alcohol hypoglycemia. VII. Further studies on the refractoriness of obese subjects. Metabolism 1968;17:977-87.
35. Asensio C, Cettour-Rose P, Theander-Carrillo C, Rohner-Jeanrenaud F, Muzzin P. Changes in glycemia by leptin administration or high- fat feeding in rodent models of obesity/type 2 diabetes suggest a link between resistin expression and control of glucose homeostasis. Endocrinology 2004;145:2206-13.
36. Janke J, Engeli S, Gorzelniak K, Luft FC, Sharma AM. Resistin gene expression in human adipocytes is not related to insulin resistance. Obes.Res. 2002;10:1-5.
37. Lee JH, Chan JL, Yiannakouris N et al. Circulating resistin levels are not associated with obesity or insulin resistance in humans and are not regulated by fasting or leptin administration: cross-sectional and interventional studies in normal, insulin-resistant, and diabetic subjects. J.Clin.Endocrinol.Metab 2003;88:4848-56.
38. Azuma K, Katsukawa F, Oguchi S et al. Correlation between serum resistin level and adiposity in obese individuals. Obes.Res. 2003;11:997-1001.



Chapter 9

The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity; a human intervention study

Joline WJ Beulens

Luc JC van Loon

Frans J Kok

Maurice Pelsers

Thomas Bobbert

Joachim Spranger

Anders Helander

Henk FJ Hendriks

Submitted

ABSTRACT

Objective

To investigate whether moderate alcohol consumption increases plasma high-molecular weight (HMW) adiponectin and/or muscle substrate oxidation.

Methods

Eleven lean (BMI: 18-25 kg/m^2) and eight overweight (BMI≥ 27 kg/m^2) men consumed 100 mL whisky (~32 g alcohol/day) or water daily for four weeks in a randomized controlled crossover trial. After each treatment period muscle biopsies and fasting blood samples were collected.

Results

Adiponectin concentrations increased ($p<0.001$) by 12.5% after four weeks of moderate alcohol consumption. Moderate alcohol consumption tended to increase HMW ($p= 0.07$) by 57% and mean-molecular weight (MMW) adiponectin ($p=0.07$) by 12.5%, but not low-molecular weight (LMW) adiponectin. Skeletal muscle citrate synthase, cytochrome c oxidase and β-Hydroxyacyl-CoA dehydrogenase (β-HAD) activity were not changed after moderate alcohol consumption. Particularly plasma HMW adiponectin correlated positively with activities of skeletal muscle citrate synthase ($r= 0.64$; $p=0.009$), cytochrome c oxidase ($p= 0.59$; $p=0.009$) and β-HAD ($r=0.46$; $p=0.056$), while such correlation was not present for LMW adiponectin. Insulin sensitivity and intramyocellular triglycerides were not affected by moderate alcohol consumption.

Conclusion

Moderate alcohol consumption increases adiponectin concentrations, and in particular HMW adiponectin. Concentrations of particularly HMW adiponectin were positively associated with skeletal muscle oxidative capacity. Four weeks of moderate alcohol consumption, however, did not affect insulin sensitivity and oxidative capacity.

INTRODUCTION

Type 2 diabetes is a major disease burden in developed and developing countries and its prevalence is expected to double the next 20 years (1). Insulin resistance is a key feature of this disease and considered the initial step in the development of type 2 diabetes (2). Dysregulation of muscular fatty acid oxidation and increased intramyocellular triglyderide (IMTG) content may play a significant role in the development of insulin resistance (3). Impaired mitochondrial oxidative phosphorylation is indeed also associated with insulin resistance (4) and type 2 diabetes (5).

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes compared to abstention (6). This association could be explained by improved insulin sensitivity after moderate alcohol consumption (7;8). The underlying mechanism for this improvement is not completely understood, but several pathways can be involved.

Firstly, moderate alcohol consumption increases plasma adiponectin concentrations, which could precede changes of insulin sensitivity (8). Adiponectin improves insulin sensitivity by increasing muscular fatty acid oxidation and/or decreasing IMTG storage (9). A recent study of Civitarese et al. showed that adiponectin may also improve muscle oxidative capacity (10). Plasma adiponectin forms multimers and can be present as a trimer, hexamer or as high-molecular weight (HMW) form (11). As the HMW isoform binds most avidly to its receptor, it was suggested that HMW adiponectin is more relevant to the etiology of insulin resistance than total adiponectin (12).

Secondly, moderate alcohol consumption acutely increases energy expenditure, diet induced thermogenesis, and decreases lipolysis and whole-body lipid oxidation (13;14). As ethanol is preferentially oxidized, these changes are mainly due to hepatic production of acetate, the end-product of ethanol oxidation. This, in turn, is converted to acetyl-CoA in peripheral tissue such as muscle that highly express acetyl-CoA synthetase (15). Over time, such acute changes could cumulatively affect oxidative capacity and thereby affect insulin sensitivity.

Therefore the present study investigates whether moderate alcohol consumption affects plasma adiponectin oligomer concentrations and skeletal muscle oxidative capacity. The relation between the different adiponectin oligomers and skeletal muscle (fat) oxidative capacity was studied as well.

METHODS

Subjects

We recruited twenty healthy, non-smoking men, aged 18 to 40 years from the pool of volunteers of TNO Quality of Life (Zeist, the Netherlands) and by advertisements in local newspapers. Subjects reported their alcohol intake, physical activity, diet, medical history and family history of alcoholism and were considered healthy based on pre-study laboratory tests, their medical history and a physical examination. Eligible subjects complied with the following criteria: alcohol consumption between 10 and 28 units/week, normal Dutch eating habits, less than eight hrs/week of intense exercise and no family history of alcoholism. The subjects were divided in a lean subgroup (BMI 18-25 kg/m2, n=11) and an overweight subgroup (BMI ≥27 kg/m2, n=9) to explore whether BMI modifies the effect of moderate alcohol consumption. Subjects gave written informed consent after the study was carefully explained. University Medical Center Utrecht Medical Ethics Committee approved the research protocol and we conducted the study according to the Declaration of Helsinki (2000) and the International Conference on Harmonisation Guideline for Good Clinical Practice.

Study design

The study was performed according to a randomized, partially diet-controlled, crossover trial, consisting of two four-week periods preceded by one week wash-out. Subjects were randomized based on BMI-group to the sequence whisky (Famous Grouse Scotch Whisky, 40% vol, Perth, Scotland) followed by mineral water (Spa Reine, Spa, Belgium) or the other way around. They consumed two miniature bottles (50 mL each) of whisky or 100 mL mineral water daily with the evening meal, equaling 32 g alcohol/day during whisky treatment. Their diet was fully controlled during the last seven days of each treatment period when all food was supplied by TNO and the subjects were not allowed to eat or drink anything but the foods supplied, except tap water, tea or coffee. We adjusted energy content of the diet to body weight and physical activity level of each participant. The composition of the diet was based on the Dutch Food Consumption Survey of 1998 (16) and consisted of 37 energy% fat, 15 energy% protein, and 48 energy% carbohydrates, excluding energy from alcohol. During the entire study (including wash-out periods)

subjects were not allowed to drink any alcoholic beverages, besides those supplied by TNO. Body weight was measured every visit. Compliance was checked by daily questionnaires, ten measurements of urinary ethyl glucuronide, a direct metabolite of ethanol (17), for each participant, and HDL cholesterol concentrations.

Muscle biopsy collection

Subjects visited TNO on the second last day of each treatment period after an overnight fast for muscle biopsy collection. They were asked not to engage in strenuous physical activity three days before the muscle biopsy. After a 30 min rest, an anesthetic (1% xylocaine) was injected locally in skin, soft tissue below, and in the muscle fascia in the middle region of the vastus lateralis muscle. Thereafter, a small incision (4 mm) was made through the skin and the fascia at ~15 cm above the patella. A Bergström needle was inserted to a depth of ~2–3 cm below the entry of the fascia, and a muscle sample (~60 mg) was obtained by suction.

Oral glucose tolerance test, and blood and urine sampling

On the last day of each treatment period subjects visited TNO after an overnight fast for an oral glucose tolerance test (OGTT) using a 75 g glucose load and blood sampling. Blood was collected from the antecubital vein in tubes containing clot activator (for serum) and in ice-chilled tubes containing ethylenediaminetetraacetic acid (EDTA) for plasma (Vacutainer Systems, Becton Dickinson, Plymouth, UK). The blood was centrifuged for 15 min at 2.000 g at 4°C, within 15 to 30 minutes after collection. The first void of morning urine was collected every five days (five times in both the alcohol and water drinking periods) for determination of urinary ethyl glucuronide. Samples were stored at -80 °C.

Biochemical analyses

Serum glucose was determined by hexokinase method (Roche Reagens, Mannheim, Germany) and serum insulin by immunoenzymomatric assay (Tosho, Japan). Adiponectin concentrations were determined by radioimmunoassay method (Linco Research, Inc., St. Charles, Missouri, USA) and HDL cholesterol homogenous enzymatic colorimetric (Roche reagens, Mannheim, Germany). HbA1C was determined by Turbidimetric Inhibition Immunoassay (Roche Diagnostics, Mannheim, Germany). Ethyl glucuronide in urine was

determined by LC-MS according to Sarkola et al (17). The lower limit of the quantification of the method was 0.5 mg/L.

Activities of citrate synthase, β-3-hydroxyacyl-CoA dehydrogenase (β-HAD) and cytochrome c oxidase were determined using standard procedures (18). Determination of muscle fibre IMTG content was performed by oil red O staining together with immunolabelled cellular constituents as previously described (19). The proportion of type I, IIa and IIx muscle fibres was determined by ATPase staining (20). To assess intramyocellular glycogen content we used the modified PAS stain as recently described (21), allowing direct, fibre-type-specific determination of muscle glycogen content. Muscle fibre-type-specific oxidative capacity was estimated by determining succinate dehydrogenase activity in the muscle cross-sections using histochemical analyses (22).

Adiponectin oligomers were determined by SDS-PAGE performed according to the standard Laemmli's method as previously described by Bobbert et al. (23). Densitometry analysis of adiponectin oligomers was done with AIDA image analysis software. After adjusting for background activity, density of specific adiponectin oligomers bands were measured. Relative distribution of adiponectin oligomers were calculated by dividing band density through total density. Percentage of adiponectin oligomers were multiplied with total adiponectin levels to calculate absolute oligomer values.

Sample size calculation

Sample size was calculated with a power of 80% and accepting a 2-sided α of 5% based on 3-hydroxy fatty-acyl CoA dehydrogenase (β-HAD) and citrate synthase activity (24;25). A sample size of 20 persons would be sufficient to detect a ~15% difference of β-HAD and citrate synthase in this crossover trial.

Statistical analysis

Data were analyzed using the SAS statistical software package (SAS/STAT Version 8, SAS Institute, Cary, NC, USA). Insulin sensitivity (ISI) was calculated based on the OGTT according to Matsuda (26). Treatment effects were assessed by analysis of variance using a mixed model including BMI, treatment order, period, treatment, and the interaction between BMI and treatment. Residuals were tested for normality and homogeneity of variance. Subgroup analysis based on BMI was performed in case of an interaction

between BMI and treatment ($p < 0.15$). Changes were calculated by distracting mean of whisky from mineral water. Correlation coefficients were computed according to Pearson or Spearman to assess associations between changes in outcome measures. Two-sided p-values below 0.05 were considered statistically significant.

RESULTS

Table 1 shows the characteristics of our study population. One overweight men dropped out of the study due to a treatment unrelated reason and 19 men are thus included in the analysis. BMI of lean and obese subgroups differed profoundly, which is reflected in significant differences of total and LDL cholesterol, glucose and insulin concentrations between the two groups. Urinary ethyl glucuronide was assessed ten times during the study for each participant (five times in both the whisky and water drinking periods). One urine sample from the whisky drinking period was negative for ethyl glucuronide and no positive urine samples were observed during the water drinking period, indicating good overall compliance to study treatments. Means ±sd over different days of urine collection ranged from 0 ±0 mg/L to 0.02 ±0.07 mg/L for the water-drinking and from 8.6 ±8.2 mg/L to 17.0 ±19.2 mg/L for the whisky-drinking period. Another indication for good compliance was the 11% increase ($p< 0.001$) of fasting serum HDL cholesterol after whisky as compared to mineral water consumption. On average, subjects slightly lost weight during the water treatment (-0.6 ±0.1 kg), which was different ($p= 0.004$) from the whisky drinking period, in which no weight loss occurred (0.1 ±0.1 kg).

Table 1: Baseline characteristics (mean ±sd) among lean and overweight men.

Variable	Lean	Range	Overweight	Range
N	11		8	
Age (years)	21± 2[1]	18- 24	28 ± 6	21- 36
BMI (kg/m^2)	21.4 ± 2.0[1]	18.3- 25.1	30.1 ± 3.4	27.5- 35.5
Waist circumference (cm)	78 ± 7[1]	69- 90	100 ± 6	92- 108
Glucose (mmol/l)	4.9 ± 0.2[1]	4.6- 5.2	5.4 ± 0.4	4.9- 5.8
Insulin (IU/l)	4.7 ± 1.2[1]	3.6- 7.3	11.0 ± 5.4	3.6- 19.3
HOMA-index	1.1 ± 0.3[1]	0.8- 1.5	2.6 ± 1.3	0.8- 5.0
Total cholesterol (mmol/l)	4.0 ± 0.5[1]	3.1- 4.8	5.1 ± 0.8	4.4- 6.8
HDL cholesterol (mmol/l)	1.2 ± 0.2	1.0- 1.6	1.1 ± 0.2	0.9- 1.4
LDL cholesterol (mmol/l)	2.3 ± 0.5[1]	1.8- 3.3	3.4 ± 0.7	2.4- 4.7
Triacylglycerols (mmol/l)	1.0 ± 0.3	0.5- 1.8	1.3 ± 0.5	0.6- 1.9
Alkaline phosphatase (U/l)	71 ± 11	54- 86	81 ± 14	65- 108
Aspartate aminotransferase (U/l)	23 ± 7	16- 36	24 ± 3	20- 27
Alanine aminotransferase (U/l)	18 ± 5[1]	12- 28	29 ± 11	19- 50
Gamma-glutamyl transpeptidase (U/l)	21 ± 8	12-37	26 ± 7	18- 37

[1] $p< 0.05$ lean compared to overweight group

Insulin sensitivity, adiponectin and enzyme activities

Table 2 shows results on insulin sensitivity, adiponectin and muscle enzyme activities. Insulin sensitivity was not changed after four weeks of moderate alcohol consumption, but adiponectin concentrations increased from 7.9 ±0.2 mg/L after water to 9.0 ±0.2 mg/L after whisky consumption. Moderate alcohol consumption tended to increase HMW by 57% and MMW adiponectin by 12.5%, but not LMW adiponectin. HbA1c concentrations decreased ($p= 0.023$) from 4.9 ±0.02 % after water to 4.8 ±0.02% after whisky consumption.

