Influence of moderate alcohol consumption on emotional and physical well-being

Ilse C. Schrieks

Thesis committee

Promotor
Prof. Dr R.F. Witkamp
Professor of Nutrition and Pharmacology
Wageningen University

Co-promotor
Dr H.F.J. Hendriks
Consultant for TNO, Zeist

Other members
Prof. Dr J.C.M. van Trijp, Wageningen University
Dr K.J. Mukamal, Harvard Medical School, Boston MA, U.S.A.
Prof. Dr C.R. Markus, Maastricht University
Dr J.W.J. Beulens, UMC Utrecht / VUmc Amsterdam

This research was conducted under the auspices of the Graduate School VLAG (Advanced studies in Food Technology, Agrobiotechnology, Nutrition and Health Sciences).

Influence of moderate alcohol consumption on emotional and physical well-being

Ilse C. Schrieks

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University
by the authority of the Academic Board,
in the presence of the
Thesis Committee appointed by the Academic Board
to be defended in public
on Monday 8 June 2015
at 11 a.m. in the Aula.

Ilse C. Schrieks
Influence of moderate alcohol consumption on emotional and physical well-being,
212 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)
With references, with summaries in Dutch and English

ISBN 978-94-6257-290-4

Abstract

Background and aim: Moderate alcohol consumption has been suggested to contribute to emotional well-being. However, the effects of moderate alcohol consumption on emotional well-being in common drinking situations and the influence of alcohol on physical well-being remain unclear. The aims of this thesis were 1) to further explore the acute effects of moderate alcohol consumption on emotional well-being and the association between habitual alcohol consumption and emotional well-being and 2) to provide more insight into physiological markers that may be related to alcohol-induced emotional well-being.

Methods: We compared the acute effects of alcohol (20-30 g) vs. alcohol-free drinks on mood, food reward and mental stress in three randomized crossover trials. To explore the short-term effects of alcohol on physiological markers of emotional well-being, we conducted four randomized crossover trials of 3-6 weeks in which 25-41 g alcohol/day, or no alcohol was consumed. In addition, we conducted a meta-analysis of 14 randomized intervention trials with at least 2 weeks of alcohol intervention. Finally, the association between long-term alcohol consumption and health-related quality of life was investigated with a bidirectional, longitudinal analysis among 92,448 U.S. women of the Nurses' Health Study II cohort.

Results: Moderate alcohol consumption in an unpleasant ambiance resulted in higher happiness scores in women as compared to the consumption of alcohol-free drinks. Consumption of 20 gram alcohol increased subsequent intake and rewarding value of savoury foods in men, as measured by an increased implicit wanting and explicit liking of savoury foods. When alcohol was consumed by male volunteers immediately after a mental stressor, a reduced response of the stress hormones ACTH and cortisol, the inflammatory marker IL-8, and the percentage of monocytes in blood were observed. Furthermore, alcohol consumption was found to attenuate meal-induced NF-κB and to increase total antioxidant capacity in men. Four weeks of moderate alcohol consumption reduced circulating fetuin-A, while increasing urinary F2-isoprostanes in men. In women, short-term moderate alcohol consumption did not reduce fetuin-A but it tended to increase insulin sensitivity. Habitual moderate alcohol consumption was associated with a higher physical health-related quality of life 2 years later. Vice versa, higher physical health-related quality of life was associated with a higher alcohol intake 2 years later. Moderate alcohol consumption was not associated with mental health-related quality of life in either direction, although moderate alcohol consumption was associated with higher scores on the scales for social functioning and vitality.

Conclusions: Moderate alcohol consumption may acutely improve emotional well-being by improving mood, increasing food reward and reducing mental stress. In the short-term, moderate alcohol consumption may attenuate meal-induced oxidative stress and circulating

fetuin-A in men. In women, moderate alcohol consumption may improve insulin sensitivity. Habitual moderate alcohol consumption may be associated with a small increase in physical health related quality of life but not with mental health related quality of life in women.

Table of Contents

Chapter 1

General introduction

Alcohol consumption has been part of most cultures for thousands of years. As a consequence, we have become familiar with the possible negative consequences of drinking alcohol as well. At the same time, a combination of its mood effects and its predominant positive social and cultural associations have resulted in a rather wide-spread societal acceptance of alcohol consumption in moderation. Both short en long-term health effects of alcohol consumption have been researched for quite some time. In particular, the influence of alcohol consumption on chronic diseases, such as cardiovascular disease and type 2 diabetes, has received most attention during the last decades (1-3). However, alcoholic beverages are commonly not consumed for their long-term effects but most often because of their mood effects, such as relaxation and enhanced mood (4). In contrast to the large body of evidence on chronic diseases, the effects of alcohol on long-term emotional and physical well-being and their possible relations are less well understood (5, 6).

Well-being, mood and physical health

The World Health Organization (WHO) defines health as 'a complete state of physical, mental and social well-being and not merely the absence of disease or infirmity' (7). However, research has thus far often been focused on measuring the absence of disease or physical impairment, or markers which are indicative for risk and health deterioration. More in agreement with the WHO definition of health, research is gaining more interest during the last decades in measuring positive indicators of physical and mental health. For example, the study of subjective well-being is a growing research field (8).

Subjective well-being encompasses three components: evaluative well-being, hedonic well-being and eudemonic well-being. Evaluative well-being is defined as satisfaction with life, such as thoughts about the quality of life and contentment with life. Hedonic well-being (sometimes referred to as affective well-being) includes negative and positive feelings, mood or emotions. Eudemonic well-being refers to judgements about the meaning and purpose of life (9, 10).

Measures of subjective well-being are often divided in trait levels of well-being (e.g. life satisfaction) and transient (state level) affect, moods and emotions. Both life satisfaction and positive affect have been associated with better physical health outcomes (11, 12). For example, Steptoe and Wardle (2005) showed that people with higher positive affect have a lower fibrinogen response on stress (13). Positive affect has also been related to a lower incidence of stroke and more effective immune functions (12). Life satisfaction has been related to a lower risk on mortality from cardiovascular disease and renal failure (14). Likewise, subjective well-being has been related to an attenuated stress induced response in cortisol (11). These data suggest that well-being is directly related to a better immune function and responsivity to stress.

Long term effects of moderate alcohol consumption

Chronic alcohol intake has profound effects on health: excessive alacohol consumption has clear detrimental health effects, whereas moderate alcohol consumption (see text box for definitions) has been shown to contribute to a number of positive health outcomes (15).

Health benefits of moderate alcohol consumption

The association between alcohol and mortality has been extensively researcvvhed. The results of a meta-analysis of 34 prospective studies showed a J-shaped association: mortality risk is lower in people drinking moderately, but it is higher in heavy alcohol consumers (16). Furthermore, a large prospective study in women showed that moderate alcohol consumption may contribute positively to successful aging (i.e. being free of 11 major chronic diseases and having no major impairments in cognitive, physical and mental health) (17).

What is moderate?

Moderate drinking is defined in the US Dietary Guidelines as no more than one drink daily for women and two for men (9). In the Netherlands, alcohol consumption is also advised to be no more than one standard unit per day for women and two standard units per day for men (10). However, there is no universal definition of one glass or one standard unit. In the United States, one glass contains approximately 14 to 15 g alcohol depending on the type of beverage, whereas in the Netherlands one standard unit contains 10 g alcohol irrespective of the type of beverage (i.e. 250 mL beer, 100 mL wine and 35 mL spirits).

An apparently protective effect of moderate alcohol consumption has been shown for specific diseases: moderate drinking is associated with a reduced risk for certain diseases, including cardiovascular diseases (1), type 2 diabetes (2) and dementia (18), in comparison to abstention or heavy alcohol consumption.

These observations are supported by intervention studies revealing several potential biological mechanisms for the beneficial effects of alcohol on cardiovascular disease and type 2 diabetes. Moderate alcohol consumption increases HDL cholesterol and stimulates reverse cholesterol transport (19). Furthermore, it increases paraoxonase activity, it stimulates fibrinolysis (20) and decreases coagulation (21). Additionally, higher adiponectin levels (22, 23) and anti-inflammatory effects (24, 25) may contribute to the protective effects of alcohol.

Parallel to the research on the association between alcohol and physical health, there is a large body of research on the association between alcohol and psychosocial health. Moderate alcohol consumption has been suggested to be beneficial for emotional and social well-being. It may contribute to a reduced psychological distress, an increased sociability and an enhanced mood (4). Furthermore, cross-sectional studies suggest an association between

moderate alcohol consumption and higher health-related quality of life (26-31), subjective well-being (32), subjective health (33) and life satisfaction (28). However, these associations may be biased by reverse causation, and prospective data is scarce (34).

Health risks of moderate alcohol consumption

Moderate alcohol consumption can also have harmful effects on health. Alcohol consumption increases the relative risk on breast cancer among women in a linear fashion by 8% per extra glass/day. This means that even at a low habitual alcohol intake, breast cancer risk is increased (35) compared to abstinence from alcohol. Additionally, alcohol consumption increases the risk on cancers of the upper digestive and respiratory tract in a dose-dependent manner (oral and pharyngeal cancer, oesophageal squamous cell carcinoma and laryngeal cancer) (36).