β-HAD, cytochrome c oxidase and citrate synthase activity were not significantly changed after moderate alcohol consumption. However, a borderline significant interaction between alcohol consumption and BMI was observed for cytochrome c oxidase ($p= 0.072$) and citrate synthase ($p= 0.102$) activity. Among lean men, moderate alcohol consumption tended to increase cytochrome c oxidase and citrate synthase activity by respectively 23 and 26%, while no change among overweight men was observed. Succinate dehydrogenase activity in mixed muscle fibers decreased ($p=0.03$) by 15% after moderate alcohol consumption. Despite a 15 to 20 % difference between both treatments of IMTG content, this did not reach statistical significance. Similarly, glycogen content did not differ between whisky and mineral water treatment.

Table 2: Mean ±SEM insulin sensitivity index, HbA1c, adiponectin oligmer concentrations and enzyme activities after 4 weeks consumption of whisky or mineral water among 19 lean or overweight men.

	Mineral water	Whisky	% Change	P-value
Insulin sensitivity index[1]	10.6 ± 1.3	9.6 ± 1.3	- 9.4	0.61
Adiponectin (mg/l)	7.9 ± 0.2	9.0 ± 0.2	12.5	0.0008
High-molecular weight (mg/l)	0.7 ± 0.1	1.1 ± 0.1	57.1	0.074
Mean-molecular weight (mg/l)	4.0 ± 0.2	4.5 ± 0.2	12.5	0.065
Low-molecular weight (mg/l)	3.2 ± 0.3	3.5 ± 0.3	9.4	0.442
HbA1c (%)	4.9 ± 0.02	4.8 ± 0.02	-2.0	0.023
Muscle 3-hydroxy fatty acyl CoA dehydrogenase (mol/µg protein)	4.22 ± 0.48	4.37 ± 0.49	3.6	0.827
Muscle citrate synthase (mol/µg protein)	0.66 ± 0.05	0.75 ± 0.05	13.6	0.262
Muscle cytochrome C oxidase (mol/µg protein)	1.48 ± 0.12	1.67 ± 0.12	12.8	0.262
Intramyocellular triglycerides[2]				
Type 1 (A.U.)	0.027 ± 0.004	0.022 ± 0.004	-18.5	0.339
Type 2 (A.U.)	0.010 ± 0.002	0.008 ± 0.002	-20.0	0.489
Mixed (A.U.)	0.019 ± 0.003	0.016 ± 0.002	-15.8	0.429
Succinate dehydrogenase activity[2]				
Type 1 (A.U.)	0.084 ± 0.005	0.075 ± 0.004	-10.7	0.122
Type 2 (A.U.)	0.057 ± 0.003	0.052 ± 0.003	-8.8	0.221
Mixed (A.U.)	0.073 ± 0.003	0.062 ± 0.003	-15.1	0.028
Glycogen[2]				
Type 1 (A.U.)	0.048 ± 0.006	0.044 ± 0.006	-8.3	0.587
Type 2 (A.U.)	0.056 ± 0.006	0.052 ± 0.006	-7.1	0.655
Mixed (A.U.)	0.052 ± 0.006	0.048 ± 0.006	-7.7	0.661

[1]Insulin sensitivity according to Matsuda & DeFronzo (1999)

[2]A.U.: arbitrary units

BMI effects

Table 3 shows differences between lean and overweight men. Insulin sensitivity was significantly higher among lean than overweight subjects. Adiponectin concentrations were not different between lean and overweight men, nor were concentrations of HMW, MMW and LMW adiponectin. Both citrate synthase and cytochrome c oxidase activity were lower among overweight than lean men, while intramyocellular lipid content tended to be higher among overweight than lean men.

Table 3: Mean ±SEM insulin sensitivity index, HbA1c, adiponectin oligmer concentrations and enzyme activities after 4 weeks consumption of whisky or mineral water among 19 lean or overweight men.

	Lean group	Overweight group	% difference	P-value
Insulin sensitivity index[1]	13.1 ± 1.2	6.2 ± 1.5	-52.7	0.007
Adiponectin (mg/l)	8.8 ± 0.2	8.1 ± 0.2	-8.0	0.699
High-molecular weight (mg/l)	1.0 ± 0.1	0.8 ± 0.1	-20.0	0.569
Mean-molecular weight (mg/l)	4.5 ± 0.2	4.0 ± 0.2	-11.1	0.552
Low-molecular weight (mg/l)	3.3 ± 0.2	3.3 ± 0.3	0	0.998
HbA1c (%)	4.9 ± 0.02	4.9 ± 0.02	0	0.643
Muscle 3-hydroxy fatty acyl CoA dehydrogenase (mol/µg protein)	4.94 ± 0.45	3.65 ± 0.53	-26.1	0.177
Muscle citrate Synthase (mol/µg protein)	0.83 ± 0.05	0.58 ± 0.05	-30.1	0.043
Muscle cytochrome C oxidase (mol/µg protein)	1.92 ± 0.11	1.23 ± 0.13	-35.9	0.075
Intramyocellular triglycerides[2]				
Type 1 (A.U.)	0.019 ± 0.004	0.030 ± 0.004	57.9	0.092
Type 2 (A.U.)	0.007 ± 0.002	0.010 ± 0.002	42.9	0.219
Mixed (A.U.)	0.013 ± 0.002	0.022 ± 0.002	69.2	0.089
Succinate dehyrogenase activity[2]				
Type 1 (A.U.)	0.083 ± 0.004	0.076 ± 0.004	-8.4	0.405
Type 2 (A.U.)	0.058 ± 0.003	0.051 ± 0.004	-12.1	0.342
Mixed (A.U.)	0.071 ± 0.003	0.063 ± 0.004	-11.3	0.268
Glycogen[2]				
Type 1 (A.U.)	0.052 ± 0.005	0.039 ± 0.007	-25.0	0.222
Type 2 (A.U.)	0.061 ± 0.005	0.045 ± 0.007	-26.2	0.177
Mixed (A.U.)	0.055 ± 0.005	0.043 ± 0.007	-21.8	0.238

[1]Insulin sensitivity according to Matsuda & DeFronzo (1999)

[2]A.U.: arbitrary units

Correlations

After whisky consumption, HMW correlated positively with activities of citrate synthase (r= 0.64, p=0.004), cytochrome c oxidase (r=0.59, p=0.009) and β-HAD (r= 0.46; p=0.056). MMW adiponectin tended to correlate with activities of citrate synthase (r=0.44, p=0.07), cytochrome c oxidase (r=0.32, p=0.20) and β-HAD (r=0.44; p=0.07), while no such relations were present for LMW adiponectin. A similar pattern was observed for the

correlations of adiponectin oligomers and HDL cholesterol (HWM: r=0.55, p=0.014; MMW: r=0.55, p=0.014; LMW: r=0.21, p=0.38). Insulin sensitivity correlated modestly with all oligomers of adiponectin after whisky consumption ($r \approx 0.44$; $p<0.05$). Differences of ISI correlated inversely with differences in type 1 (r= -0.63; p=0.006), type 2 (r= -0.63; p= 0.007) and mixed muscle fibers IMTG content (r= -0.66; p= 0.004), but not with adiponectin or its oligomers.

DISCUSSION

This study showed that moderate alcohol consumption increases adiponectin concentrations, with a particular increase of HMW adiponectin concentrations. However, the latter was not accompanied by changes of whole-body insulin sensitivity or muscle oxidative capacity. Concentrations of HMW and MMW adiponectin correlated positively with markers of skeletal muscle oxidative capacity.

Strengths of this study are its randomized, controlled, partially diet-controlled cross-over design. In addition, we assessed compliance to study treatments several times throughout the study and observed no significant deviations. It therefore seems unlikely that our results are confounded by diet or lifestyle. Our study was, however, limited by slightly small sample size for certain contrasts such as analyses in subgroups of lean and overweight men or of IMTG content, which needs to be taken into account when interpreting these results.

This study showed that moderate alcohol consumption increases adiponectin concentrations, consistent with previous reports from our group (8) and observational studies (27). In addition, we now observed that this alcohol-induced increase of adiponectin may be oligomer specific. Moderate alcohol consumption particularly increased HMW adiponectin, MMW adiponectin to a lesser extent while LMW adiponectin remained unchanged. These results are consistent with the study of Bobbert et al. showing a similar pattern of changes in adiponectin oligomers after a relatively mild life-style intervention of moderate weight reduction (23). Studies with thiazolidinedione or more rigorous weight reduction show qualitatively similar, but more pronounced results (28). Consistent with Pajvani et al., we observed modest correlations between adiponectin oligomers and insulin sensitivity. HDL cholesterol concentrations correlated particularly with HMW adiponectin as previously observed by Bobbert et al. (23). In addition, we now show that HMW and MMW adiponectin are correlated with markers of skeletal muscle oxidative capacity (e.g. β-HAD, citrate synthase, cytochrome c oxidase). This is in line with reports of increased fat oxidation after adiponectin infusion (9) and a recent study of Civitarese et al. showing that adiponectin increases skeletal muscle oxidative capacity (10). Our findings confirm their results and show that these relations may be specific to HMW adiponectin. Altogether, these results may provide an underlying mechanism for the proposed importance of HMW

adiponectin in the etiology of insulin resistance (12).

We did not observe that the alcohol-induced increase of adiponectin and specifically HMW adiponectin significantly changed muscle oxidative capacity, IMTG content and insulin sensitivity. Our results on insulin sensitivity are inconsistent with a previous controlled trial of Davies et al. (7) and observational studies (29). This disparity may relate to the duration of our intervention. Because changes of adiponectin and HMW adiponectin could precede changes of insulin sensitivity, four weeks may have been too short to detect effects on insulin sensitivity.

We hypothesized that changes of insulin sensitivity would be mediated by a decrease of IMTG content and increased muscle oxidative capacity, but we could not demonstrate this. Apart from human studies investigating acute metabolic effects of moderate alcohol consumption (13;14), this is to our knowledge the first human study investigating the chronic effect on muscle oxidative capacity. Several animal experiments showed inconsistent results of the chronic effect of ethanol on oxidative capacity in either liver, muscle or other tissue (30), but these studies used high doses of ethanol and are therefore not comparable to our study of moderate consumption.

However, as some subtle differences were present, we cannot completely rule out the hypothesis of changes of IMTG content and/or oxidative capacity. Although the difference in IMTG content was not significant, we did observe a 15 to 20% lower IMTG content after whisky than water consumption. This difference is of similar magnitude as previous observations after a weight loss and physical activity intervention (31). It could therefore physiologically be relevant. However, apart from IMTG content other metabolic changes in muscle such as oxidative capacity are functionally related to insulin sensitivity (32). Our results on oxidative capacity were somewhat disparate. Moderate alcohol consumption tended to increase skeletal muscle citrate synthase activity among lean men, while succinate dehydrogenase activity declined after moderate alcohol consumption in mixed muscle fibers. This disparity as measured by citrate synthase and succinate dehydrogenase could simply be due to the measurement of ex vivo oxidative capacity instead of in vivo activity or due to chance. Alternatively, moderate alcohol consumption could differentially affect capacity of enzymes in different oxidative pathways such as the citric acid or glyoxylate cycle (33). However, more research is warranted to address these questions.

In conclusion, this study showed that moderate alcohol consumption increases adiponectin concentrations and in particular HMW adiponectin concentrations. Four weeks moderate alcohol consumption did not affect whole-body insulin sensitivity and muscle oxidative capacity. Concentrations of HMW and MMW adiponectin are positively associated with skeletal muscle oxidative capacity. Possibly changes of HMW adiponectin concentrations may affect insulin sensitivity after longer time periods.

Acknowledgements

The skillful help of Rene Koopman, Martijn Brouwers and Anna Georgieva is gratefully acknowledged. We thank the volunteers for their enthusiastic participation. The research described in this paper was funded by the Dutch Foundation for Alcohol Research.

REFERENCES

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-53.
2. Nijpels G. Determinants for the progression from impaired glucose tolerance to non-insulin-dependent diabetes mellitus. Eur.J.Clin.Invest 1998;28 Suppl 2:8-13.
3. Petersen KF, Shulman GI. Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. Am.J.Cardiol. 2002;90:11G-8G.
4. Pitteloud N, Mootha VK, Dwyer AA et al. Relationship between testosterone levels, insulin sensitivity, and mitochondrial function in men. Diabetes Care 2005;28:1636-42.
5. Mootha VK, Lindgren CM, Eriksson KF et al. PGC-1alpha-responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. Nat.Genet. 2003;34:267-73.
6. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
7. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.
8. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
9. Yamauchi T, Kamon J, Waki H et al. The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat.Med. 2001;7:941-6.
10. Civitarese AE, Ukropcova B, Carling S et al. Role of adiponectin in human skeletal muscle bioenergetics. Cell Metab 2006;4:75-87.
11. Waki H, Yamauchi T, Kamon J et al. Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. J.Biol.Chem. 2003;278:40352-63.
12. Hara K, Horikoshi M, Yamauchi T et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.
13. Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. Am.J.Clin.Nutr. 2003;77:91-100.
14. Siler SQ, Neese RA, Hellerstein MK. De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. Am.J.Clin.Nutr. 1999;70:928-36.
15. Antoshechkin AG. Physiological model of the stimulative effects of alcohol in low-to-moderate doses. Ann.N.Y.Acad.Sci. 2002;957:288-91.
16. Dutch Food Composition Table 2001. 2001. Den Haag.
17. Sarkola T, Dahl H, Eriksson CJ, Helander A. Urinary ethyl glucuronide and 5-hydroxytryptophol levels during repeated ethanol ingestion in healthy human subjects. Alcohol Alcohol 2003;38:347-51.
18. Lowry OH, Passonneau JV. A Flexible System of Enzymatic Analysis. New York: Academic, 1972.
19. Koopman R, Schaart G, Hesselink MK. Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids. Histochem.Cell Biol. 2001;116:63-8.
20. Mabuchi K, Sreter FA. Actomyosin ATPase. II. Fiber typing by histochemical ATPase reaction. Muscle Nerve 1980;3:233-9.