The possible harmful effects of alcohol increase when consumption rises above moderation. With high intakes of alcohol (≥45 g/d) the risk on colorectal cancer increases with 40% (37). Also, a higher risk on liver cirrhosis and liver cancer (hepatocellular cancer) has been associated with excessive alcohol intake (38). Furthermore, alcohol consumption is positively associated with accidents and violence (39).

Acute effects of moderate alcohol consumption

Pharmacokinetics of alcohol

Alcohol is rapidly absorbed after ingestion. Several factors influence the rate of absorption, such as the amount and concentration ingested, and the presence and properties of food in the stomach. Although some absorption occurs in the stomach (~5%), alcohol is primarily absorbed in the intestine. After absorption from the intestine, alcohol is transported via the portal vein to the liver, where a portion is directly metabolized. During this process, often called first-pass metabolism, alcohol is converted into acetaldehyde by alcohol dehydrogenase (ADH). Acetaldehyde is highly toxic and rapidly converted into acetate by aldehyde dehydrogenase (ALDH) (40, 41). Most of the acetate is released in the blood and oxidized to CO_2 and H_2O in the citric acid cycle (Figure 1.1). A small portion of acetate is converted to carbohydrates, lipids or proteins and incorporated in tissues (42).

When ingested on an empty stomach, peak blood concentrations are reached 30-60 min after alcohol consumption. When alcohol consumption is combined with food, the absorption rate of alcohol is slower. As alcohol shows capacity limited (‘zero-order’) metabolism, more alcohol can now be metabolized during first-pass metabolism, resulting in lower blood alcohol concentrations (BAC). As a consequence, a moderate dose of alcohol may result in an approximately two times lower BAC when consumed together with a meal (40, 41). The type of beverage also influences the BAC curve. Consumption of an equal amount of alcohol as spirits, wine or beer, showed that spirits induce the highest and earliest peak BAC, followed

by wine and then by beer (43). Alcohol contains a polar hydroxyl group, which makes it highly soluble in water. Therefore, the distribution of alcohol over the body mainly depends on the water content of organs and tissues (44).

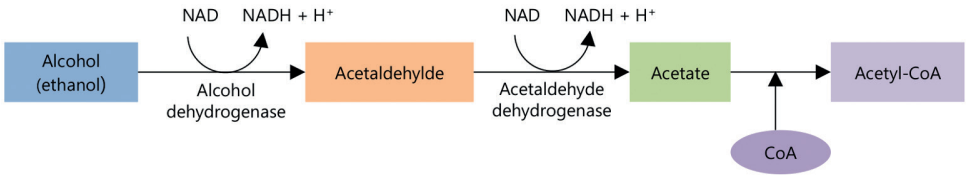


Figure 1.1. Alcohol metabolism by alcohol dehydrogenase and acetaldehyde dehydrogenase

Acute effects on psychological well-being

The acute influence of moderate alcohol consumption on behaviour follows a biphasic pattern (Figure 1.2). During rising BACs primarily stimulatory effects occur, such as increased arousal, cheerfulness, social interaction and loss of inhibitions. During declining BACs more sedative effects are reported, such as relaxation and sleepiness (41, 45-47). Although stimulation and sedation are opposite effects, they can occur at the same time. This occurs mainly around peak BACs (46). The experience of stimulation and sedation also depends on the dose. Higher intakes tend to induce more sedation, while lower intakes induce more stimulation (48).

These behavioural effects of alcohol consumption are mainly a result of alcohol's interaction with the neuronal systems of the brain. Alcohol generally inhibits central nervous system activity. It primarily influences the gamma-aminobutyric acid (GABA) and glutaminergic neurotransmitter systems. Alcohol stimulates the activity of GABA, the main inhibitory neurotransmitter, and inhibits that of glutamate, the main excitatory neurotransmitter. Additionally, it enhances serotonergic, dopaminergic and opioid neurotransmission (49). Also, a role of endocannabinoids is suggested in the behavioural effects of alcohol (50, 51). An experiment in rats showed that after oral alcohol self-administration, the dialysate 2-arachidonoylglycerol (2-AG) concentrations increased and followed the same pattern as the blood alcohol concentration (52).

Furthermore, alcohol consumption has been shown to acutely increase heart rate and skin conductance level during rising BACs (53-55). In addition, epinephrine levels were increased immediately after moderate drinking (56). This suggests that alcohol increases sympathetic nervous system activity during the initial stages following intake, which may be related to the higher arousal during rising BACs (57). Parasympathetic activity is decreased by alcohol shortly after consumption. However, from previous research it is not clear if this effect is limited to the rising part of the plasma curve (54).

The tension-reducing effects of moderate alcohol doses have been attributed to a decrease in

hypothalamic-pituitary-adrenal (HPA) axis activity after consumption. This would be in line with alcohol's enhancing effects on GABA and its inhibiting effects on glutamate neurotransmission, resulting in a net inhibition of HPA-axis activity. The effect of alcohol on HPA-axis activity seems to be dose-dependent, with lower dosages of alcohol consumption reducing HPA-axis activity while higher doses (>0.8 g/kg) having a stimulating effect on HPA-axis activity (58).

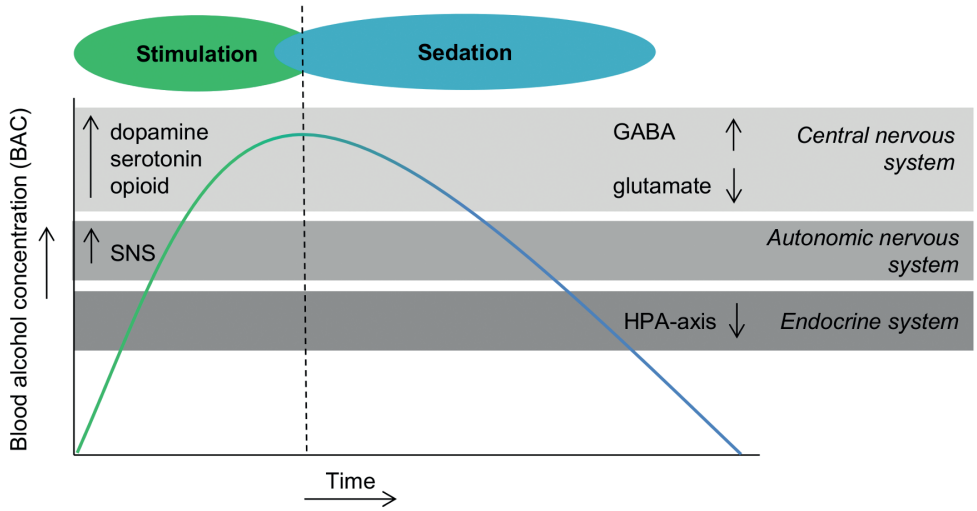


Figure 1.2. The acute biphasic behavioural and neuroendocrine effects of moderate alcohol consumption.

The stimulatory effects of alcohol have been related to increased activity of the dopaminergic, serotonergic and opioid neurotransmitter systems and increased sympathetic nervous system (SNS) activity. The sedative effects are caused by stimulation of gamma-aminobutyric acid (GABA) and inhibition of glutamate neurotransmission (46, 54). Moderate alcohol consumption attenuates hypothalamic-pituitary-adrenal (HPA) axis activity(58).

Rewarding effects of alcohol

The stimulatory effects of alcohol contribute to the rewarding properties of alcohol. People who experience relatively more stimulating effects and less sedative effects after drinking report more euphoria and liking of alcohol, and are thought to be more vulnerable to the addictive properties of alcohol. Indeed, heavier drinkers and binge drinkers experience more stimulant-like effects as compared to light drinkers. This may increase the risk for drinking alcohol again and for developing alcohol-related problems (45, 59, 60). Although some sedative effects, like reduction of tension, can be experienced as pleasant, these are in general considered less pleasant than the stimulatory effects. Sedative effects can also motivate drinking behaviour. However, generally people start drinking primarily because of the stimulatory effects but when they become dependent on alcohol they drink more to reduce anxiety and tension feelings (46).

The activation of the brain's mesocorticolimbic reward circuit is primarily causing the

stimulatory effects of alcohol. It is likely that multiple neurotransmitter systems are involved in the rewarding effects of alcohol, including the dopaminergic, opioidergic, glutamatergic, GABAergic and serotonergic systems (61). The addictive properties of alcohol are believed to be mainly driven by its enhancing effect on dopamine signalling, since dopamine is the main neurotransmitter mediating the motivation to drink (46, 62). According to the incentive sensitization theory of addiction, reward comprises two components: 'liking' and 'wanting'. These can be divided both psychologically and neurologically. Psychologically, wanting refers to the motivation to approach and obtain a stimulus, and liking refers to the pleasantness of the stimulus or the pleasure derived from the stimulus. Neurologically, the dopamine system is mainly responsible for wanting, whereas opioid, GABA and endocannabinoid neurotransmission are assumed to be mainly involved in liking. The sensitization of wanting and the enhancement of dopamine are suggested to be responsible for compulsive drug use and the development of addiction (63-65).