21. Schaart G, Hesselink RP, Keizer HA, van KG, Drost MR, Hesselink MK. A modified PAS stain combined with immunofluorescence for quantitative analyses of glycogen in muscle sections. Histochem.Cell Biol. 2004;122:161-9.
22. Gosker HR, van MH, van Dijk PJ et al. Skeletal muscle fibre-type shifting and metabolic profile in patients with chronic obstructive pulmonary disease. Eur.Respir.J. 2002;19:617-25.
23. Bobbert T, Rochlitz H, Wegewitz U et al. Changes of adiponectin oligomer composition by moderate weight reduction. Diabetes 2005;54:2712-9.
24. Bruce CR, Anderson MJ, Carey AL et al. Muscle oxidative capacity is a better predictor of insulin sensitivity than lipid status. J.Clin.Endocrinol.Metab 2003;88:5444-51.
25. Kempen KP, Saris WH, Kuipers H, Glatz JF, van d, V. Skeletal muscle metabolic characteristics before and after energy restriction in human obesity: fibre type, enzymatic beta-oxidative capacity and fatty acid-binding protein content. Eur.J.Clin.Invest 1998;28:1030-7.
26. Matsuda M, DeFronzo RA. Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999;22:1462-70.
27. Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am.J Clin.Nutr. 2005;81:780-6.
28. Pajvani UB, Hawkins M, Combs TP et al. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. J.Biol.Chem. 2004;279:12152-62.
29. Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB, Jr., Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care 2000;23:1630-6.
30. Marcinkeviciute A, Mildaziene V, Crumm S, Demin O, Hoek JB, Kholodenko B. Kinetics and control of oxidative phosphorylation in rat liver mitochondria after chronic ethanol feeding. Biochem.J. 2000;349:519-26.
31. He J, Goodpaster BH, Kelley DE. Effects of weight loss and physical activity on muscle lipid content and droplet size. Obes.Res. 2004;12:761-9.
32. van Loon LJ, Goodpaster BH. Increased intramuscular lipid storage in the insulin-resistant and endurance-trained state. Pflugers Arch. 2006;451:606-16.
33. Kokavec A, Crowe SF. Alcohol consumption in the absence of adequate nutrition may lead to activation of the glyoxylate cycle in man. Med.Hypotheses 2002;58:411-5.

Chapter 10

General discussion

This thesis described population-based studies investigating the association between alcohol consumption and risk of cardiovascular disease (CVD) and type 2 diabetes (DM2) in populations at increased risk of those diseases. In addition, the influence of genetic variation in alcohol dehydrogenase (*ADH1C*) on and the underlying physiological mechanism for those associations was investigated. This chapter summarizes these findings and discusses their relevance based on their internal and external validity. General conclusions, public health implications and recommendations for future research are based on this discussion.

MAIN FINDINGS

The main findings of the studies described in this thesis are summarized in table 1.

Cardiovascular disease

Similar to studies in the general population, we observed that moderate alcohol consumption is associated with a decreased risk of CHD among hypertensive men. Alcohol consumption was not associated with total or cardiovascular mortality, while an inverse association was found for CHD mortality in this group of hypertensive men. The associations of total and ischemic stroke with moderate alcohol consumption were indeterminate due to few cases of stroke.

Two randomized cross-over trials were conducted to investigate functional consequences of increased HDL cholesterol concentrations. The first showed that moderate alcohol consumption increased cholesterol efflux capacity from J774 macrophages by 17.5%, which may be mediated by ABCA1. Cholesterol efflux capacity from Fu5AH cells, highly expressing SR-BI, increased by only 4.6%. Lp-PLA2 activity remained unchanged after moderate alcohol consumption in a second trial.

Type 2 diabetes

Similar to studies in male populations, we showed that moderate alcohol consumption is associated with a decreased risk of DM2 among older women. This association was independent from beverage type. In two nested case-control studies, however, we showed that genetic variation in alcohol dehydrogenase may modify this association. Among moderate drinkers, the ADH1C*2 allele, conferring slower ethanol oxidation, is dose-dependently associated with an increased risk of DM2.

Table 1: Summary of the main findings of the studies described in this thesis

Study type	Population	Exposure	Outcome	Outcome measure	Estimate (95%-CI)[1]	Chapter
Cardiovascular disease						
Cohort-study	Hypertensive men	Alcohol: 15.0- 29.9 g/day	Myocardial Infarction	Hazard Ratio	0.72 (0.54; 0.97)	2
Randomized crossover trial	Middle-aged men	Alcohol: 40 g/day	Cholesterol efflux	Mean difference (%)	17.5 (2.3; 34.1)	3
Randomized crossover trial	Lean and overweight, young men	Alcohol: 40 g/day	Lp-PLA2 activity	Mean difference (%)	-2.9 (-7.9; 2.0)	4
Type 2 diabetes						
Cohort-study	Middle-aged women	Alcohol: 20.0- 29.9 g/day	Incident type 2 diabetes	Hazard ratio	0.61 (0.41- 0.92)	5
Case-control	Moderate drinking women and men	ADH1C*1*2	Incident type 2 diabetes	Odds ratio	1.63 (1.02-2.61)	6
		ADH1C*2*2	Incident type 2 diabetes	Odds ratio	2.38 (1.08-5.22)	6
Randomized crossover trial	Lean and overweight, young men	Alcohol: 40 g/day	Insulin sensitivity	Mean difference (%)	-2.1 (-9.2; 5.1)	7
			Adiponectin	Mean difference (%)	10.2 (5.1; 15.3)	7
Randomized crossover trial	Men with increased waist circumference	Alcohol: 40 g/day	Abdominal fat	Mean difference (%)	1.0 (-3.8; 5.8)	8
			Subcutaneous fat	Mean difference (%)	-4.2 (-10.1; 1.6)	8
			Adiponectin	Mean difference (%)	9.7 (4.5; 14.9)	8
			Insulin sensitivity	Mean difference (%)	-7.0 (-18.8; 13.3)	8
Randomized crossover trial	Lean and overweight, young men	Alcohol: 32 g/day	Adiponectin	Mean difference (%)	12.5 (6.5; 20.5)	9
			HMW adiponectin	Mean difference (%)	57.1 (-4.7; 89.6)	9
			Oxidative capacity	Mean difference (%)	13.6 (-10.3; 35.2)	9
			Insulin sensitivity	Mean difference (%)	-10.0 (-48.8; 28.7)	9

[1] Alcohol consumption versus non-alcoholic control condition

In order to explore the underlying mechanism for these associations, randomized controlled trials were performed. These consistently showed an increase of adiponectin concentrations after moderate alcohol consumption, which may be correlated with changes of insulin sensitivity. A subsequent trial showed that this increase of adiponectin occurred independently from alcohol-induced changes of body weight or body fat stores. In addition, we showed that moderate alcohol consumption may particularly increase high-molecular weight adiponectin. Unfortunately we could not replicate the previously observed improvements of insulin sensitivity after moderate alcohol consumption. Therefore we aimed to investigate changes of (fat) oxidative capacity that could precede changes of insulin sensitivity, but could not demonstrate consistent significant improvements of oxidative capacity.

In conclusion, our findings on CVD and population-based studies of DM2 are very consistent with previous knowledge and our a priori hypotheses on the association with alcohol consumption and its underlying mechanism. We showed that the association between alcohol consumption and DM2 may be causal and explained by increased adiponectin concentrations. However, we could not confirm improvements of insulin sensitivity after moderate alcohol consumption.

METHODOLOGICAL CONSIDERATIONS

This thesis relied on observational studies and randomized controlled trials, each subject to certain limitations. Although these have been described in the discussions of chapters two to nine, several critical aspects will be commented upon here to validate our findings.

Observational studies

Selection bias

A potential source of bias of any observational study could result from differences in study participation depending on certain characteristics. For our studies on alcohol consumption self-selection bias may have occurred, as heavier drinkers could be less likely to participate in a study than abstainers or moderate drinkers. Both cohort studies described in this thesis are conducted in specific study populations of US health professionals and Dutch women undergoing a population-based program of breast cancer screening.

We compared the prevalence of heavy drinking (i.e. more than five drinks/day for men and two drinks/day for Dutch women) with population statistics of the source population (1;2). The 4% prevalence of heavier drinking in the Health Professionals Follow-up Study and 8% prevalence among Prospect-women is only slightly lower than ~6% of US male college graduates aged >35 years and ~10% among Dutch women aged >50 years. However, comparing to the prevalence in the overall US male population (~9%) and Dutch females (~11%) differences are larger. Thus self-selection bias within the cohorts is limited and these studies provide valid risk estimates for the moderate range of consumption. They, however, may have limited generalizability for heavier drinking to the general population.

In chapter two and six a sub-population of an entire cohort was selected that may be subject to selection bias. In our study among hypertensive men, selection on this characteristic inevitably leads to certain differences with the source population and cannot be regarded selection bias. The nested case-control studies in chapter six only included those providing blood samples. Several key characteristics of those providing blood samples were compared to the entire cohort with no significant differences observed.

Information bias

A second potential source of bias in any cohort study can occur from errors in

measurement of study information (i.e. information bias), leading to either differential or non-differential misclassification. A specific source of differential misclassification is recall bias. However, given the prospective designs of our studies, it is very unlikely that cases recalled certain health behaviors differentially from non-cases.

In addition, observational studies on alcohol consumption can be subject to certain specific biases. This concerns 'sick-quitters' and was raised first by Shaper et al. (3). They observed an inverse association between alcohol consumption and CVD mortality, no longer present in a sub-cohort initially free from heart disease and related disorders. This suggests that the inclusion of 'sick-quitters' among non-drinkers accounts for the high incidence of disease among non-drinkers compared to moderate drinkers. Therefore in our study on alcohol consumption and risk of DM2, we used a reference category of lifetime abstainers excluding potential bias from 'sick-quitters'. In our study on coronary heart disease among hypertensive men a sensitivity analysis excluding abstainers that reported having decreased their alcohol consumption was performed with consistent results, making it unlikely that our results are biased by sick-quitters.

Apart from these sources of differential misclassification, non-differential misclassification may have occurred in our studies. Misclassification in exposure or outcome would have influenced our results to greatest extent and are therefore specifically discussed here.

In our observational studies, alcohol consumption was assessed by a food frequency questionnaire (FFQ). Despite obvious limitations, the food frequency questionnaire is accepted as a standard tool for dietary assessment in large studies on diet and chronic disease. However, recent studies showing poor comparability of FFQ with doubly labeled water and inconsistencies of associations with biomarkers or food records, have raised considerable questions regarding the use of FFQ's (4). Most FFQ's, as those used in our studies (5-8), are validated against food records or 24-hour recalls showing only modest correlations for most nutrients ranging from 0.4 to 0.6. For alcohol consumption, however, agreement between methods often is considerably higher with correlation coefficients of ~0.9 for the questionnaires used in our studies. This suggests that for alcohol intake a FFQ may be a reasonably valid method for ranking subjects according to their intake. These high correlation coefficients could also reflect very large variability in alcohol intake compared to other nutrients. However, given the conscious decision to consume alcohol, it

is likely that recall of alcohol consumption may be better compared to other nutrients. Feunekes et al. reviewed the validity of alcohol intake assessment using FFQ's and indeed concluded that FFQ's are valid to rank subjects according to their alcohol intake, although alcohol intake usually was underreported. This underreporting was, however, less for methods that separately addressed specific alcoholic beverages, as the FFQ's used in our studies. In addition, they showed that underreporting was proportional, i.e. independent of amount of alcohol intake.

Despite this, we cannot exclude misclassification of alcohol consumption in our studies. Therefore a sensitivity analysis was performed for non-differential misclassification of alcohol intake based on 3000 simulation iterations using probabilistic models (9;10). As expected from correction of non-differential misclassification, this showed a lower median relative risk of 0.56 as observed in the original study (0.72), but a wider 95% simulation interval from 0.08 to 0.75, reflecting decreased precision. It is therefore unlikely that such non-differential misclassification would affect our results.

Finally, assessment of outcome (CVD and DM2) was based on self-reports of physician diagnosed disease. These self-reports were further confirmed in two studies, making it unlikely for misclassification to occur to a large extent. Unfortunately, we were not able to validate self-reports of DM2 in chapter five. However, restricting our analyses to clinical cases of DM2 (i.e. using anti-diabetic medication or insulin) showed similar results.

Confounding

As in any observational study, our results could be influenced, at least in part, by differences in factors other than alcohol consumption that are associated with both exposure and disease, i.e. confounding. We therefore adjusted our risk estimates for a number of important CVD or DM2 risk factors (age, BMI, smoking, family history of CVD and DM2, education) and nutritional factors (e.g. energy intake, saturated or trans fatty acids). In addition, several sensitivity analyses were performed excluding particular sources of confounding such as smokers or heavier drinkers or adjusting for waist circumference. Nevertheless, we cannot rule out residual confounding. Therefore, a sensitivity analysis for an unmeasured confounder using 4000 iterations based on probabilistic models was performed (9;10). This showed that even a confounder of a similar magnitude as smoking could not completely account for the association between alcohol consumption and risk of

CVD. Although residual confounding may attenuate the observed association, it is unlikely that a confounder of this magnitude would have so far remained undetected. For DM2, we observed an association with alcohol consumption of a similar magnitude as for CVD. This sensitivity analysis could therefore also hold true for risk of DM2.

Intervention studies

Study design

Our intervention studies were performed according to a randomized, controlled cross-over design, either with or without partial diet control. This design allows within subject comparison of treatments and (statistical) control for period or carry-over effects. As alcohol intake has obvious behavioral effects, the intervention studies were unblinded and could be subject to placebo effects. Our outcome measurements, however, were assessments of biomarkers of disease in blood, urine or muscle tissue that are unlikely to be affected by placebo effects. In theory, knowledge of the study treatment could affect other health behaviors of our participants that could influence our results. However, as all participants received both intervention and control treatment, this is not likely to occur. In addition, participants were instructed not to change their usual lifestyle and daily questionnaires did not reveal any significant deviation from those instructions. A strength of our trials is its conduct according to Good Clinical Practice, providing quality assurance for the results.

Treatment

We used habitual alcoholic beverages and their most comparable control beverage as treatment in order to mimic habitual alcohol consumption. Despite efforts to select comparable study treatments, slight differences in other components than ethanol could be present. Both population-based studies and a randomized controlled intervention comparing beer, wine and liquor with water showed that effects of alcohol consumption on CVD, DM2 or mediating markers such as HDL cholesterol were independent from beverage type and due to ethanol itself. Although we cannot exclude that certain components (B vitamins in beer or antioxidants in wine) may affect specific markers such as homocystein or anti-oxidative capacity, the outcome measures used in our studies are unlikely to be affected by such components. Therefore choice of beverages will not have influenced our results to a large extent.

Dose of alcohol used in the studies range from 30 to 40 gram alcohol per day, equaling three to four units. This dose of alcohol is based on meta-analyses on the association between alcohol consumption and risk of CVD or DM2 (11;12) and chosen to be at the nadir of these associations. Admittedly, the dose used is relatively high when considering a public health message for alcohol consumption. However, the trials aim to provide an underlying mechanism for the observed associations and are not meant to directly translate in a public health message. Because most interventions with alcohol are limited to relatively short treatment period, a dose at or just above the upper limit for moderate consumption was chosen to ensure sufficient contrast between treatments to observe relevant differences.

In any intervention study compliance to study treatments is key to obtain valid results. In addition, our participants should also refrain from using any other alcoholic beverages apart from study treatments. Compliance was checked daily without any significant deviations. Increases of HDL cholesterol concentrations also provide indication of good compliance in our studies. Strength of two studies was the measurement of ethyl glucuronide, a breakdown product of alcohol intake present in urine up to 36 hours after consumption (13), confirming that participants complied well with study treatments.

Subjects and sample size

Participants in our intervention studies were males, either middle-aged or younger and ranging from very lean to obese. For ethical reasons every study only included moderate drinkers and in case of a family history of alcoholism, subjects were excluded from the study. This may limit generalizability of our results to women or abstainers.

As in any intervention study, the number of subjects included in the study is crucial for statistical power to detect relevant differences. Sample size calculations for our studies were mainly based on previous interventions with moderate alcohol consumption performed in the same research unit. For most studies we assumed a 15% difference to be relevant and achievable with our intervention. This choice is based on previous trials with alcohol consumption, showing differences of this magnitude. A sample size of 20, however, is sufficient to detect smaller differences (~10%) for markers like HDL cholesterol or adiponectin, while for certain markers in muscle like IMTG a sample size of 20 may not be sufficient to detect these changes. Therefore one study may have been slightly

underpowered for certain contrasts. As we were unable to detect significant differences on insulin sensitivity, one may argue that studies were underpowered for this assessment. For insulin sensitivity, a sample size 20 was sufficient to detect a 15% difference based on a previous study within the same research unit. In most studies we observed small differences of insulin sensitivity (ca. 3-8%) making it unlikely that our results are caused by small sample size. Based on results of this thesis and a previous study (14) it now seems more realistic to achieve a 10% increase of insulin sensitivity with moderate alcohol consumption, obviously requiring slightly larger samples (~ 25-30).

Measures

The trials described in this thesis included several different outcome measurements, most importantly biological markers of disease in serum or plasma, insulin sensitivity and muscle enzyme activities. Biological markers of disease were determined using previously validated assays. Intra-assay coefficients of variation were 1.8% for adiponectin and 6.4% for Lp-PLA2 activity, and inter-assay coefficients of variation were 6.2% for adiponectin and 2.2% for Lp-PLA2 activity. The cholesterol efflux assay was performed under previously validated conditions and standardized to pooled serum as reference (15). Because we used whole serum in our study, individual variation in lipoprotein composition determines the capacity of cholesterol efflux. This should be considered when interpreting these results.

Insulin sensitivity was assessed either by the hyperinsulinemic, euglycemic clamp technique or an oral glucose tolerance test. The clamp-technique is regarded as the golden standard for measuring insulin sensitivity (16). The oral glucose tolerance test is the most commonly used test to assess insulin sensitivity, but may be less appropriate to use in small scale trials as ours (16). We chose to use the oral glucose tolerance test in a first explorative study and in case insulin sensitivity was not a primary outcome of the study. However, both using the clamp technique or the oral glucose tolerance test we observed similar results. Therefore it seems unlikely that the method used to assess insulin sensitivity has affected our results to a large extent.

Finally, we assessed activities of oxidative capacity of enzymes involved in fat or carbohydrate metabolism according to previously described methods. These assays are all performed under optimal conditions for enzyme activity and therefore assessed maximal capacity of these enzymes. This may differ from actual physiological activity, which needs to be taken into account when interpreting these results.

EXTERNAL VALIDITY AND CONCLUSIONS

Cardiovascular disease

The association between alcohol consumption and CVD and its underlying mechanism are well established in the general population. We have now shown that moderate alcohol consumption is also inversely associated with myocardial infarction among hypertensive men. In contrast to the general population (11) and previous studies among hypertensive patients (17;18), we did not find significant associations with cardiovascular and total mortality. This may be due to background cardiovascular risk of the populations. Hypertensive patients are at increased risk of particularly stroke compared with the general population (19). As cardiovascular mortality is a composite endpoint of CHD and other CVD such as stroke, in this population it may include relatively more other outcomes such as stroke. Stroke is less strongly associated with alcohol consumption (20), which may explain the non-significant association with cardiovascular mortality in this population of hypertensive men.

The seemingly contradictory associations for MI and cardiovascular mortality may also reflect the underlying biological mechanism of the associations. In line with MI we also observed a significant inverse association of alcohol consumption with CHD mortality. Furthermore, the associations with CHD were independent from anti-hypertensive medication (as a proxy for severity of hypertension) and similar to the association observed among non-hypertensive men (21). This suggests that the association between alcohol consumption and CHD is not mediated by blood pressure. Altogether these results are in line with the described effects of moderate alcohol consumption on the atherosclerotic process by increasing HDL cholesterol and fibrinolysis.

Results from our trials are also in line with this notion, but show that moderate alcohol consumption may also improve functional properties of HDL cholesterol such as cholesterol efflux. Consistent with previous studies using the SR-BI receptor (22;23), we showed that this increased cholesterol efflux may be mediated by ABCA1, a receptor of particular importance in the atherosclerotic process. Furthermore, the observed effect size (+17.5%) suggests that ABCA1 may be a more relevant mechanism for the protective effects of moderate alcohol consumption than SR-BI (+4.6%). A more recent study, however, comparing the contribution of both SR-BI and ABCA1 to cholesterol efflux in one cell

system showed that a large proportion of cholesterol efflux was not accounted for by SR-BI nor ABCA1 (24). This study questions the relevance of the ABCA1 transporter for the effects of moderate alcohol consumption. Possibly other transporters such as the recently discovered ABCG1 transporter, also expressed in J774 macrophages (24), or another yet unidentified receptor may be more important to (alcohol-induced) cholesterol efflux.

In contrast to increased paraoxonase activity (25), we could not demonstrate that moderate alcohol consumption decreased Lp-PLA2 activity, despite profound effects on lipoproteins (18% increase of HDL cholesterol and 8% decrease of LDL cholesterol concentrations). First, this could be explained by the fact that Lp-PLA2 is mainly associated with LDL cholesterol, while paraoxonase is associated with HDL cholesterol (26). Because moderate alcohol consumption particularly affects HDL cholesterol concentrations, increased paraoxonase activity may be more important in the protective effects of moderate alcohol consumption than Lp-PLA2 activity. Second, a different biological role of paraoxonase and Lp-PLA2 may explain these differences. Initially, Lp-PLA2 was thought to have an atheroprotective effect attributable to the degradation of platelet activating factor similar to other molecules like paraoxonase (26). Recent studies suggest a proatherogenic role for Lp-PLA2, attributed to hydrolysis of oxidatively modified LDL that results in the accumulation of proinflammatory products (26) and Lp-PLA2 is therefore proposed a an inflammatory marker. Possibly the inflammatory pathway is less relevant for the cardioprotective of moderate alcohol consumption, as suggested by recent studies (27;28).

Type 2 diabetes

For DM2, the inverse association between alcohol consumption and DM2 was mainly described for male populations and younger women. The evidence was less substantive than for CVD, but pointed towards a causal association. However, particularly a plausible mechanism needed further investigation. The research described in this thesis has further substantiated this association in several ways.

First, we showed a 20 to 30% risk reduction for DM2 among older women as previously shown in men and younger women. The U-shaped association for lifetime alcohol consumption is in line with the meta-analysis of Koppes et al. (12). These results add to the consistency of the association between alcohol consumption and risk of DM2.

Second, this inverse association between alcohol consumption and risk of DM2 was

modified by a common polymorphism in alcohol dehydrogenase. According to the theory of 'Mendelian' randomization, this indicates that the association between alcohol consumption and DM2 may indeed be causal (29). However, in contrast with CVD (30), carrying of the ADH1C*2 allele conferring slower metabolism was dose-dependently associated with an increased risk of DM2 among moderate drinkers. This suggests different mechanisms underlying the associations with moderate drinking. For CVD the benefit from moderate alcohol consumption may be mediated by ethanol itself. Because slower ethanol oxidation could result in decreased concentrations of downstream metabolites such as acetate (31;32), our results indicate that these could mediate the association between alcohol consumption and DM2. The strongest data relating ethanol metabolites to DM2 risk perhaps involve acetate and its effects on peripheral tissue. Acetaldehyde is the main toxic metabolite of ethanol oxidation (33). However, because slow metabolism decreases production of acetaldehyde, it seems less likely that acetaldehyde is the active metabolite affecting risk of DM2. Further studies are warranted to investigate influence of these metabolites.

Third, several controlled trials provided evidence for the underlying mechanism, which adds to the plausibility of the association between alcohol consumption and DM2. These trials consistently showed a ~10% increase of adiponectin concentrations, which is in line with results from observational studies (34;35). In addition, we showed that moderate alcohol consumption may particularly increase high-molecular weight adiponectin, which is thought to specifically predict insulin sensitivity (36). Changes of other markers such as decreased acylation-stimulating protein and two-hour glucose concentrations, and increased ghrelin concentrations were also in line with the hypothesis of improved insulin sensitivity. Only in a first study the increase of adiponectin concentrations was highly correlated with changes of insulin sensitivity. Altogether these results are in line with our a priori hypothesis and point towards improved insulin sensitivity.

Unfortunately, we did not observe significant improvements of insulin sensitivity after moderate alcohol consumption in any of our studies, which is generally considered as the underlying mechanism for the DM2 risk reduction. These results are consistent with several short-term interventions (37-39). However, one trial reported a borderline significant improvement of insulin sensitivity in an insulin-resistant subgroup (40) and cross-sectional studies consistently show inverse associations between alcohol consumption and insulin

sensitivity (41;42). The most convincing study so far showed a dose-dependent increase of insulin sensitivity in a diet-controlled, eight week cross-over study among 63 postmenopausal women (14).

The disparity between the study of Davies et al. and our results on insulin sensitivity could be due to measures used for insulin sensitivity. Davies et al. relied on fasting measures of glucose, insulin and triglycerides to calculate an insulin sensitivity index (14), while our measures relied only on glucose and insulin concentrations during a clamp or glucose tolerance test. Both fasting insulin and triglycerides decreased significantly in their study, while most studies show an elevation or no change of triglycerides after moderate alcohol consumption (43). This could be due to the population of postmenopausal women, that show different lipoprotein changes after moderate alcohol consumption of decreased concentrations of LDL cholesterol and triglycerides (44;45). Therefore, this measure used in that specific population may explain the disparity between our results. Indeed unpublished results from our group among postmenopausal women, show a similar decrease ($p< 0.05$) of triglyderides (wine: 1.0 ±0.05; juice 1.2 ±0.05) and fasting insulin (wine: 5.8 ±0.2; juice: 6.7 ±0.2) after six weeks of wine consumption. Consequently, insulin sensitivity was increased ($p< 0.05$) after wine compared with juice consumption both when assessed by HOMA-index (wine: 1.43 ±0.06; juice: 1.65 ±0.06) and McAuley index (wine: 9.1 ±0.2; juice: 8.3 ±0.2) as in the trial by Davies et al. (14;46). Still, Davies et al. and our study showed a significant decrease of fasting insulin concentrations, while these remained unchanged in our studies among men. Therefore other experimental differences than study population and measures of insulin sensitivity may also explain these results; for example a longer study period.

In a subsequent study we thus focused on changes preceding possible improvements of insulin sensitivity. Ethanol oxidation acutely affects whole body lipid oxidation and energy expenditure (47;48), which is mainly mediated by production of acetate. We hypothesized that improvements of substrate oxidation could precede changes of insulin sensitivity. We were, however, not able to demonstrate these effects. To our knowledge, this is the first human study investigating the chronic effect of moderate alcohol consumption on muscle oxidative capacity and cannot be compared to previous animal studies with high doses of alcohol (49;50). Still, some subtle differences in citrate synthase or succinate dehydrogenase were present and the non-significant, 15 to 20% difference in IMTG content

is of similar magnitude as previously shown for lifestyle interventions (51). Therefore, we cannot completely rule out effects of moderate alcohol consumption on muscle metabolism. However, larger trials and longer interventions may be needed to investigate this.

In summary, our trials consistently show that moderate alcohol consumption increases adiponectin concentrations and in particular high-molecular weight adiponectin. In addition, several other markers of insulin sensitivity (adipokines, two-hour glucose concentrations) changed in line with our hypothesis of improved insulin sensitivity. Unfortunately, this did not result in increased insulin sensitivity after moderate alcohol consumption. Altogether, these results provide evidence for an underlying mechanism of previously reported improvements of insulin sensitivity. However, results of a trial of Davies et al. (14) and unpublished results from our group suggest that larger trials and longer interventions are required to affect insulin sensitivity. Naturally, it is also possible that other mechanisms are more important to explain the association between alcohol consumption and DM2. Moderate alcohol consumption has several acute, postprandial effects (lowering of glucose concentrations, vasodilatation) that over time could result in improved metabolic profiles and a subsequent risk reduction of DM2.