Acute effects on psychological distress

Because of the ability of alcohol to reduce tension, there has been a lot of interest in the influence of alcohol consumption on the stress response. In 1980, Levenson et al. proposed the stress-response dampening model of alcohol (53). They showed that alcohol consumption prior to a stressor (either electric shock or speech) reduced anxiety and heart rate during the stress response as compared to the placebo condition. Furthermore, a reduced activity of the HPA-axis has been reported after alcohol consumption, as measured by ACTH and cortisol hormones (66, 67). However, also an increased activation of the HPA axis by alcohol has been shown (68). The inconsistency in outcomes may be due to the higher dosages (0.75 and 1.1 mL/kg) used in the latter study. Furthermore, Balodis et al. (2011) showed that the alcohol induced-reductions in the cortisol and tension score response to stress were of the same order of magnitude as those seen after the anticipation of alcohol only. This indicates that it is of high importance to take into account the expectation effects in addition to the pharmacological effects of alcohol consumption on stress response dampening (67).

Aims and outline of this thesis

Previous research shows that the acute effects of alcohol consumption on well-being are valid for low to moderate doses of alcohol and that these occur in a biphasic pattern. Previous data also suggest that the higher the stimulatory effects after consumption are, the more rewarding the drinking experience is. The tension reducing effects of alcohol were also effective during psychological distress.

However, several knowledge gaps remain about the effects of moderate alcohol consumption on well-being and psychological distress during situations that are representative for more common consumption patterns, such as alcohol consumption with a meal and after

psychological stress. Additionally, long-term effects of alcohol on well-being have thus far mainly been measured by cross-sectional studies which demands for further substantiation by prospective observational studies. Furthermore, it is not understood to what extent acute psychological effects of alcohol consumption influence physical well-being.

We thus aimed to:

1. further explore the acute effects of moderate alcohol consumption on emotional well-being and the association between habitual alcohol consumption and emotional well-being.
2. provide more insight in the influence of moderate alcohol consumption on physiological determinants and health outcomes related to emotional well-being.

How the chapters in this thesis contribute to these aims is shown in Figure 1.3.

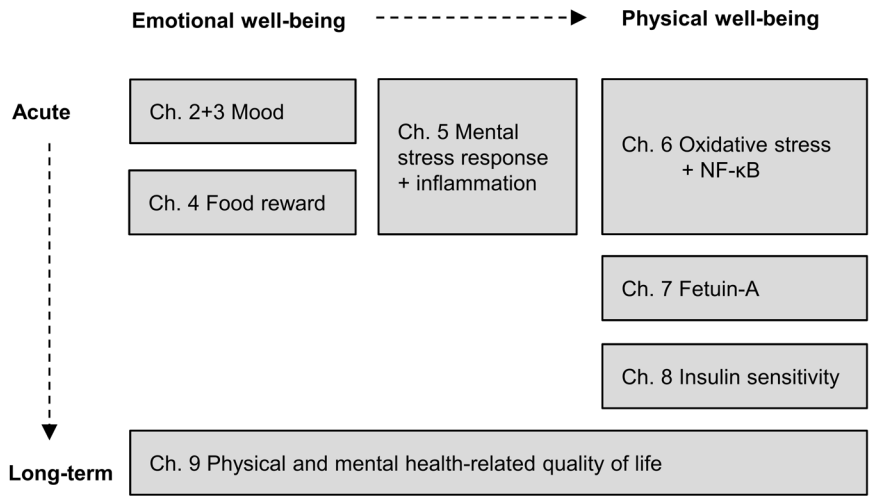


Figure 1.3. Schematic overview of the aims of this thesis and the contribution of the chapters to these aims.

Acute moderate alcohol consumption and emotional well-being

Alcohol is commonly consumed around mealtimes. Therefore, we have investigated the influence of moderate alcohol consumption with dinner on mood and autonomic nervous system activity in women in **chapter 2**. In addition, we investigated whether alcohol influenced mood differently when consumed during a current pleasant or unpleasant mood. To bring people in these mood states, we developed a mood induction model by creating a pleasant or

unpleasant meal ambiance.
In **chapter 3**, we further examined the effects of mood changes induced by the meal ambiance and moderate alcohol consumption on plasma endocannabinoids (2-arachidonoylglycerol (2-AG), anandamide (AEA)) and some N-acylethanolamine (NAE) congeners. Endocannabinoids have been suggested to play a role in the behavioural effects of alcohol. However, the effect of moderate alcohol consumption on plasma endocannabinoids in humans is not clear yet, especially not in relation to alcohol's induced mood changes.

Despite the well-known rewarding properties of alcohol, the influence of alcohol on the reward response of subsequent food intake has not been measured before. When alcohol is consumed before or with a meal, it increases food intake. In **chapter 4** we investigated whether alcohol increases food intake by increasing food reward in men. In addition, we explored the role of gut or oral nutrient sensing in alcohol's effect on food reward.

Although several studies showed a stress-dampening effect of alcohol when consumed before a mental stressor, data on the influence of moderate alcohol consumption on the stress response when consumed after the stressor are limited. Therefore, we evaluated the physiological stress response of a mental stressor followed by alcohol consumption in men in **chapter 5**. We also explored the effect of alcohol consumption on the stress-induced immune response.

These first three studies were all performed in a controlled setting (human intervention trials) and focused on the acute effects of moderate alcohol consumption on different aspects of emotional well-being: mood, reward and psychological distress.

Acute and short-term moderate alcohol consumption and physical well-being
Emotional well-being has been related to a better immune function and stress-buffering capacity. To further explore the effect of moderate alcohol consumption on physiological determinants of emotional well-being, we investigated the influence of moderate alcohol consumption with a meal on postprandial levels oxidative capacity and NF-κB (nuclear transcription factor playing an important role in inflammation), and urinary F2-isoprostanes in men in **chapter 6**. This study was a randomized intervention trial and focused on the acute and short-term effects of alcohol on physical well-being.

There is a clear association between habitual moderate alcohol consumption and a lower risk of type 2 diabetes. However, the acute effects of moderate drinking on inflammation and insulin sensitivity, two factors that might explain this relation, are not completely understood. To determine the effects of several weeks of moderate alcohol consumption on inflammation markers and insulin sensitivity, we conducted two studies. In **chapter 7** we evaluated alcohol's

influence on fetuin-A in three randomized intervention studies in both men and women. Additionally, we conducted a meta-analysis on intervention studies to evaluate the influence of short-term alcohol consumption (≥2 weeks) on insulin sensitivity and glycemic status (**chapter 8**).

Habitual alcohol consumption and emotional and physical well-being

A positive association between habitual moderate alcohol consumption and well-being has been suggested by cross-sectional studies. In **chapter 9** we investigated the bidirectional associations between alcohol consumption and mental and physical health-related quality of life in women by the use of longitudinal analyses.

References

1. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ. 2011;342:d671.
2. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, et al. Alcohol as a Risk Factor for Type 2 Diabetes: A systematic review and meta-analysis. Diabetes Care. 2009;32(11):2123-32.
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(3):719-25.
4. Peele S, Brodsky A. Exploring psychological benefits associated with moderate alcohol use: a necessary corrective to assessments of drinking outcomes? Drug Alcohol Depend. 2000;60(3):221-47.
5. Cooper ML. Motivations for alcohol use among adolescents: Development and validation of a four-factor model. Psychol Assess. 1994;6(2):117.
6. Cooper ML, Frone MR, Russell M, Mudar P. Drinking to regulate positive and negative emotions: a motivational model of alcohol use. J Pers Soc Psychol. 1995;69(5):990.
7. World Health Organization. Preamble to the constitution of the WHO as adopted by the International Health Conference, New York, 19–22 June 1946. Geneva: WHO. 1948.
8. de Chavez AC, Backett-Milburn K, Parry O, Platt S. Understanding and researching wellbeing: Its usage in different disciplines and potential for health research and health promotion. Health Educ J. 2005;64(1):70-87.
9. Steptoe A, Deaton A, Stone AA. Subjective wellbeing, health, and ageing. The Lancet. 2015;385:640-48.
10. Jovanovic V. Personality and subjective well-being: One neglected model of personality and two forgotten aspects of subjective well-being. Personality and Individual Differences. 2011 04;50(5):631-5.
11. Howell RT, Kern ML, Lyubomirsky S. Health benefits: Meta-analytically determining the impact of well-being on objective health outcomes. Health Psychology Review. 2007;1(1):83-136.
12. Dockray S, Steptoe A. Positive affect and psychobiological processes. Neuroscience & Biobehavioral Reviews. 2010;35(1):69-75.
13. Steptoe A, Wardle J, Marmot M. Positive affect and health-related neuroendocrine, cardiovascular, and inflammatory processes. Proc Natl Acad Sci U S A. 2005;102(18):6508-12.
14. Chida Y, Steptoe A. Positive Psychological Well-Being and Mortality: A Quantitative Review of Prospective Observational Studies. Psychosom Med. 2008;70(7):741-56.
15. Ferreira MP, Willoughby D. Alcohol consumption: the good, the bad, and the indifferent. Applied Physiology, Nutrition, and Metabolism. 2007;33(1):12-20.
16. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med. 2006;166(22):2437-45.
17. Sun Q, Townsend MK, Okereke OI, Rimm EB, Hu FB, Stampfer MJ, et al. Alcohol consumption at midlife and successful ageing in women: a prospective cohort analysis in the nurses' health study. PLoS medicine. 2011;8(9):e1001090.
18. Peters R, Peters J, Warner J, Beckett N, Bulpitt C. Alcohol, dementia and cognitive decline in the elderly: a systematic review. Age Ageing. 2008;37(5):505-12.
19. Sierksma A, Gaag MS, Tol A, James RW, Hendriks HF. Kinetics of HDL cholesterol and paraoxonase activity in

moderate alcohol consumers. Alcohol Clin Exp Res. 2002;26(9):1430-5.

20. 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):1003-6.

21. Dimmitt S, Rakic V, Puddey I, Baker R, Oostryck R, Adams M, et al. The effects of alcohol on coagulation and fibrinolytic factors: a controlled trial. Blood Coagulation Fibrinol. 1998;9(1):39-46.

22. Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, Heine RJ, et al. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-α, and insulin sensitivity. Diabetes Care. 2004;27(1):184-9.

23. Beulens JW, Rimm EB, Hu FB, Hendriks HF, Mukamal KJ. Alcohol consumption, mediating biomarkers, and risk of type 2 diabetes among middle-aged women. Diabetes Care. 2008;31(10):2050-5.

24. Hendriks HFJ. Moderate Alcohol Consumption and Insulin Sensitivity: Observations and Possible Mechanisms. Ann Epidemiol. 2007;17(5):S40-2.

25. Romeo J, Warnberg J, Nova E, Diaz LE, Gomez-Martinez S, Marcos A. Moderate alcohol consumption and the immune system: a review. Br J Nutr. 2007;98 Suppl 1:S111-5.

26. Van Dijk AP, Toet J, Verdurmen J. The relationship between health-related quality of life and two measures of alcohol consumption. Journal of Studies on Alcohol and Drugs. 2004;65(2):241.

27. Saito I, Okamura T, Fukuhara S, Tanaka T, Suzukamo Y, Okayama A, et al. A cross-sectional study of alcohol drinking and health-related quality of life among male workers in Japan. Journal of occupational health. 2005;47(6):496-503.

28. Chan AM, von Mühlen D, Kritz-Silverstein D, Barrett-Connor E. Regular alcohol consumption is associated with increasing quality of life and mood in older men and women: the Rancho Bernardo Study. Maturitas. 2009;62(3):294-300.

29. Saarni SI, Joutsenniemi K, Koskinen S, Suvisaari J, Pirkola S, Sintonen H, et al. Alcohol consumption, abstaining, health utility, and quality of life--a general population survey in Finland. Alcohol Alcohol. 2008;43(3):376-86.

30. Valencia-Martín JL, Galan I, Guallar-Castillón P, Rodriguez-Artalejo F. Alcohol drinking patterns and health-related quality of life reported in the Spanish adult population. Prev Med. 2013;57(5):703-7.

31. Stranges S, Notaro J, Freudenheim JL, Calogero RM, Muti P, Farinaro E, et al. Alcohol drinking pattern and subjective health in a population-based study. Addiction. 2006;101(9):1265-76.

32. Lang I, Wallace RB, Huppert FA, Melzer D. Moderate alcohol consumption in older adults is associated with better cognition and well-being than abstinence. Age Ageing. 2007;36(3):256-61.

33. Poikolainen K, Vartiainen E, Korhonen HJ. Alcohol intake and subjective health. Am J Epidemiol. 1996;144(4):346-50.

34. Byles J, Young A, Furuya H, Parkinson L. A Drink to Healthy Aging: The Association Between Older Women's Use of Alcohol and Their Health-Related Quality of Life. J Am Geriatr Soc. 2006;54(9):1341-7.

35. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW,Jr, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer. 2002;87(11):1234-45.

36. Poli A, Marangoni F, Avogaro A, Barba G, Bellentani S, Bucci M, et al. Moderate alcohol use and health: a consensus document. Nutrition, Metabolism and Cardiovascular Diseases. 2013;23(6):487-504.

37. Cho E, Smith-Warner SA, Ritz J, van den Brandt, Piet A, Colditz GA, Folsom AR, et al. Alcohol intake and colorectal cancer: a pooled analysis of 8 cohort studies. Ann Intern Med. 2004;140(8):603-13.

38. International Agency for Research on Cancer, IARC Working Group on the Evaluation of Carcinogenic Risks to Human. IARC monographs on the evaluation of carcinogenic risks to humans: Consumption of Alcoholic Beverages; 2012.

39. Corrao G, Bagnardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. Prev Med. 2004;38(5):613-9.

40. Mumenthaler MS, Taylor JL, O'Hara R, Yesavage JA. Gender differences in moderate drinking effects. Alcohol Res Health. 1999;23(1):55-64.

41. Rajendram R, Hunter R, Peters T. Alcohol absorption, metabolism and physiological effects. Encyclopaedia of Human Nutrition. 2005:48-57.

42. Buemann B, Astrup A. How does the body deal with energy from alcohol? Nutrition. 2001;17(7):638-41.

43. Mitchell MC, Teigen EL, Ramchandani VA. Absorption and peak blood alcohol concentration after drinking beer, wine, or spirits. Alcoholism: Clinical and Experimental Research. 2014;38(5):1200-4.

44. Ramchandani V, Bosron W, Li T. Research advances in ethanol metabolism. Pathologie Biologie. 2001;49(9):676-82.

45. King A, de Wit H. Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. Arch Gen Psychiat. 2011;68(4):389-99.

46. Hendler RA, Ramchandani VA, Gilman J, Hommer DW. Stimulant and Sedative Effects of Alcohol. Curr Top Behav Neurosci. 2013;13:489-509.

47. Tucker JA, Vuchinich RE, Sobell MB. Alcohol's effects on human emotions: A review of the stimulation/ depression hypothesis. Int J Addict. 1982;17(1):155-80.

48. Holdstock L, de Wit H. Individual Differences in the Biphasic Effects of Ethanol. Alcohol Clin Exp Res. 1998;22(9):1903-11.

49. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, et al. Effects of moderate alcohol consumption on the central nervous system. Alcohol Clin Exp Res. 1998;22(5):998-1040.

50. Perra S, Pillolla G, Melis M, Muntoni A, Gessa G, Pistis M. Involvement of the endogenous cannabinoid system in the effects of alcohol in the mesolimbic reward circuit: electrophysiological evidence in vivo. Psychopharmacology. 2005;183(3):368-77.

51. Perra S, Pillolla G, Luchicchi A, Pistis M. Alcohol Inhibits Spontaneous Activity of Basolateral Amygdala Projection Neurons in the Rat: Involvement of the Endocannabinoid System. Alcohol Clin Exp Res. 2008;32(3):443-9.

52. Caillé S, Alvarez-Jaimes L, Polis I, Stouffer DG, Parsons LH. Specific Alterations of Extracellular Endocannabinoid Levels in the Nucleus Accumbens by Ethanol, Heroin, and Cocaine Self-Administration. The Journal of Neuroscience. 2007;27(14):3695-702.

53. Levenson RW, Sher KJ, Grossman LM, Newman J, Newlin DB. Alcohol and stress response dampening: Pharmacological effects, expectancy, and tension reduction. J Abnorm Psychol. 1980;89(4):528-38.

54. Romanowicz M, Schmidt JE, Bostwick JM, Mrazek DA, Karpyak VM. Changes in Heart Rate Variability Associated With Acute Alcohol Consumption: Current Knowledge and Implications for Practice and Research. Alcohol Clin Exp Res. 2011;35(6):1092-105.

55. Spaak J, Tomlinson G, McGowan CL, Soleas GJ, Morris BL, Picton P, et al. Dose-related effects of red wine and alcohol on heart rate variability. Am J Physiol Heart Circ Physiol. 2010;298(6):H2226-31.

56. Spaak J, Merlocco AC, Soleas GJ, Tomlinson G, Morris BL, Picton P, et al. Dose-related effects of red wine and alcohol on hemodynamics, sympathetic nerve activity, and arterial diameter. Am J Physiol Heart Circ Physiol.

2008;294(2):H605-12.

57. Kreibig SD. Autonomic nervous system activity in emotion: A review. Biol Psychol. 2010;84(3):394-421.

58. Mick I, Spring K, Uhr M, Zimmermann US. Alcohol administration attenuates hypothalamic–pituitary–adrenal (HPA) activity in healthy men at low genetic risk for alcoholism, but not in high-risk subjects. Addict Biol. 2013;18(5):863-71.

59. Holdstock L, King AC, de Wit H. Subjective and Objective Responses to Ethanol in Moderate/Heavy and Light Social Drinkers. Alcohol Clin Exp Res. 2000;24(6):789-94.