GENERAL CONCLUSIONS

Moderate alcohol consumption is also inversely associated with CHD among hypertensive men, but for total and cardiovascular mortality such risk reduction was not observed. Increased cholesterol efflux mediated by ABCA1 may be an important underlying mechanism for this risk reduction, but anti-inflammatory effects by decreasing Lp-PLA2 activity do not seem to be involved. Moderate alcohol consumption is also inversely associated with risk of DM2 among older women. The interaction between *ADH1C* genotype and alcohol consumption suggests that this association may be causal, but due to downstream metabolites such as acetate instead of ethanol itself. Increased concentrations of adiponectin, particularly the high-molecular weight form, may explain this association. Unfortunately, we could not confirm increased insulin sensitivity after moderate alcohol consumption. This could be due to study population, measure of insulin sensitivity and intervention period.

In conclusion, research presented in this thesis has further substantiated the evidence

for a causal relation between alcohol consumption and CVD and DM2 by showing consistency of the associations in different populations, providing an underlying mechanism and showing effect modification by the *ADH1C* gene for DM2.

PUBLIC HEALTH IMPLICATIONS

The results presented in this thesis again underline that moderate alcohol consumption has protective effects for CVD. In addition, this research shows that moderate alcohol consumption may also protect against DM2 and that this relation may be causal. Therefore moderate alcohol consumption could be part of a healthy lifestyle.

Despite this, the current Dutch Nutritional guidelines do not include advice on alcohol consumption (52). It appears reasonable, though, to suggest that moderate alcohol consumption can be part of a healthy lifestyle, as included in US and UK recommendations (53;54). It would therefore be sensible to include such recommendation toward alcohol consumption in the Dutch Nutritional Guidelines as well. Naturally such recommendation would not imply an advice to abstainers to take up moderate alcohol consumption. Abstainers usually do so for a reason (e.g. religion, family history of alcoholism) and it therefore is undesirable for them to start drinking. However, for those consuming alcohol, such recommendation can provide clear information on what level of alcohol consumption is considered moderate. Both the UK and US recommendations on alcohol consumption have defined such moderate amounts and they are identical when quantified as grams per day (53;54). In addition, a recommendation could be included on which drinking patterns are considered healthy and in which situations abstinence is required (e.g. pregnancy, driving). In particular, a more conservative policy should be applied for adolescents, as is currently a key objective of Dutch alcohol policy (55).

In addition to the general population, this thesis also showed that moderate alcohol consumption may also fit in a healthy lifestyle for hypertensive patients. However, because alcohol consumption was not associated with a risk reduction of total or cardiovascular mortality, more caution is required to formulate guidelines on alcohol consumption for those patients. In addition, recommendations regarding alcohol use for hypertensive patients must be made on individual basis while considering a patients' cardiovascular risk profile and the risks and benefits of their drinking behavior (e.g. medication use). However, our

results show that hypertensive men who drink moderately and safely may not need change their drinking habits.

FUTURE RESEARCH

Although certain specific questions regarding alcohol consumption and CVD may remain unanswered, the association and underlying mechanism for the relation between alcohol consumption and CVD are largely known. However, specific studies can provide more certainty to formulate clear public health recommendations regarding alcohol consumption.

- A randomized controlled trial with a longer intervention period and more convincing outcome measures (intima media thickness or CHD events) would provide a more definitive answer to the question of causality. It is possible to design this trial in such a way that it would be ethical to perform. Such a study could be performed according to a randomized parallel design with an intervention of one unit of alcohol per day against a non-alcoholic control condition. In order to limit any possible addictive effects of regular alcohol use, one day of abstention per week and in-study checks for addictive effects could be used. A population of moderate drinkers with a slightly increased cardiovascular risk (and thus most likely to benefit) could be included and those with a family history of alcoholism or other addictive behaviors should be excluded. A trial with an intervention of six months to a year could be feasible to perform, but whether a longer term intervention is actually feasible to conduct successfully is questionable. Finally, given the current evidence, one may argue that it is unethical to advice abstinence to moderate drinkers for such time periods (according to the principle of equipoise). However, this is a matter of debate on how to evaluate current evidence.
- A more general point of attention for future research would be a more thorough investigation of drinking patterns that also include the heavier range of consumption. It could be particularly interesting to see what patterns may predict evolving towards alcohol misuse and alcoholism, which may provide evidence for policy.

For DM2, current evidence on the association between alcohol consumption and DM2 and particularly its underlying evidence is not yet complete. The current evidence points toward a causal relation between moderate alcohol consumption and risk of DM2 and this thesis

has provided a more solid basis for these conclusions. To come to a more firm conclusion several questions remain to be answered:

- The interaction between alcohol consumption and *ADH1C* polymorphism should be confirmed in other studies.
- The underlying mechanism of a risk reduction is not completely understood, but several candidate markers and mechanisms such as insulin sensitivity, adiponectin, glycemic control and inflammation have been proposed. We are unaware of any studies that explored to what extent such candidate markers explain the association between alcohol consumption and DM2, as is shown for HDL cholesterol and CVD. Such study could provide further direction for the strength of several proposed mechanisms.
- Although improved insulin sensitivity seems a very plausible mechanism for a risk reduction of DM2 with moderate alcohol consumption, studies presented in this thesis were unable to confirm this. Larger trials with longer treatment periods can provide a more definitive answer and could further confirm changes of oxidative capacity and IMTG content.
- Finally, other mechanisms may account for the inverse association between alcohol consumption and DM2. Moderate alcohol consumption acutely affects substrate oxidation and energy expenditure, lowers blood glucose concentrations and causes vasodilatation. These postprandial metabolic changes could cumulatively lead to an improved metabolic profile and thereby increase insulin sensitivity and reduce risk of DM2. A randomized controlled trial, that relates postprandial metabolic changes after moderate alcohol consumption to its chronic effects on insulin sensitivity or other markers of DM2 could help to elucidate this relation.

REFERENCES

1. National Institute on Alcohol Abuse and Alcoholism of the National Institutes of Health. Percent reporting heavy alcohol use in the past month by age group and demographic characteristics: NSDUH (NHSDA), 1994–2002. Accessed at htttp://www.niaaa.nih.gov/Resources/DatabaseResources/QuickFacts/Alcohol Consumption/dkpat21.htm. on October 7, 2006.
2. Rijksinstituut voor Volksgezondheid en Milieu. Het Nationaal Kompas: Alcoholgebruik: Omvang van het probleem: Hoeveel mensen hebben een overmatig alcoholgebruik? Accessed at http://www.rivm.nl/vtv/object_document/o1190n19086.html. On October 7, 2006.
3. Shaper AG, Wannamethee G, Walker M. Alcohol and mortality in British men: explaining the U-shaped curve. Lancet 1988;2:1267-73.
4. Kristal AR, Peters U, Potter JD. Is it time to abandon the food frequency questionnaire? Cancer Epidemiol Biomarkers Prev 2005;14:2826-8.
5. Giovannucci E, Colditz G, Stampfer MJ et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol 1991;133:810-7.
6. Ocke MC, Bueno-de-Mesquita HB, Goddijn HE et al. The Dutch EPIC food frequency questionnaire. I. Description of the questionnaire, and relative validity and reproducibility for food groups. Int J Epidemiol 1997;26 Suppl 1:S37-S48.
7. Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, Van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26 Suppl 1:S49-S58.
8. Rimm EB, Giovannucci EL, Stampfer MJ, Colditz GA, Litin LB, Willett WC. Reproducibility and validity of an expanded self-administered semiquantitative food frequency questionnaire among male health professionals. Am J Epidemiol 1992;135:1114-26.
9. Fox M. Epidemiologic Method Research; Boston University Sensitivity Analysis Tools. Boston university 2006. Accessed at http://www.bu.edu/dbin/sph/departments/epidemiology/ research/epidemiologic_methods_research.php on October 7, 2006.
10. Fox MP, Lash TL, Greenland S. A method to automate probabilistic sensitivity analyses of misclassified binary variables. Int J Epidemiol 2005;34:1370-6.
11. Grobbee DE, Rimm EB, Keil U, Renaud S. Alcohol and the Cardiovascular System. In: MacDonald I, ed. Health issues related to alcohol consumption. Bodmin: Blackwell Sciences Ltd 1999:125-79.
12. Koppes LL, Dekker JM, Hendriks HF, Bouter LM, Heine RJ. Moderate alcohol consumption lowers the risk of type 2 diabetes: a meta-analysis of prospective observational studies. Diabetes Care 2005;28:719-25.
13. Sarkola T, Dahl H, Eriksson CJ, Helander A. Urinary ethyl glucuronide and 5-hydroxytryptophol levels during repeated ethanol ingestion in healthy human subjects. Alcohol Alcohol 2003;38:347-51.
14. Davies MJ, Baer DJ, Judd JT, Brown ED, Campbell WS, Taylor PR. Effects of moderate alcohol intake on fasting insulin and glucose concentrations and insulin sensitivity in postmenopausal women: a randomized controlled trial. JAMA 2002;287:2559-62.

15. Sakr SW, Williams DL, Stoudt GW, Phillips MC, Rothblat GH. Induction of cellular cholesterol efflux to lipid-free apolipoprotein A-I by cAMP. Biochim Biophys Acta 1999;1438:85-98.
16. Monzillo LU, Hamdy O. Evaluation of insulin sensitivity in clinical practice and in research settings. Nutr Rev 2003;61:397-412.
17. Malinski MK, Sesso HD, Lopez-Jimenez F, Buring JE, Gaziano JM. Alcohol consumption and cardiovascular disease mortality in hypertensive men. Arch Intern Med 2004;164:623-8.
18. Palmer AJ, Fletcher AE, Bulpitt CJ et al. Alcohol intake and cardiovascular mortality in hypertensive patients: report from the Department of Health Hypertension Care Computing Project. J Hypertens 1995;13:957-64.
19. Chobanian AV, Bakris GL, Black HR et al. Seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure. Hypertension 2003;42:1206-52.
20. Mukamal KJ, Ascherio A, Mittleman MA et al. Alcohol and risk for ischemic stroke in men: the role of drinking patterns and usual beverage. Ann Intern Med 2005;142:11-9.
21. Beulens JWJ, Rimm EB, Ascherio A, Hendriks HFJ, Mukamal KJ. Alcohol consumption and risk or coronary heart disease and stroke among hypertensive men. Circulation 2006;113:E315-E316.
22. Sierksma A, Vermunt SH, Lankhuizen IM et al. Effect of moderate alcohol consumption on parameters of reverse cholesterol transport in postmenopausal women. Alcohol Clin Exp Res 2004;28:662-6.
23. van der Gaag MS, van Tol A, Vermunt SH, Scheek LM, Schaafsma G, Hendriks HF. Alcohol consumption stimulates early steps in reverse cholesterol transport. J Lipid Res 2001;42:2077-83.
24. Duong M, Collins HL, Jin W, Zanotti I, Favari E, Rothblat GH. Relative contributions of ABCA1 and SR-BI to cholesterol efflux to serum from fibroblasts and macrophages. Arterioscler Thromb Vasc Biol 2006;26:541-7.
25. van der Gaag MS, van Tol A, Scheek LM et al. Daily moderate alcohol consumption increases serum paraoxonase activity; a diet-controlled, randomised intervention study in middle-aged men. Atherosclerosis 1999;147:405-10.
26. Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. Arterioscler Thromb Vasc Biol 2005;25:923-31.
27. Maraldi C, Volpato S, Kritchevsky SB et al. Impact of inflammation on the relationship among alcohol consumption, mortality, and cardiac events: the health, aging, and body composition study. Arch Intern Med 2006;166:1490-7.
28. Mukamal KJ, Jensen MK, Gronbaek M et al. Drinking frequency, mediating biomarkers, and risk of myocardial infarction in women and men. Circulation 2005;112:1406-13.
29. Clayton D, McKeigue PM. Epidemiological methods for studying genes and environmental factors in complex diseases. Lancet 2001;358:1356-60.
30. Hines LM, Stampfer MJ, Ma J et al. Genetic variation in alcohol dehydrogenase and the beneficial effect of moderate alcohol consumption on myocardial infarction. N Engl J Med 2001;344:549-55.
31. Sarkola T, Iles MR, Kohlenberg-Mueller K, Eriksson CJ. Ethanol, acetaldehyde, acetate, and lactate levels after alcohol intake in white men and women: effect of 4-methylpyrazole. Alcohol Clin Exp Res 2002;26:239-45.

32. Visapaa JP, Gotte K, Benesova M et al. Increased cancer risk in heavy drinkers with the alcohol dehydrogenase 1C*1 allele, possibly due to salivary acetaldehyde. Gut 2004;53:871-6.
33. Lieber CS. Alcoholic fatty liver: its pathogenesis and mechanism of progression to inflammation and fibrosis. Alcohol 2004;34:9-19.
34. Pischon T, Girman CJ, Rifai N, Hotamisligil GS, Rimm EB. Association between dietary factors and plasma adiponectin concentrations in men. Am J Clin Nutr 2005;81:780-6.
35. Shai I, Rimm EB, Schulze MB, Rifai N, Stampfer MJ, Hu FB. Moderate alcohol intake and markers of inflammation and endothelial dysfunction among diabetic men. Diabetologia 2004;47:1760-7.
36. Hara K, Horikoshi M, Yamauchi T et al. Measurement of the high-molecular weight form of adiponectin in plasma is useful for the prediction of insulin resistance and metabolic syndrome. Diabetes Care 2006;29:1357-62.
37. Cordain L, Bryan ED, Melby CL, Smith MJ. Influence of moderate daily wine consumption on body weight regulation and metabolism in healthy free-living males. J Am Coll Nutr 1997;16:134-9.
38. Cordain L, Melby CL, Hamamoto AE et al. Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. Metabolism 2000;49:1473-8.
39. Zilkens RR, Burke V, Watts G, Beilin LJ, Puddey IB. The effect of alcohol intake on insulin sensitivity in men: a randomized controlled trial. Diabetes Care 2003;26:608-12.
40. Sierksma A, Patel H, Ouchi N et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-alpha, and insulin sensitivity. Diabetes Care 2004;27:184-9.
41. Bell RA, Mayer-Davis EJ, Martin MA, D'Agostino RB, Jr., Haffner SM. Associations between alcohol consumption and insulin sensitivity and cardiovascular disease risk factors: the Insulin Resistance and Atherosclerosis Study. Diabetes Care 2000;23:1630-6.
42. Kiechl S, Willeit J, Poewe W et al. Insulin sensitivity and regular alcohol consumption: large, prospective, cross sectional population study (Bruneck study). BMJ 1996;313:1040-4.
43. Rimm EB, Williams P, Fosher K, Criqui M, Stampfer MJ. Moderate alcohol intake and lower risk of coronary heart disease: meta-analysis of effects on lipids and haemostatic factors. BMJ 1999;319:1523-8.
44. Baer DJ, Judd JT, Clevidence BA et al. Moderate alcohol consumption lowers risk factors for cardiovascular disease in postmenopausal women fed a controlled diet. Am J Clin Nutr 2002;75:593-9.
45. van der Gaag MS, Sierksma A, Schaafsma G et al. Moderate alcohol consumption and changes in postprandial lipoproteins of premenopausal and postmenopausal women: a diet-controlled, randomized intervention study. J Womens Health Gend Based Med 2000;9:607-16.
46. McAuley KA, Williams SM, Mann JI et al. Diagnosing insulin resistance in the general population. Diabetes Care 2001;24:460-4.
47. Raben A, Agerholm-Larsen L, Flint A, Holst JJ, Astrup A. Meals with similar energy densities but rich in protein, fat, carbohydrate, or alcohol have different effects on energy expenditure and substrate metabolism but not on appetite and energy intake. Am J Clin Nutr 2003;77:91-100.
48. Siler SQ, Neese RA, Hellerstein MK. De novo lipogenesis, lipid kinetics, and whole-body lipid balances in humans after acute alcohol consumption. Am J Clin Nutr 1999;70:928-36.