60. King AC, Houle T, de Wit H, Holdstock L, Schuster A. Biphasic Alcohol Response Differs in Heavy Versus Light Drinkers. Alcohol Clin Exp Res. 2002;26(6):827-35.

61. Koob GF, Le Moal M. Drug addiction, dysregulation of reward, and allostasis. Neuropsychopharmacology. 2001;24(2):97-129.

62. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? Brain Research Reviews. 1998;28(3):309-69.

63. Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Res Rev. 1993;18(3):247-91.

64. Robinson TE, Berridge KC. Incentive-sensitization and addiction. Addiction. 2001;96(1):103-14.

65. Robinson TE. & Berridge. KC. Addiction, Annual Review of Psychology. 2003;54:25-53.

66. Dai X, Thavundayil J, Gianoulakis C. Response of the Hypothalamic-Pituitary-Adrenal Axis to Stress in the Absence and Presence of Ethanol in Subjects at High and Low Risk of Alcoholism. Neuropsychopharmacology. 2002;27(3):442-52.

67. Balodis IM, Wynne-Edwards KE, Olmstead MC. The stress–response-dampening effects of placebo. Horm Behav. 2011;59(4):465-72.

68. Schuckit MA, Gold E, Risch C. Plasma cortisol levels following ethanol in sons of alcoholics and controls. Arch Gen Psychiatry. 1987;44(11):942-5.

Chapter 2

The biphasic effects of moderate alcohol consumption with a meal on ambiance-induced mood and autonomic nervous system balance: A randomized crossover trial

Ilse C. Schrieks
Annette Stafleu
Victor L. Kallen
Marc Grootjen
Renger F. Witkamp
Henk F.J. Hendriks

PLoS One. 2014; 9(1):e86199

Abstract

Background: The pre-drinking mood state has been indicated to be an important factor in the mood effects of alcohol. However, for moderate alcohol consumption there are no controlled studies showing this association. Also, the mood effects of consuming alcohol combined with food are largely unknown. The aim of this study was to investigate the effects of moderate alcohol combined with a meal on ambiance-induced mood states. Furthermore effects on autonomic nervous system activity were measured to explore physiological mechanisms that may be involved in changes of mood state.

Methods: In a crossover design 28 women (age 18-45 y, BMI 18.5-27 kg/m^2) were randomly allocated to 4 conditions in which they received 3 glasses of sparkling white wine (30 g alcohol) or alcohol-free sparkling white wine while having dinner in a room with either a pleasant or unpleasant created ambiance. Subjects filled out questionnaires (B-BAES, POMS and postprandial wellness questionnaire) at different times. Skin conductance and heart rate variability were measured continuously.

Results: Moderate alcohol consumption increased happiness scores in the unpleasant, but not in the pleasant ambiance. Alcohol consumption increased happiness and stimulation feelings within 1 hour and increased sedative feelings and sleepiness for 2.5 hour. Skin conductance was increased after alcohol within 1 hour and was related to happiness and stimulation scores. Heart rate variability was decreased after alcohol for 2 hours and was related to mental alertness.

Conclusions: Mood inductions and autonomic nervous system parameters may be useful to evaluate mood changes by nutritional interventions. Moderate alcohol consumption elevates happiness scores in an unpleasant ambiance. However, drinking alcohol during a pleasant mood results in an equally positive mood state.

Trial registration: Clinicaltrials.gov NCT01426022

Introduction

Alcoholic beverages are amongst the oldest beverages and are commonly consumed because of their anticipated mood effects. Motivation for moderate alcohol consumption may vary from enhancing pleasure to reducing tension (1, 2). Mood and emotions are distinct phenomena, although the two are closely related. Whereas emotions are of short duration and often directed towards an object, moods are generally lasting longer and not object-related. Mood comprises a mixture of hedonic (pleasure-displeasure) and arousal (sleepy-activated) values (3).

The effect of moderate alcohol consumption on mood may depend on several interacting factors, including the rise or decline of blood alcohol concentration (BAC) (4-6), the pre-drinking mood state of the drinker (7) and factors such as the social context of drinking (8, 9), and expectancy of mood effects (10, 11).

Alcohol has been shown to change mood in a biphasic pattern. During rising BACs alcohol has stimulating effects, reported by feelings of euphoria and elation, while during declining BACs alcohol has sedative effects, reported by relaxation and sleepiness (4-6). In Western countries alcohol is often consumed with a meal. Food consumption may also affect mood. Previous studies showed an increased calmness and sleepiness after food intake (12-15). Furthermore, food consumption may influence the mood effects of alcohol because it delays gastric emptying and alcohol absorption, resulting in a lower peak BAC (16, 17). Thus, the combination of food and alcohol consumption may affect mood differently from that of alcohol consumption only. To date, only two studies investigated the mood effects of combined food and alcohol consumption, and their results were inconsistent (18, 19). Lloyd and Rogers (1997) found that alcohol with a meal improved mood by increasing confidence and decreasing tension and confusion, while Markus et al. (2004) did not find any effect on mood.

Furthermore, the pre-drinking mood state has been indicated to play an important role in the mood effects of alcohol (7, 11). However, the effects of different pre-drinking moods on postprandial mood changes have not yet been compared in an intervention study. The most accurate method to measure mood is by self-report of feelings as mood is a subjective state. Nevertheless, specific physiological parameters associated with mood changes are increasingly being used in studies generally to validate subjective outcomes or to explore physiological mechanisms involved in mood regulation. However, for yet another reason this may be highly relevant, as incorporating the physiological dynamics underlying both mood effects and pharmacological activities of psychotropic substances like alcohol may be instrumental to further unravel the effects of such enhancers on mood. With respect to alcohol, the relevant physiological parameters are likely to include measures of autonomic nervous system (ANS) activity as moderate alcohol consumption has been shown to increase the activity of the sympathetic division of the ANS (being related to emotional arousal), and to decrease the activity of the parasympathetic division of the ANS, associated with relaxation

(10, 20-25).
The primary aim of this study was to evaluate the biphasic effects of moderate alcohol consumption with a meal on ambiance-induced mood, as measured by subjective responses and ANS activity. The secondary aim was to study the association between the effects of moderate alcohol consumption on subjective responses of mood and on ANS activity. We hypothesized that moderate alcohol consumption with a meal would amplify the pre-drinking mood state, resulting in a more positive mood in a positive induced mood state and in a more negative mood in a negative induced mood state. Furthermore, the induced mood was expected to influence the effect of alcohol on ANS activity, specifically by decreasing sympathetic outflow and increasing parasympathetic outflow in a positive induced mood and opposite effects in a negative induced mood.

Materials and Methods

Ethics statement

The study was conducted at TNO (The Netherlands Organization for Applied Scientific Research) in Zeist, The Netherlands, and was performed according to the International Conference on Harmonisation Guidelines for Good Clinical Practice. The study also complied with the Declaration of Helsinki and was approved by an independent Medical Ethics Committee (METOPP, Tilburg, The Netherlands). Written informed consent was obtained from all subjects. The study is registered at ClinicalTrials.gov (NCT): NCT01426022. The protocol for this trial and supporting CONSORT checklist are available as supporting information (Protocol S1 and Checklist S1).

Subjects

Healthy women (28 women aged 18-43 years, BMI 22.1 ± 1.7 kg/m^2) participated in the study (Figure 2.1). The subjects were recruited from a pool of volunteers at TNO in Zeist, The Netherlands. Eligible subjects did not use any medication, habitually consumed alcohol (3-20 glasses/week) and had no (family) history of alcoholism. We chose women who were taking oral contraceptives, thus expecting to reduce possible effects of the menstrual cycle on mood. They were not tested in the week they were not taking oral contraceptives. The calculated sample size (power analysis) was 24 subjects, where α was 0.05 (two-sided), β was 0.80 and the effect size was 1.2, based on a previous study with Profile of Mood States (POMS) as outcome measure (14). Twenty-eight subjects were included to guarantee sufficient power, even in case drop out may occur.

Experimental protocol

The study used a randomized single-blind crossover design, with the intervention factors alcohol and ambiance. Subjects consumed 3 glasses of sparkling white wine (30 g alcohol;

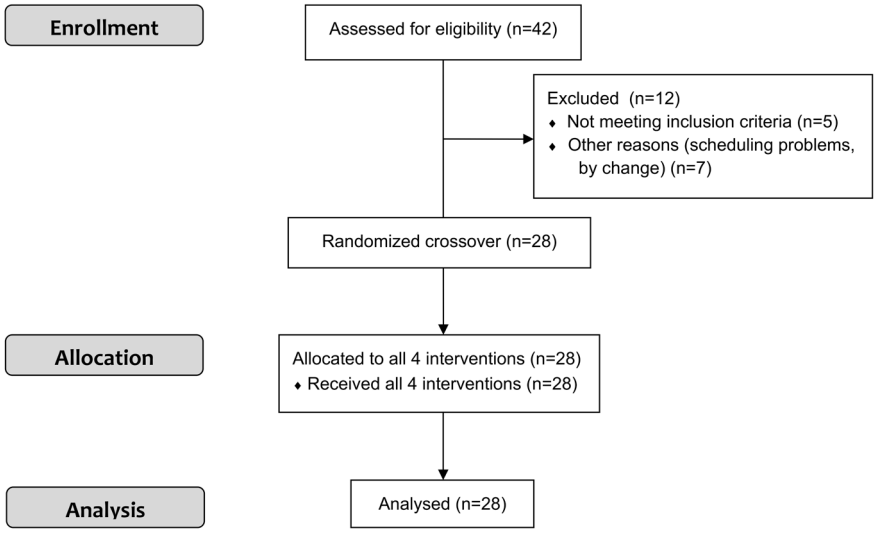


Figure 2.1. Flow chart (CONSORT).

Prosecco Santa Chiara, Italy) or alcohol-free sparkling white wine (<2 g alcohol; Vita Nova Sparkling Secco, The Netherlands) with a meal in either a pleasant or an unpleasant ambiance. The blood alcohol concentration was expected not to exceed 0.5‰, which is the Dutch legal limit of driving. Therefore, we considered consumption of 30 g alcohol together with a meal as a moderate dose.

Each subject participated in all 4 experimental conditions, which occurred at least one week apart. Subjects were equally divided in 4 groups with different intervention orders according to a Latin square design (Figure 2.2). Allocation to intervention order was randomized according to body fat percentage and age by a computer-generated randomization scheme. Randomization and intervention order allocation were performed by statisticians of TNO.

Subjects were kept ignorant to the ultimate study aim; instead they were informed that the aim was to investigate the effect of different meal settings and alcohol on hormones and satiety. In addition, they were informed that the alcohol content of the beverages could vary per intervention day. Subjects were blinded to the alcohol intervention. They were instructed to refrain from drinking alcohol on the evening preceding each test day, to eat their standard breakfast and lunch on standard times on each test day and to refrain from eating and drinking anything except water 2 hours before testing. After connecting the electrodes for skin conductance and electrocardiography (ECG) measurements, a baseline measurement was performed for 2 min in a seated position. From that moment onwards, skin conductance and ECG were collected continuously until the end of the study day. Subjects relaxed for at least 15 min in a room where soft music was played before they went to the test rooms with

either a pleasant or an unpleasant ambiance. In these rooms, subjects filled out computer questionnaires about their mood. Afterwards, they were asked to consume their first glass of sparkling white wine or alcohol-free white wine within 5 min. This moment is considered t=0. Immediately following their wine consumption the participants were served a meal consisting of a macaroni dish (2004 kJ, Apetito B.V., Denekamp, The Netherlands) with two more glasses of wine. The meal and 2 glasses of wine had to be finished in 15 min. BAC was measured with a breathalyzer (Alcotest 7410, Dräger Nederland, Zoetermeer, The Netherlands) before the first drink and 30 min, 60 min, 120 min and 150 min afterwards. Mood questionnaires were filled out before the first drink and at 20 min, 50 min, 110 and 140 min after. After the last questionnaire, a second baseline (recovery) measurement of 2 min was carried out for skin conductance and ECG.

Minor changes were made in the study procedure after the second study day to reduce potential stress from e.g. number of blood collections. These changes were approved by the Medical Ethics Committee METOPP.

Order of interventions	Study day 1	2	3	4
1	A	B	**C**	D
2	**C**	A	D	B
3	B	D	A	**C**
4	D	**C**	B	A

Figure 2.2. Randomisation of the interventions according to a Latin Square design.

A: Unpleasant ambiance + 3 glasses of sparkling white wine

B: Pleasant ambiance + 3 glasses of sparkling white wine

C: Unpleasant ambiance + 3 glasses of alcohol-free sparkling white wine

D: Pleasant ambiance + 3 glasses of alcohol-free sparkling white wine

Mood induction by meal ambiance

Rooms with a pleasant or an unpleasant meal ambiance were created by environmental factors as lighting, music, cleanness, decoration and a film scene. The pleasant ambiance room was colourfully decorated, light was soft, and music was playing. The unpleasant ambiance was created by having very bright lighting, no music, a filled dustbin next to the table, no decoration and plastic cutlery and serving dish. Ambient temperature was similar in both rooms. The ambiances were enhanced by showing the participants either a happy or sad film scene from the animation films ‘Bambi’ (Walt Disney, 1942) and ‘The Lion King’ (Walt Disney, 1994). The scenes were approximately 2.5 min long and were shown during the first glass of wine or alcohol-free wine. The happy scenes were either the scene of ‘Bambi on the ice’ or the Lion King scene of ‘hakuna matata’. The sad scenes were the scene of ‘Bambi’s mother dying’ (26) or the ‘Lion Kings father dying’ (27). Subjects watched each film scene once. Subjects had

dinner individually and stayed in the room until all measurements were completed.

Mood questionnaires

Mood was measured with three different questionnaires, which were filled out on the computer using adaptive VAS software (28). Subjects practised the questionnaires once to familiarize.

Profile of Mood States (POMS)

Changes in mood were measured using the short version of the 'Profile of Mood States' (POMS) questionnaire (29). The questionnaire was computer based and asked participants to answer questions on a five-point interval scale ranging from 'strongly disagree' to 'strongly agree'. The POMS comprises 40 items for five different subscales for mood. The subscale Anger (7 items, score range 7-35), Depression (8 items, score range 8-40), Fatigue (6 items, score range 6-30) and Tension (6 items, score range 6-30) refer to a negative mood state, whereas the subscale Vigor (5 items, score range 5-25) refers to a positive mood state. We added two more positive mood subscales, Happiness (4 items, score range 4-20) and Calmness (4 items, score range 4-20) from the Brunel mood scale to make the questionnaire more balanced for negative and positive moods (30).

Postprandial wellness questionnaire

The postprandial wellness questionnaire has been developed and described by Boelsma et al. (2010) to measure the combined effects of satiety feelings and wellness after a meal (visual analogue scale, 100-unit) (15). The recorded items were pleasantness, satisfaction, relaxation, sleepiness, physical energy, mental alertness, hunger, fullness, desire to eat and prospective food consumption. We added the items experienced body temperature and thirst.

Biphasic Alcohol Effects Scale (B-BAES)

The stimulation and sedative effects of alcohol were measured with the brief version of the Biphasic Alcohol Effects Scale (B-BAES) (31, 32). The questionnaire comprises of two subscales, sedation and stimulation, with three items per subscale. The participants were asked to indicate 'the extent to which the adjectives described their feelings' on a visual analogue scale (100-unit) from 'not at all' to 'extremely'. The questions were translated to Dutch and participants had to answer them on a visual analogue scale instead of on an 11-point scale as in the original questionnaire. The B-BAES stimulation and sedation scores were calculated as the average of the scores on the three items within the subscale.

Autonomic nervous system measurements

Skin conductance and ECG data were collected and stored using a wireless multi-channel ambulatory system (Mobi-8, TMS International, The Netherlands), designed to measure different electro-physiological signals at the same time in both ambulatory and stationary

conditions. Data were analysed with BioTrace + Software for NeXus version 1.2.0.0 (Mind Media B.V., The Netherlands).

Skin conductance level

As primary parameter of sympathetic activity, skin conductance level was measured continuously with two Ag/AgCL electrodes, which were positioned on the middle phalanx of the ring finger and on the middle phalanx of the index finger of the non-dominant hand after removal of any hand jewelry. Mean skin conductance level was calculated from 2 min time frames. The same frames were used for calculation of the ECG time-domain derivatives.

Heart rate variability parameters

Heart rate and heart rate variability parameters were measured using continuous ECG monitoring. Three electrodes were attached on the upper body for the ECG measurement (pre-cordial lead, sampling rate 512 Hz).

From the original ECG time series, inter beat intervals were calculated from R-R intervals. These are the intervals between two successive R-spikes of the QRS-complex in the ECG. Based on the inter beat intervals, the heart rate and root mean square of successive differences (RMSSD) were calculated. RMSSD is a time-domain measure that reflects the short-term variation of the inter beat intervals and is strongly related to parasympathetic nervous system activity (33, 34). In addition, frequency-domain measurements were determined from a power spectral density analysis computed by a 1024 point Fast Fourier Transformation (FFT) on the inter beat intervals. The following frequency-domain measurements were calculated: low frequency power (LF power, 0.04-0.15 Hz), high frequency power (HF power, 0.15-0.40 Hz) and the LF:HF ratio. HF power is an index of parasympathetic nervous system activity and strongly related to RMSSD. Both are mainly influenced by vagal activity. Heart rate variability analysis was performed according to the Task Force Guidelines (35). Time frames of 6 min were selected at time points when the questionnaires were filled out (4 time frames at -5 min, 25 min, 55 min and 115 min) to be able to compare the outcomes of the questionnaires with the physiological responses. Each 6 min time frame was subdivided in 2 min time frames for calculation of skin conductance, heart rate and the time-domain heart rate variability parameter RMSSD. Inter beat intervals were manually checked and segments with ectopic beats were not used for analysis. As FFT's mathematically require longer time series (e.g. as compared to time domain based parameters like RMSSD), heart rate variability parameters quantified within the frequency domain were calculated from the 6 min time frames. Consequently, skin conductance level, heart rate and RMSSD were calculated from 2 min time frames, whereas LF power, HF power and LF:HF ratio were calculated from 6 min time frames.