49. Marcinkeviciute A, Mildaziene V, Crumm S, Demin O, Hoek JB, Kholodenko B. Kinetics and control of oxidative phosphorylation in rat liver mitochondria after chronic ethanol feeding. Biochem J 2000;349:519-26.
50. Piquet MA, Nogueira V, Devin A et al. Chronic ethanol ingestion increases efficiency of oxidative phosphorylation in rat liver mitochondria. FEBS Lett 2000;468:239-42.
51. He J, Goodpaster BH, Kelley DE. Effects of weight loss and physical activity on muscle lipid content and droplet size. Obes Res 2004;12:761-9.
52. Gezondheidsraad. Voedingsnormen energie, eiwitten, vetten en verteerbare koolhydraten. publicatie nr 2001/19. 2001. Den Haag, Gezondheidsraad.
53. Inter-Departmental Working Group. Sensible Drinking. 1995. Wetherby, England, Department of Health.
54. U.S.Department of Agriculture and U.S.Department of Health and Human Services. Dietary Guidelines for Americans, 2005. 6th edition. 2005. Washington DC, US Government Printing Office.
55. Ministerie van Volksgezondheid, Welzijn en Sport. Dossier: Alcohol. Accessed at http://www minvws nl/dossiers/alcohol/ on October 7, 2006.

Summary

SUMMARY

Already since decades it is apparent that moderate alcohol consumption is associated with a decreased risk of cardiovascular disease (CVD) compared with both abstainers and heavier drinkers. More recently a similar risk reduction with moderate alcohol consumption for type 2 diabetes mellitus (DM2) was observed in several prospective studies. Despite overwhelming evidence for CVD, the debate around causality still continues, while for DM2 the evidence is not yet complete. This thesis therefore aims to further substantiate the evidence for a causal relation between alcohol consumption and CVD and DM2 by investigating consistency of the associations in different populations, an underlying mechanism and effect modification by the alcohol dehydrogenase 1C (*AHD1C*) gene for DM2.

Cardiovascular disease

The relation and underlying mechanism of moderate alcohol consumption with cardiovascular disease has been extensively studied the past decades. These results show a protective effect of moderate alcohol consumption for cardiovascular disease of about 20 to 30% and the association seems to be U-shaped for both coronary heart disease and CVD. Beverage type does not influence the association and the protective effect therefore seems to be caused by ethanol per se instead of other components in the drinks, but drinking pattern does affect risk of CVD. These results are consistent for the general population and also observed among diabetic patients, who are at increased risk of developing CVD. For hypertensive patients, also at increased risk of CVD, limited data exists, particularly on non-fatal CVD events. This may have contributed to inconsistencies in guidelines on alcohol consumption for these patients. **In chapter 2**, we therefore aim to further quantify the association between alcohol consumption and cardiovascular disease among hypertensive men. Among 11,982 US hypertensive men, a risk of myocardial infarction of 0.72 (95%-CI: 0.54- 0.97) is observed for men consuming two to three alcoholic beverages (15.0- 29.9 grams of alcohol) per day. Alcohol consumption, however, is not associated with total or cardiovascular mortality and it is difficult to determine associations with stroke due to few cases. With coronary heart disease mortality, though, an inverse association is observed.

The inverse association between moderate alcohol consumption and CVD is explained

by an increase of HDL cholesterol for about 50%, but effects on fibrinolysis, blood clotting and glucose tolerance are also involved. **In chapter 3 and 4** we focus on functional properties of HDL cholesterol to further explain the underlying mechanism of the cardioprotective effects. In two randomized controlled crossover trials, we show that moderate alcohol consumption increases cholesterol efflux mediated by ABCA1 by 17.5% (95%-CI: 2.3- 34.1), while lipoprotein-associated phospholipase A2 activity remains unchanged (-2.9%; 95%-CI: -7.9; 2.0).

Type 2 diabetes

Similar to CVD moderate alcohol consumption is associated with a decreased risk of DM2. This risk reduction of about 30% seems attributable to alcohol per se instead of other components of alcoholic drinks. Drinking pattern also influences risk of DM2. The evidence for the association between alcohol consumption and DM2 is less substantive than for CVD, but points towards a causal relation. Therefore **in chapter 5**, we aim to investigate the relation between alcohol consumption and DM2 among older women. This association has not been investigated previously for this population, while the risk of DM2 increases with age and the majority of DM2 patients are females. Our results show a relative risk of 0.61 (95-% CI: 0.41- 0.92) for consuming two to three alcoholic beverages (20.0 to 29.9 grams of alcohol) per day among 16,330 women aged 49-70 years. Lifetime alcohol consumption is associated with DM2 in a U-shaped fashion.

Because polymorphisms are randomly distributed throughout the population, the finding that genotype, affecting ethanol oxidation, modifies the association of alcohol intake with diabetes would support the hypothesis that the association is causal. **In chapter 6**, we therefore investigate whether a common polymorphism in the *AHD1C* gene, conferring slower ethanol oxidation, modifies the association between alcohol consumption and DM2. We indeed observe a significant interaction between alcohol consumption and *ADH1C* genotype on risk of DM2 in two nested case-control studies from the Nurses' Health Study and Health Professionals Follow-up Study. Among moderate drinkers (>5 g/day for women and >10 g/day for men) carrying of the *ADH1C*2* allele, conferring slower ethanol oxidation, increases risk of DM2. Odds ratios are 1.63 (1.02-2.61) for heterozygotes and 2.38 (1.08-5.22) for homozygotes of the *ADH1C*2* allele. These results suggest that the association between alcohol consumption and DM2 may indeed be causal. Because slower

ethanol oxidation can result in decreased concentrations of downstream metabolites such as acetate, our results indicate that these could mediate the association between alcohol consumption and DM2 instead of ethanol itself.

Improved insulin sensitivity with moderate alcohol consumption is generally proposed as an underlying mechanism for the risk reduction of DM2. **In chapter 7, 8 and 9**, results from three randomized, controlled crossover trials are described that investigate an underlying mechanism. These three studies consistently show that moderate alcohol consumption increases plasma adiponectin concentrations by about 10%. In chapter 8 we show that this increase of adiponectin occurs independently from changes of body weight or fat distribution. Our study in chapter 9 shows that this alcohol-induced increase of adiponectin concentrations may be oligomer specific, as particularly high-molecular weight adiponectin is increased by 57.1% (95%-CI: -4.7; 89.6) after moderate alcohol consumption. Only in chapter 7, this increase of adiponectin is positively correlated with changes of insulin sensitivity. Changes of other markers (such as other adipokines and two-hour glucose concentrations) are also in line with the hypothesis of improved insulin sensitivity. Unfortunately, we do not observe significant improvements of insulin sensitivity after moderate alcohol consumption in any of our studies, as previously shown in observational studies and one randomized controlled trial. This disparity is probably due to our study populations of men compared to postmenopausal women in other studies combined with the measures of insulin sensitivity used. Longer intervention periods may be needed for increased adiponectin concentrations to result in improved insulin sensitivity.

General Conclusions

Moderate alcohol consumption is also inversely associated with CHD among hypertensive men, but for total and cardiovascular mortality such risk reduction is not observed. Thus, hypertensive men who drink moderately and safely may not need change their drinking habits, but recommendations should be carefully made on individual basis. Increased cholesterol efflux mediated by ABCA1 may be an important underlying mechanism for this risk reduction, whereas anti-inflammatory effects of decreased Lp-PLA2 activity seems not involved.

The inverse association of moderate alcohol consumption with risk of DM2 is expanded to a population of older women. The interaction between *ADH1C* genotype and

alcohol consumption suggests that this association may be causal, but due to downstream metabolites such as acetate instead of ethanol itself. Increased concentrations of adiponectin, particularly the high-molecular weight form, may explain this association. Unfortunately, we cannot confirm increased insulin sensitivity after moderate alcohol consumption. This can be due to study population, measure of insulin sensitivity and intervention period. Possibly, longer interventions are needed for increased adiponectin concentrations to improve insulin sensitivity.

Altogether, research presented in this thesis further substantiates the evidence for a causal association between alcohol consumption and CVD and DM2 by showing consistency of the associations in different populations, providing an underlying mechanism and showing effect modification by the *ADH1C* gene for DM2.

Samenvatting

SAMENVATTING

Het is bekend dat matige alcoholconsumptie samenhangt met een verminderd risico op hart- en vaatziekten in verhouding tot geheelonthouding en overmatige consumptie. Kort geleden hebben verschillende onderzoeken laten zien dat matig drinken ook gerelateerd is aan een verlaagd risico op diabetes mellitus type 2. Ondanks veelvuldig bewijs, is er nog steeds discussie of het verband tussen matige alcoholconsumptie en hart- en vaatziekten oorzakelijk is. Voor type 2 diabetes is het bewijs nog niet compleet. Dit proefschrift heeft daarom als doel het bewijs voor een mogelijk oorzakelijk verband van alcoholconsumptie met hart- en vaatziekten en type 2 diabetes verder te onderbouwen. Dit wordt gedaan door consistentie van deze relatie in verschillende populaties te onderzoeken, een plausibel onderliggend mechanisme te onderzoeken en een interactie na te gaan tussen alcoholconsumptie en het alcoholdehydrogenasegen.

Matige alcoholconsumptie
Matige alcoholconsumptie kan afhankelijk van de onderliggende betekenis op verschillende manieren gedefinieerd worden. In dit proefschrift wordt met matig drinken gedoeld op de hoeveelheid die samengaat met het laagste risico op ziekte en sterfte. Dit is ongeveer 10 tot 30 gram per dag, ofwel een tot drie alcoholische consumpties.

Hart- en vaatziekten

De relatie en het onderliggende mechanisme tussen alcoholconsumptie en hart- en vaatziekten is uitgebreid onderzocht. Dit onderzoek laat zien dat matig drinken het risico op hart- en vaatziekten met ongeveer 20 tot 30 procent kan verlagen. Het type alcoholische drank (bier, wijn of sterke drank) heeft geen invloed op deze relatie. Het effect lijkt dus veroorzaakt te worden door alcohol zelf.

Hart- en vaatziekten
Hart- en vaatziekten is de verzamelnaam voor aandoeningen aan het hart- en vaatsysteem, zoals het hartinfarct (myocard infarcten), beroerten (cerebrovasculair accident), ischemisch attack (TIA) en vaatlijden van de grote vaten. Deze ziekten zijn op dit ogenblik doodsoorzaak nummer 1 binnen de westerse wereld.

Drinkpatronen, zoals aantal dagen per week dat men alcohol consumeert, hebben wel invloed op deze relatie. De risicoreductie met matige alcoholconsumptie geldt voor de normale populatie, maar is ook bij diabetespatiënten aangetoond. Voor patiënten met verhoogde bloeddruk, die een verhoogd risico hebben op hart- en vaatziekten, waren nog niet veel gegevens bekend. Dit kan er mede toe geleid hebben dat de richtlijnen voor alcoholconsumptie voor mensen met hoge bloeddruk soms tegenstrijdig zijn. In **hoofdstuk**

2 is daarom onderzocht wat de relatie is tussen alcoholconsumptie en risico op hart- en vaatziekten bij 11,982 mannen met verhoogde bloeddruk. Dit onderzoek laat zien dat het risico op een hartinfarct met ongeveer 30% verlaagd is voor mannen die twee tot drie alcoholische dranken per dag dronken. Alcoholconsumptie was bij deze mannen niet gerelateerd aan sterfte door alle oorzaken of sterfte door hart- en vaatziekten.

De relatie tussen alcoholconsumptie en het risico op hart- en vaatziekten wordt voor 50% verklaard door een stijging van HDL-cholesterol, maar een effect op bloedstolling speelt ook een rol. In **hoofdstuk 3 en 4** hebben we ons gericht op de functionele gevolgen van een HDL-cholesterolstijging. Twee gerandomiseerde interventieonderzoeken laten zien dat matige alcoholconsumptie cholesterolefflux via de ABCA1-transporter verhoogt met ongeveer 17%. Een ontstekingsremmend effect door daling van Lp-PLA2 activiteit lijkt niet betrokken te zijn bij het beschermende effect van matige alcoholconsumptie op hart- en vaatziekten.

Cholesterolefflux
Cholesterolefflux is het transporteren van cholesterol uit de cel, zodat het afgevoerd kan worden uit het lichaam. Het goede 'HDL' cholesterol is nauw betrokken bij dit proces. ABCA1 is een eiwit dat zorg draagt voor dit transport van cholesterol uit de cel.

Lp-PLA2
Lp-PLA2 is een eiwit wat gebonden is aan cholesterol en dit cholesterol om kan zetten in een vorm die ontstekingen in de vaatwand kan veroorzaken. Dit verhoogt dan het risico op een hartinfarct.

Type 2 diabetes

Zoals voor hart- en vaatziekten is matige alcoholconsumptie ook gerelateerd aan een verlaagd risico op type 2 diabetes. Deze risicoreductie bedraagt ongeveer 30% en lijkt te worden veroorzaakt door alcohol zelf. Drinkpatronen beïnvloeden ook het risico op type 2 diabetes. Het bewijs voor deze relatie is minder substantieel dan voor hart- en vaatziekten, maar wijst op een oorzakelijk verband. In **hoofdstuk 5** is de relatie tussen alcoholconsumptie en type 2 diabetes onderzocht in een populatie van oudere vrouwen, voor wie deze relatie nog onbekend was. Dit is onderzocht omdat het risico op type 2 diabetes toeneemt bij het ouder worden en is het merendeel van diabetespatiënten vrouw. Ook in deze populatie lieten wij een risicoreductie op type 2

Type 2 diabetes
Diabetes mellitus, ofwel suikerziekte, wordt gekenmerkt door herhaaldelijk verhoogde bloedglucose (= suiker) concentraties. Bij ouderdomsdiabetes wordt dit voornamelijk veroorzaakt doordat het lichaam onvoldoende op afgifte van insuline reageert (= insulineresistentie).

diabetes van ongeveer 30% zien voor vrouwen die twee tot drie alcoholische dranken per dag dronken.

Men gaat ervan uit dat wanneer genetische informatie de relatie tussen alcoholconsumptie en type 2 diabetes beïnvloedt dit een bewijs vormt voor een oorzakelijk verband. In **hoofdstuk 6** is daarom onderzocht of genetische variatie in alcoholdehydrogenase (ADH1C) het verband tussen alcoholconsumptie en het risico op diabetes beïnvloedt. Er is inderdaad een significante interactie gevonden tussen alcoholconsumptie en het ADH1C gen voor het risico op type 2 diabetes onder Amerikaanse mannen en vrouwen. Onder mensen die matig dronken, bleek het dragen van het ADH1C*2 allel, wat gerelateerd is aan langzamere alcoholafbraak, gerelateerd te zijn aan een hoger risico op diabetes. Deze resultaten suggereren dat de relatie tussen alcoholconsumptie en het risico op diabetes inderdaad oorzakelijk is. Omdat langzamere alcohol afbraak resulteert in hogere bloedalcoholconcentraties maar lagere concentraties van de afbraakproducten, wijzen deze resultaten er ook op dat de relatie tussen alcoholconsumptie en diabetes niet veroorzaakt wordt door alcohol zelf maar zijn afbraakproducten zoals acetaat.

Alcoholdehydrogenase
Het alcoholdehydrogenase gen reguleert de aanmaak van het eiwit alcohol dehydrogenase. Dit eiwit is betrokken bij de afbraak van alcohol. Er bestaan verschillende vormen van dit gen die de snelheid van alcoholafbraak bepalen. Bij dragers van verschillende vormen van dit gen wordt alcohol hetzij snel, gemiddeld of langzaam afgebroken.

In vervolgstudies is nader onderzocht wat het onderliggende mechanisme kan zijn voor het verband tussen alcoholconsumptie en diabetes. Een verhoogde insulinegevoeligheid met matige alcoholconsumptie werd veelal verondersteld als mechanisme. In **hoofdstuk 7, 8 en 9** worden de resultaten van drie interventieonderzoeken beschreven die nader ingaan op dit onderliggende mechanisme. Deze drie onderzoeken laten consistent zien dat matige alcoholconsumptie adiponectineconcentraties in het bloed met ongeveer 10% verhoogt. In hoofdstuk 8 laten we zien dat deze toename in adiponectineconcentraties niet afhankelijk is van verandering in lichaamsgewicht en vetverdeling. Het onderzoek in hoofdstuk 9 laat vervolgens zien dat

Insulinegevoeligheid
Insulinegevoeligheid betekent gevoeligheid van weefsels zoals de spieren voor insuline.
Insuline transporteert glucose (=suiker) naar de weefsels. Wanneer deze gevoeligheid afneemt spreekt men van insulineresistentie. Dit kan verhoogde bloedglucoseconcentraties veroorzaken.

matige alcoholconsumptie mogelijk specifiek de hoogmoleculaire vorm van adiponectine verhoogt. Er wordt verondersteld dat dit de biologisch actieve vorm van adiponectine is. Alleen in het onderzoek in hoofdstuk 7 is een significant positief verband gevonden tussen adiponectine en insulinegevoeligheid. Andere veranderingen zoals een daling van de twee-uur glucoseconcentraties na matige alcoholconsumptie waren consistent met onze hypothese van verhoogde insulinegevoeligheid. Helaas hebben we in geen van deze studies aan kunnen tonen dat matige alcoholconsumptie insulinegevoeligheid verbetert in tegenstelling tot eerdere onderzoeken. Dit kan mogelijk verklaard worden door de onderzoekspopulatie van mannen ten opzichte van postmenopausale vrouwen, maar ook de duur van de interventie kan een rol spelen. Mogelijk duurt het langer dan vier weken voordat verhoogde adiponectine concentraties resulteren in verhoogde insulinegevoeligheid.

Adiponectine
Het eiwit adiponectine wordt geproduceerd en afgegeven door het vetweefsel. Dit eiwit beïnvloedt het vet- en glucose metabolisme in het lichaam. Hoge adiponectine concentraties in het bloed verhogen insulinegevoeligheid en hangen samen met een lager risico op diabetes en hart- en vaatziekten.

Algemene conclusies

Matige alcoholconsumptie is niet alleen geassocieerd met een verlaagd risico op hart- en vaatziekten in de gezonde populatie, maar ook bij mannen met verhoogde bloeddruk. Mannen met verhoogde bloeddruk die verantwoord en matig drinken, hoeven hun drinkgewoonten dus niet aan te passen, maar zulke adviezen dienen uiteraard zorgvuldig en op individuele basis gegeven te worden. Een toename van cholesteroltransport uit de cel is mogelijk een belangrijk onderliggend mechanisme dat de risicoreductie voor hart- en vaatziekten kan verklaren. Een ontstekingsremmend effect door verlaging van Lp-PLA2 activiteit lijkt minder van belang te zijn.

Het inverse verband tussen matige alcoholconsumptie en risico op diabetes dat eerder is gevonden bij mannen en jongere vrouwen, geldt ook voor oudere vrouwen. Dit verband wordt echter beïnvloed door genetische variatie in alcoholdehydrogenase. Dit suggereert dat de relatie oorzakelijk is, maar mogelijk veroorzaakt wordt door afbraakproducten van alcohol in plaats van alcohol zelf. Hogere adiponectineconcentraties, in het bijzonder de hoogmoleculaire vorm, bij matige alcoholconsumptie kunnen de risicoreductie voor diabetes verklaren. Helaas is verhoogde insulinegevoeligheid bij matige alcoholconsumptie

niet bevestigd in dit proefschrift. Dit kan mogelijk verklaard worden door de onderzoekspopulatie of de duur van de interventie.

Samenvattend, dit proefschrift levert bewijs voor een oorzakelijk verband tussen matige alcoholconsumptie en een verminderd risico op hart- en vaatziekten en type 2 diabetes door consistentie van associaties in verschillende populaties te laten zien, een plausibel onderliggend mechanisme te identificeren en door effectmodificatie door het alcoholdehydrogenasegen voor diabetes aan te tonen.

Dankwoord

Dankwoord

Het is een beetje onverwacht, maar bijzonder prettig om na drie jaar hard werken al zo ver te zijn dat ik de laatste woorden van dit proefschrift op papier kan gaan zetten. Uiteraard was ik nooit zo ver gekomen zonder de hulp van velen. Daarom wil ik nu van de gelegenheid gebruik maken om een aantal mensen te bedanken voor hun bijdrage, maar vooral ook voor het plezier waarmee ik aan dit boekje heb kunnen werken.

Graag wil ik beginnen met mijn copromotor, Dr. H.F.J. Hendriks. Beste Henk, al uit ons eerste emailcontact over een mogelijke promotieplek bij TNO bleek je groot enthousiasme voor het alcoholonderzoek en mede hierdoor was de keuze voor een plek bij TNO snel gemaakt. Daarnaast wil ik je bedanken voor je persoonlijke, ontspannen en enthousiaste wijze waarop je nieuwe ideeën, theorieën en resultaten hebt benaderd. Hierdoor heb ik niet alleen met veel plezier aan mijn onderzoek gewerkt, maar daarnaast heb je mij de ruimte en het vertrouwen gegeven om voor ons onderzoek contacten te leggen buiten de eigen groep. Ik heb hierdoor de mogelijkheid gekregen op uiteenlopende gebieden veel te leren. Zonder jouw inbreng was dit proefschrift niet op deze manier tot stand gekomen. Uiteraard wil ik ook mijn promotoren, Prof. Dr. Ir. F.J. Kok en Prof. Dr. Ir. G. Schaafsma, bedanken. Beste Frans, bij mijn aanstelling op deze plek ben je nauw betrokken geweest. Toen ik anderhalf jaar na mijn afstuderen niet op mijn plek zat bij de industrie en contact met je opnam over een promotieplek stond de deur direct open. Zonder jouw inbreng was dit proefschrift dus sowieso niet tot stand gekomen. Tijdens het promotieonderzoek was je meer op afstand betrokken, maar op de kritische momenten heb je steeds een doorslaggevende rol gespeeld. Ik waardeer dit enorm. Beste Gertjan, ook jij was op afstand bij het onderzoek betrokken. Desondanks was je altijd sterk geïnteresseerd in mijn nieuwe bevindingen en altijd positief kritisch in de benadering van de gezondheidseffecten van matige alcoholconsumptie. Tijdens de besprekingen hierover wist je mij dan ook regelmatig te verrassen met alternatieve verklaringen en nieuwe zienswijzen. Ook jou wil ik bedanken voor de prettige, inspirerende en constructieve samenwerking.

During my PhD-project, I was given the opportunity to work for six months at Harvard School of Public Health. Dear Eric, I would like to thank you again for the possiblity to work within your group and the nutrition department of HSPH. It was very inspiring to work within this research environment. In part because of your pleasant approach toward research, my stay was a very enjoyable experience and took my Phd-project to a new level and direction.
Dear Ken, I would also like to thank you for enabling me to work at HSPH. Despite a busy schedule, you've always taken time to discuss whatever aspect of research that came up. I much enjoy our conversations by email, phone or in person about alcohol research,

sensitivity analyses, epidemiology or whatever else. I would also like to thank you for your hospitality during my stay. This definitely made me feel more at home in Boston. I'm very glad that you are able to be present for the defence of my thesis.

Ondanks de belangrijke rol van mijn begeleiders, had er waarschijnlijk geen getal op papier gestaan zonder de hulp van velen bij het opzetten en uitvoeren van de studies en natuurlijk al helemaal niet zonder proefpersonen. Daarom zou ik allereerst de mannen en vrouwen die zich twee tot drie maanden van alcohol onthouden hebben willen bedanken voor hun inzet.
Daarnaast uiteraard mijn collega's uit de Metabole Unit die nauw betrokken zijn geweest bij de alcoholstudies; door jullie aanwezigheid was de sfeer altijd top tijdens de studies en het onderzoek draaide steeds weer als een geoliede machine. Bedankt!
Henriëtte, ik ben blij dat je voor de alcoholstudies steeds weer als 'study-nurse' wilde optreden. Je wist alles uitstekend te organiseren, maar daarnaast heb ik ontzettend veel plezier gehad bij onze samenwerking en discussie rond de uitvoer ('return of empty cans' is namelijk echt heel belangrijk…). Enne……. zelfs na drie jaar is 'Owattenaait' mij nog echt helemaal niet gaan vervelen. Daarnaast wil ik uiteraard ook Inge en José heel hartelijk bedanken. Al snel brachten jullie mij bij dat de omgang met proefpersonen veel weg kan hebben van opvoeden van kinderen. Ik heb veel van jullie benadering geleerd. Soesila en Angelique, jullie hebben mij regelmatig vervloekt om al die 'archjes' die ik weer in mijn protocol had gezet. Ik hoop dat ik hiervoor iets heb goed kunnen maken door zelf een heleboel doppen op die archjes te draaien. Het was in ieder geval altijd weer gezellig om met jullie in het lab te staan! Eric, Christel, Hanny, ik weet niet hoe jullie het geflikt hebben, maar het is toch steeds weer gelukt om voor ieder onderzoek met voldoende deelnemers aan de start te verschijnen. Hartelijk dank hiervoor. Daarnaast wil ik ook Jolanda, Desiree, Jurgita, Linda, Ineke, Els, Marjolein, Marc, Dimitri, Carina en waarschijnlijk vele anderen die meegedraaid hebben bij de alcoholstudies heel hartelijk bedanken voor hun inzet. Ook de diëtetiek, Susanne, Petra en degenen die hen hebben geholpen wil ik hartelijk danken voor het organiseren van de gecontroleerde diëten. Diane en Linda, jullie wil ik hartelijk bedanken voor alle datasetjes, SAS tips & tricks en de gezelligheid als ik weer eens kwam binnenvallen om een vraag te stellen.
Robin, mijn steun en toeverlaat voor biochemische analyses en favoriete kamergenoot, heel erg bedankt voor je hulp bij het voorbereiden van alle analyses en opzetten van assays voor iedere 'new & exiting' biomarker die we maar konden bedenken. Ik heb er veel van geleerd. En last but not least, voor alle gezellige kopjes dubbele espresso-extra sterk (tja, waar ben je anders AIO voor…).
Tijdens mijn promotieonderzoek heb ik twee stagiaires mogen begeleiden. Emilie en Roderik, ik heb met veel plezier met jullie samengewerkt en jullie hebben beiden veel werk verzet. Ook al heb ik jullie niet kunnen overhalen tot een promotieonderzoek, ik hoop dat

ik toch iets van mijn enthousiasme voor het (alcohol)onderzoek hebben kunnen overbrengen.
Uiteraard was mijn tijd bij TNO nooit zo geslaagd zonder leuke collega's. Daarom wil ik ook al mijn andere (oud)collega's bedanken, Lisette, Gertruud, Trinette, Wim, Len, Wilrike, Esther, Susan, Aafje, Heather, Annette voor de gezelligheid en interesse in mijn promotieonderzoek. In het bijzonder zou ik Wendy Blom hier willen noemen. We hebben samen 2,5 jaar in dezelfde constructie promotieonderzoek gedaan bij TNO. Ik ben blij dat we steeds weer bij elkaar terecht konden om wat te overleggen of gewoon even bij te kletsen. Bedankt voor alle promotietips die je me het afgelopen half jaar vanuit Unilever gemaild hebt. Ik vind het leuk dat we dit jaar de rollen omdraaien en jij mijn paranimf bent.