Data analysis

Statistical analyses were performed using the SAS statistical software package (SAS version

8; SAS Institute, Cary, NC, USA). All variables were compared between interventions with a mixed analysis of variance model that included the fixed factors alcohol (alcohol vs. alcohol-free), ambiance (pleasant vs. unpleasant ambiance), time and the interaction between alcohol and ambiance, time and alcohol, time and ambiance, and time, alcohol and ambiance. The factors subject and subject by period were added to the model as random factors. A post hoc Tukey-Kramer test was used if an intervention effect occurred, to correct for multiple testing. Pearson correlations were calculated within one of the four conditions (Figure 2.2). Changes over time in subjective feelings were correlated with changes over time in autonomic nervous system measurements for each subject. A Fisher's *z* transformation was applied on the individual correlations, to correct for deviations from the normal distribution and a 95% confidence interval was calculated.

The measurements on the first study day of the first 11 subjects were considered not valid because of logistic problems that occurred, and were therefore excluded from the analyses. Except for baseline characteristics, all values are expressed as means and standard errors of the mean. Error bars in figures express standard errors of the mean.

Results

Subject baseline characteristics

Subjects were recruited and enrolled in the trial between September and December 2011 (Figure 2.1). Baseline characteristics of the participants are shown in Table 2.1.

Table 2.1. Baseline characteristics of the participants (n=28).

Variable	Value[a]
Age (y)	22 [18 – 43]
Alcohol consumption	
3-6 drinks/week	61%
7-14 drinks/week	36%
15-21 drinks/week	4%
Body weight (kg)	66 ± 6
BMI (kg/m^2)	22.1 ± 1.7
Body fat percentage (%)	24.5 ± 5.5
Heart rate (beats/min)	66 ± 9
Systolic blood pressure (mmHg)	109 ± 9
Diastolic blood pressure (mmHg)	72 ± 8

[a] Data are expressed as median and [range] for age, percentage for alcohol consumption and means ± SD for the other variables. BMI: body mass index.

Mood induction by ambiance

The study was designed to induce a positive and a negative mood by ambiance. The results of the questionnaires show that mood was influenced by ambiance, with a more positive mood in the pleasant ambiance than in the unpleasant ambiance. Scores on the subscales anger, tension and depression of the POMS were all higher in the unpleasant ambiance than in the pleasant ambiance (all $P<0.05$), with depression increasing over time in the unpleasant ambiance ($P<0.05$). The subscale happiness of the POMS and the items pleasantness, relaxation and mental alertness of the postprandial wellness questionnaire were all higher in the pleasant ambiance ($P<0.05$, $P<0.001$, $P<0.01$, $P<0.05$, respectively). There was no main effect of ambiance on the B-BAES subscales stimulation and sedation, although sedation scores were 10% lower in the pleasant ambiance ($P=0.05$).

Effects of alcohol on mood

The hypothesis was that moderate alcohol consumption would amplify the ambiance-induced mood.

Main effects of alcohol

Moderate alcohol consumption with a meal influenced mood, but these effects were different over time. Acute effects of alcohol (within 1 hour) were increased happiness and stimulation scores (Figure 2.3, $P<0.001$ and $P<0.05$) and reduced calmness and mental alertness scores (both $P<0.001$). Effects of alcohol consumption occurring or continuing after 1.5 hour were increased sleepiness ($P<0.05$) and sedation scores (Figure 2.4, $P<0.01$). These results show time-dependent effects of moderate alcohol consumption with a meal on mood, with a more positive and active mood state within 1 hour after alcohol and food consumption and a more sedated and inactive mood state occurring or continuing after 1.5 hour after alcohol and food consumption. The mean BAC was highest 30 min after alcohol consumption (0.53 ± 0.01‰). Compared to 30 min, BAC was decreased 60 min and 120 min after alcohol consumption (0.47± 0.01‰, 0.30 ± 0.01‰ and 0.24 ± 0.01‰ respectively). Thus, the positive and activation effects of alcohol on mood were more present during rising BACs, while the sedation and inactivation effects of alcohol on mood were more present during declining BACs.

Interaction effects of ambiance and alcohol

The effect of ambiance on mood was influenced by the alcohol intervention for the POMS happiness subscale and the B-BAES stimulation subscale (Figure 2.3). Happiness scores were higher when alcohol was consumed in the unpleasant ambiance than when no alcohol was consumed in both the unpleasant and pleasant ambiance (16 vs. 13 and 14 respectively, both $P<0.001$). Stimulation scores were 14% higher when no alcohol was consumed in the pleasant ambiance than in the unpleasant ambiance (54 vs. 47, $P=0.08$). There were no interaction effects between ambiance, alcohol and time.

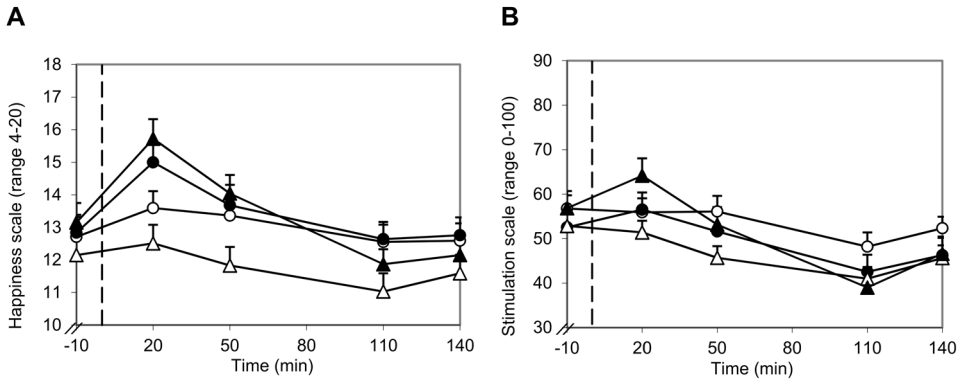


Figure 2.3. Influence of ambiance and alcohol on POMS happiness scores (A) and B-BAES stimulation scores (B).

–○– Pleasant, alcohol-free; –●– Pleasant, alcohol; –△– Unpleasant, alcohol-free; –▲– Unpleasant, alcohol; – – start alcohol consumption (t=0). (**A**) Happiness scores were higher 20 min and 50 min after alcohol consumption compared to no alcohol consumption (P<0.001 and P<0.05, respectively). Happiness scores were lower in the unpleasant ambiance without alcohol than in the pleasant and unpleasant ambiance with alcohol (P<0.001) and in the pleasant ambiance without alcohol (P<0.05). (**B**) Stimulation scores were decreased from 20 min until 50 min after alcohol consumption, which did not occur after consumption of alcohol-free drinks (P<0.05 vs. P=1.0). Stimulation scores tended to be higher in the pleasant ambiance without alcohol than in the unpleasant ambiance without alcohol (P=0.08).

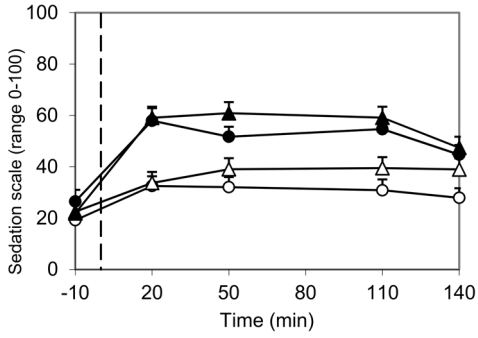


Figure 2.4. Influence of ambiance and alcohol on B-BAES sedation scores.

–○– Pleasant, alcohol-free; –●– Pleasant, alcohol; –△– Unpleasant, alcohol-free; –▲– Unpleasant, alcohol; – – start alcohol consumption (t=0). Sedation scores were higher after alcohol consumption compared to no alcohol consumption at 20 min, 50 min, 110 min (all P<0.001) and 140 min (P<0.01) after consumption. Sedation scores tended to be higher in the unpleasant ambiance (P=0.05).

Autonomic nervous system measurements

Skin conductance level

Skin conductance acutely increased 25 min after alcohol consumption (Figure 2.5). At this point skin conductance was 25% higher than after consumption of alcohol-free drinks (8.8 µS vs. 7.0 µS respectively; *P*<0.001). In the pleasant ambiance skin conductance decreased more during the time interval from 25 min until 155 min after consumption than in the unpleasant ambiance (*P*<0.05). The increased skin conductance during the first hour after alcohol consumption suggests an increase in sympathetic nervous system activity, with a gradual decline afterwards that might be associated with our findings on the sedation and inactivation effects of alcohol.