Naast mijn TNO-collega's, wil ik de coauteurs van een van de artikelen en huidige collega's uit het Julius Centrum heel hartelijk bedanken. Beste Michiel en Ronald, bedankt voor de mogelijkheid om binnen het Prospect-cohort analyses te mogen doen. Beste Yvonne en Rick, jullie ook bedankt voor de prettige samenwerking. Ik ben dan ook erg blij dat we deze samenwerking de komende jaren op intensievere basis kunnen voortzetten.
Luc, Maurice en René, heel erg bedankt voor jullie hulp bij de spierbiopten en kritische commentaren op het artikel. Dear Aad van Tol, Natalie Fournier, Jean-Louis Paul, Joachim Spranger, Thomas Bobbert and Anders Helander, thanks for your interest in our research, pleasant collaboration and (free) blood analyses!!

Natuurlijk had ik de afgelopen jaren veel collega-AIO's in Wageningen. Ook al had ik niet de mogelijkheid om iedereen vaak te treffen, ik vond het toch steeds weer erg gezellig om even op de universiteit te zijn en bij te praten. De reis naar UK/Ierland vond ik ook bijzonder geslaagd. Mariëlle en Brian, omdat we samen voor onze promotie het doorstroomprogramma doorlopen hebben, waren jullie toch wel het eerste aanspreekpunt in Wageningen. Dit was altijd weer gezellig. Ook met andere AIO's, Akke, Renate, Martinette, Gerda, Marieke, was het goed om ervaringen uit te wisselen.

Natuurlijk wil ik ook mijn vrienden, Severina, Petra, Ivonne, Maaike, Lenny en Frank heel hartelijk bedanken voor hun interesse in mijn onderzoek, maar vooral voor de nodige ontspanning. In het bijzonder wil ik Marieke en Marjolein bedanken. Marieke, ik vind het te gek dat je me wilde helpen met het ontwerpen van de kaft. Het was leuk om hier samen aan te werken en het is erg mooi geworden. Marjolein, ook al zijn onze vakgebieden verschillend, de afgelopen jaren waren we toch ook wel een beetje samen aan het promoveren. Bedankt voor een luisterend oor en gezelligheid. Ik ben dan ook blij dat jij mijn paranimf wilt zijn. Ik ben ervan overtuigd dat nog voor de zomer van 2007 de rollen zijn omgedraaid.

Tot slot zou ik mijn familie heel hartelijk willen bedanken. Arjon, Myrthe, Hugo, Geert, Janne en Corrie, ik ben ontzettend blij dat we altijd weer op elkaar kunnen terugvallen. Bedankt voor alle gezellige familieavondjes en de bezoekjes in Boston. Lieve Wendy, ik ben ontzettend blij dat je me aanbood te helpen met de opmaak van mijn boekje. Het is ontzettend mooi geworden. Maar voornamelijk ben ik blij dat ik altijd bij mijn 'grote' zus terecht kan voor wat voor zaken dan ook.

Lieve papa en mama, jullie zijn een enorme stimulans geweest voor elke stap die ik tot nu genomen heb. Zeker de afgelopen tijd heb ik mezelf vaak gelukkig geprezen met ouders waar ik altijd voor wat dan ook bij terecht kan. Bedankt voor jullie onvoorwaardelijke vertrouwen, steun en interesse bij alles wat ik doe. Mam, ik vind het bijzonder leuk dat je al mijn artikelen (probeert) te lezen.

Lieve Peter, de afgelopen jaren heb je me door dik en dun gesteund bij alles wat ik doe, zelfs een verblijf in Boston. Ik heb je daar ongelooflijk gemist, maar we hebben er samen ook een mooie tijd gehad. Bedankt dat ik steeds weer bij je terecht kan voor relativerende woorden als ik me weer eens druk maak over de kleine dingen des levens. Nu hebben we dan eindelijk wat meer tijd voor elkaar en ik hoop dat we die tijd dan ook nog jaren samen kunnen doorbrengen.

About the author

CURRICULUM VITAE

Joline Beulens was born on April 12 1976 in Made, The Netherlands. After completing secondary school at the St. Oelbert Gymnasium in Oosterhout. she started the bachelor program 'Nutrition and Dietetics' at 'Hogeschool van Amsterdam'. Within this program she performed internships at the University Medical Center Rotterdam, University of North London, and a research project at University Medical Center Utrecht (awarded by the NVVL and honorable mention Novartis award) and obtained her degree in September 1999. She enrolled in the Master-program 'Nutrition and Health' at Wageningen University and also worked in this period as a dietician in the University Medical Center Utrecht for the departments of cardiology, lung diseases and allergology. She conducted a human intervention study on α-lactalbumin and serotonin synthesis at Numico Research BV and obtained her degree with distinction in September 2001. Subsequently she started working as a Scientist Nutrition at Numico Research BV in the area of clinical nutrition.

In August 2003, Joline started her PhD-research on alcohol consumption and risk of cardiovascular disease and diabetes at Wageningen University. She was detached at TNO Quality of Life to perform human intervention studies with moderate alcohol consumption. She was also involved in epidemiological analyses on alcohol consumption and risk of type 2 diabetes and cardiovascular disease in several large prospective cohort studies. Within this project, she collaborated with several (inter)national research groups such as Julius Center for Health Sciences and Primary Care, Maastricht University and Karolinska Institute. She worked at the Harvard University School of Public Health on research after alcohol consumption and risk of cardiovascular disease among hypertensive men in collaboration with Dr Eric Rimm and Dr Ken Mukamal. Part of the research described in this thesis was awarded by the 'Foppe ten Hoor' young investigators award. Since March 2006, she was appointed as a postdoctoral fellow at Julius Center for Health Sciences and Primary Care to continue research in the area of nutrition and risk of cardiovascular disease and diabetes.

PUBLICATIONS

Published papers

1. Joline WJ Beulens, Henk FJ Hendriks. Alcohol and ischaemic heart disease. Lancet (2006); 18; 367(9514): 902.
2. Joline WJ Beulens, Roderik M van Beers, Ronald P Stolk, Gertjan Schaafsma, Henk FJ Hendriks. The effect of moderate alcohol consumption on fat distribution and adipocytokines. Obesity Research (2006); 14(1): 60-66.
3. Joline WJ Beulens, Ronald P Stolk, Yvonne T van der Schouw, Diederick E Grobbee, Henk FJ Hendriks, Michiel L Bots. Alcohol consumption and risk of diabetes mellitus type 2 among older women. Diabetes Care (2005); 28 (12): 2933- 2938.
4. Marijke J Van Trijp, Joline WJ Beulens, WJ Bos, Cuno S Uiterwaal, Diederick E Grobbee, Henk FJ Hendriks, Michiel L Bots. Alcohol consumption and augmentation index in healthy young men: the ARYA study. American Journal of Hypertension (2005); 18(6):792-6.
5. Joline WJ Beulens, Aafje Sierksma, Gertjan Schaafsma, Frans J Kok, Eduard A Struys, Cornelis Jakobs, Henk FJ Hendriks. Kinetics of homocysteine metabolism after moderate alcohol consumption. Alcohol Clin Exp Res (2005); 29(5):739-45.
6. Joline WJ Beulens, Aafje Sierksma, Arie van Tol, Natalie Fournier, Teus van Gent, Jean-Louis Paul, Henk FJ Hendriks. Moderate alcohol consumption increases cholesterol efflux mediated by ABCA1. Journal of Lipid Research (2004); 45; p. 1716-1723.
7. Joline WJ Beulens, Jacques G Bindels, Kees de Graaf, Martine Alles, Wendeline Wouters-Wesseling. Alphalactalbumin combined with a regular diet increases plasma Trp-LNAA ratio. Physiology & Behavior (2004); 81; p. 585- 593.
8. Ronald Houwing, Marja Rozendaal, Wendeline Wouters-Wesseling, Joline WJ Beulens, Eric Buskens, Jeen Haalboom. The effect of nutritional supplementation on the prevention of pressure ulcers in hip-fracture patients. Clinical Nutrition (2003); 22 (4): p. 401-405.
9. Joline WJ Beulens, Lenny MW van Venrooij, Hans CM Schreurs. [De invloed van dialysemethode op gewichtstoename na niertransplantatie. Nederlands Tijdschrift voor Diëtisten (2001); 57 (6): p. 121-124.] In Dutch

Accepted papers

1. Joline WJ Beulens, Eric B Rimm, Alberto Ascherio, Donna Spiegelman, Henk FJ Hendriks, Kenneth J Mukamal. Alcohol consumption and risk of coronary heart disease among hypertensive men. Annals of Internal Medicine

Submitted papers

1. Joline WJ Beulens, Robin van den Berg, Frans J Kok, Anders Helander, Susan HF Vermunt, Henk FJ Hendriks. The effect of moderate alcohol consumption on lipoprotein-associated phospholipase A2 activity: a randomized controlled intervention.
2. Joline WJ Beulens, Eric B Rimm, Henk FJ Hendriks, Frank B Hu, JoAnn E Manson, David J Hunter, Kenneth J Mukamal. Alcohol consumption and type 2 diabetes: influence of genetic variation in alcohol dehydrogenase.
3. Joline WJ Beulens, Emilie C de Zoete, Frans J Kok, Gertjan Schaafsma, Henk FJ Hendriks. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight young men: a diet interventions study.
4. Joline WJ Beulens, Luc JC van Loon, Frans J Kok, Maurice Pelsers, Thomas Bobbert, Joachim Spranger, Anders Helander, Henk FJ Hendriks. The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity; a human intervention study.
5. Joline WJ Beulens, Leonie M de Bruijne, Ronald P Stolk, Petra HM Peeters, Michiel L Bots, Diederick E Grobbee, Yvonne T van der Schouw. High dietary glycemic load and glycemic index increase risk of cardiovascular disease; a population-based follow-up study.
6. Robin van den Berg, Aafje Sierksma, Joline WJ Beulens, Wouter Vaes, Henk FJ Hendriks. Effect of moderate alcohol consumption on biomarkers of oxidative stress.

Abstracts

1. Joline WJ Beulens, Eric B Rimm, Alberto Ascherio, Henk FJ Hendriks, Kenneth J Mukamal. Circulation (2006); 113 (8): E315-E316; P26.
2. Joline WJ Beulens, Eric B Rimm, Frank B Hu, JoAnn E Manson, Henk FJ Hendriks, Kenneth J Mukamal. Circulation (2006); 113 (8): E321; P52.
3. Joline WJ Beulens, Gertjan Schaafsma, Frans J Kok, Henk FJ Hendriks. Alcoholism Clin Exp Res (2004); 28 (8) S1; p. 7A.

4. Joline WJ Beulens, Aafje Sierksma, Arie van Tol, Natalie Fournier, Teus van Gent, Jean-Louis Paul, Henk FJ Hendriks. Alcoholism Clin Exp Res (2004); 28 (8) S1; p. 7A.
5. Joline WJ Beulens, Jacques G Bindels, Kees de Graaf, Martine S Alles, Wendeline Wouters-Wesseling. Clinical Nutrition (2002); 21 (S1); p. 64.
6. Joline WJ Beulens, Jacqes G Bindels, Kees de Graaf, Martine S Alles, Wendeline Wouters-Wesseling. Dutch Endoneuromeeting, Doorwerth, June 4-7, 2002.
7. Ronald Houwing, Marja Rozendaal, Wendeline Wouters-Wesseling, Joline WJ Beulens, Eric Buskens, Jeen Haalboom. Clinical Nutrition (2002); 21 (S1); p. 84.
8. Joline WJ Beulens, Zandrie Hofman, George Verlaan, Rolf Smeets, Harm Kuipers, Aalt Bast. Clinical Nutrition (2002); 21 (S1); p. 17-18.
9. Hans CM Schreurs, Joline WJ Beulens, Lenny MW van Venrooij. EDTNA/ERCA Journal (2000); 26 (S1): p. 52.

EDUCATIONAL PROGRAM

DISCIPLINE SPECIFIC ACTIVITIES

Courses

ESPEN short course “Tracer methodology in metabolism”	2004
Harvard University course “Genetic epidemiology of diabetes”	2005
Harvard University course “Use of biomarkers in epidemiological research”	2005
Erasmus Summer Program course “Causal inference”	2006
Erasmus Summer Program course “Principles of genetic epidemiology”	2006

Symposia and congresses

Food Summit “Diet and the metabolic syndrome”	2004
European Society for Clinical Investigation (ESCI) Annual Scientific meeting	2004
International Society for Biomedical Research on Alcoholism (ISBRA) World Conference	2004
46th American Heart Association (AHA) Conference on Cardiovascular Disease Epidemiology and Prevention	2005
The IEA-EEF European Congress of Epidemiology	2006
NWO-voedingsdagen	2003- 2006
34th International Medical Advisory Group (IMAG) Conference	2006

GENERAL COURSES

VLAG introduction course	2004
University Utrecht course “Practical statistics for microarray data”	2004
Harvard University course “Applied survival analysis”	2005

OPTIONAL COURSES AND ACTIVITIES

Preparation of research proposal	2003
PhD-study Tour	2005
Journal Club	2003-2006
Visiting Scholar Harvard University School of Public Health (dept. of nutrition; 6 months)	2005

The studies described in this thesis were performed at TNO Quality of Life, Zeist. This work was financially supported by the Dutch Ministry of Economic Affairs, Dutch Foundation for Alcohol Research, a travel grant from the Dutch Heart Assocation and a research exchange award from the European Research Advisory Board.

The printing of this thesis was supported by:
Wageningen University, Wageningen, The Netherlands
TNO Quality of Life, Zeist, The Netherlands
Heineken International, Amsterdam, The Netherlands
Centraal Brouwerij Kantoor, Amsterdam, The Netherlands
Productschap Wijn, Den Haag, The Netherlands
Commissie Gedistilleerd van het Productschap Dranken, Schiedam, The Netherlands
Brewers of Europe, Brussels, Belgium

Design cover: Marieke Gaymans & Joline Beulens
Lay-out: Wendy Schmidt-Beulens & Joline Beulens
Printed by: Ponsen & Looijen BV, Wageningen, The Netherlands