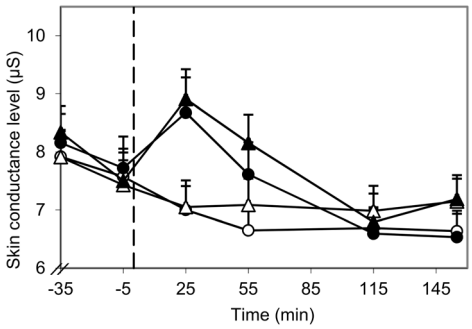


Figure 2.5. Influence of ambiance and alcohol on skin conductance.
–○– Pleasant, alcohol-free; –●– Pleasant, alcohol; –△– Unpleasant, alcohol-free; –▲– Unpleasant, alcohol; – – start alcohol consumption (t=0). Skin conductance was higher 25 min after alcohol consumption compared to no alcohol consumption (*P*<0.001). Skin conductance decreased more from 25 min until 155 in the pleasant ambiance than in the unpleasant ambiance (*P*=0.042). The values shown at time point -5, 25, 55 and 115 are averages of the mean skin conductance levels measured during three succeeding 2 min time frames.

Heart rate variability parameters

Heart rate variability responses after consumption of alcohol or alcohol-free drinks with a meal are shown in Figure 2.6. After moderate alcohol consumption ln [HF power] was decreased during all time points (25 min until 115 min after consumption) compared to no alcohol consumption (*P*<0.001, *P*<0.01 and *P*<0.001, respectively). After 115 min, ln [HF power] was still 12% lower in the alcohol intervention group than in the alcohol-free intervention group. RMSSD was similarly influenced by alcohol as HF power and showed a very similar response, with lower RMSSD 25 min, 55 min and 115 min after alcohol consumption than after consumption of alcohol-free drinks (*P*<0.001, *P*<0.01 and *P*<0.001, respectively). The decrease in HF power and RMSSD after alcohol consumption suggests an alcohol induced parasympathetic suppression.

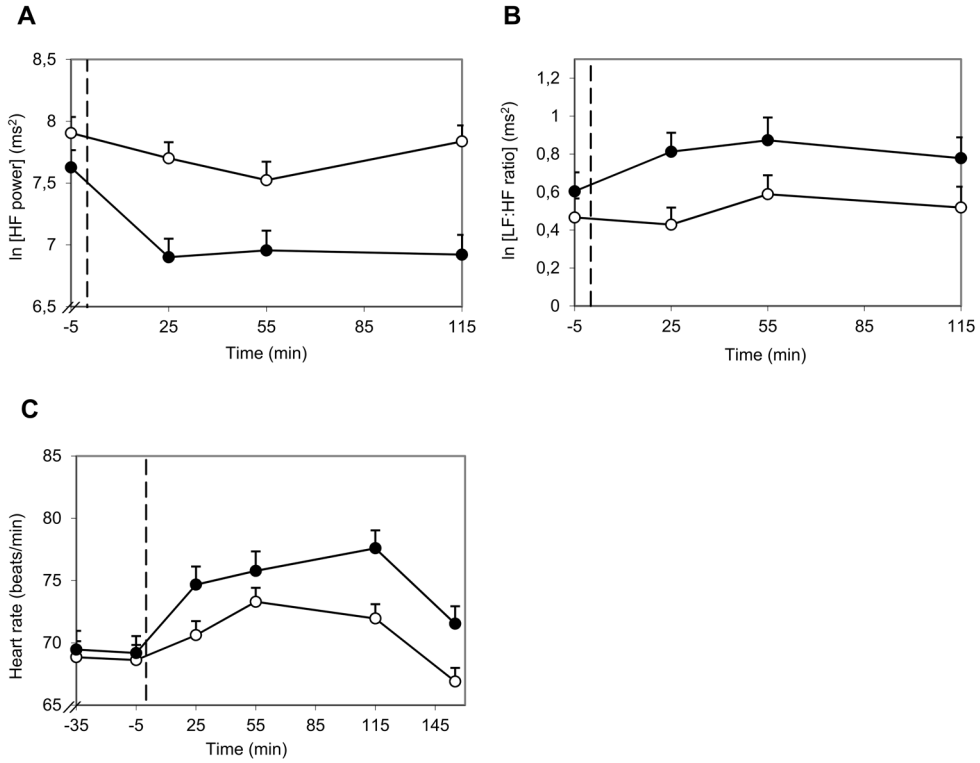


Figure 2.6. Influence of alcohol on HF power (A), LF:HF ratio (B) and heart rate (C).
–○– Alcohol-free; –●– Alcohol; – – start alcohol consumption (t=0). (**A**) HF power was lower after alcohol consumption compared to no alcohol consumption (at 25 min and 115 min $P<0.001$, at 55 min $P<0.01$). (**B**) LF:HF ratio was higher after alcohol consumption ($P<0.001$). (**C**) Heart rate was higher 25 min and 115 min after alcohol consumption compared to no alcohol consumption ($P<0.01$ and $P<0.001$, respectively). The heart rate values shown at time point -5, 25, 55 and 115 are averages.

The LF:HF ratio was higher when alcohol was consumed than when no alcohol was consumed ($P<0.001$). HF power, RMSSD and LF:HF ratio were not influenced by ambiance. Heart rate was also increased by alcohol consumption, with higher values 25 min and 115 min after alcohol consumption than after consumption of alcohol-free drinks ($P<0.001$). The heart rate response was different in the pleasant ambiance than in the unpleasant ambiance ($P<0.001$). Until 55 min after consumption, heart rate was higher in the pleasant ambiance, but from 110 min onwards heart rate was higher in in the unpleasant ambiance. However, differences were very small. The increased LF:HF ratio after alcohol consumption indicates a sympathetic overtone in the balance between sympathetic and parasympathetic activity that does not recover within 2 hours.

Relation between autonomic nervous system parameters and subjective feelings

Associations were calculated between changes in autonomic nervous system parameters and changes in subjective feelings to examine whether these were related. If correlations were higher than r=0.5 or lower than r=-0.5 and if r=0.0 was not part of the confidence interval than they were assumed significantly enough to describe (Table 2.2).

Skin conductance, the index for sympathetic activity, was positively related with the stimulation subscale and negatively related with calmness after alcohol consumption in both the pleasant and unpleasant ambiance. Skin conductance was positively related to happiness and experienced body temperature and negatively related to fatigue in the unpleasant ambiance with alcohol.

Table 2.2. Correlations between autonomic nervous system parameters and subjective feelings (n=28)[a].

Correlated variables	Intervention	Correlation	95% CI
SNS parameter – subjective feeling			
SC – Stimulation (B-BAES)	Alcohol, pleasant	0.60	(0.29, 0.79)
SC – Stimulation (B-BAES)	Alcohol, unpleasant	0.63	(0.34, 0.81)
SC – Calmness (POMS)	Alcohol, pleasant	-0.51	(-0.74, -0.07)
SC – Calmness (POMS)	Alcohol, unpleasant	-0.66	(-0.83, -0.37)
SC – Happiness (POMS)	Alcohol, unpleasant	0.75	(0.52, 0.87)
SC – Fatigue (POMS)	Alcohol, unpleasant	-0.51	(-0.74, 0.17)
SC – Anger (POMS)	No alcohol, unpleasant	0.51	(0.16, 0.74)
SC – Pleasant (PPW)	Alcohol, pleasant	0.54	(0.21, 0.76)
SC – Experienced BT (PPW)	Alcohol, unpleasant	0.52	(0.19, 0.75)
PNS parameter – subjective feeling			
HF power – Sedation (B-BAES)	Alcohol, unpleasant	-0.61	(-0.80, -0.31)
HF power – Mental alertness (PPW)	Alcohol, unpleasant	0.62	(0.33, 0.81)
HF power – Experienced BT (PPW)	Alcohol, pleasant	-0.51	(-0.74, -0.17)
HF power – Experienced BT (PPW)	Alcohol, unpleasant	-0.58	(-0.78, -0.26)
HF power – Experienced BT (PPW)	No alcohol, pleasant	-0.52	(-0.75, -0.18)
HF power – Experienced BT (PPW)	No alcohol, unpleasant	-0.55	(-0.77, -0.22)

[a] All values are mean [correlation coefficient (r)] and 95% confidence interval after Fisher's z transformation. Correlations are shown when r=0.0 is not part of the confidence interval and when r>0.5 or r<-0.5.

Abbreviations: SNS: sympathetic nervous system; PNS: parasympathetic nervous system; SC: skin conductance; HF power: high frequency power; B-BAES, brief biphasic alcohol effects scale; POMS, profile of mood states; PPW: postprandial wellness questionnaire; BT: body temperature.

HF power and RMSSD are two heart rate variability parameters that are related to parasympathetic activity. In Table 2.2 only HF power is shown, but RMSSD showed similar correlations with the listed subjective items. In the unpleasant ambiance with alcohol, HF power and RMSSD were negatively related to the sedation subscale and positively related to mental alertness. HF power and RMSSD were associated with experienced body temperature in all conditions, except for the pleasant ambiance with alcohol.

Discussion

The present study provided two major findings on the role of pre-drinking mood state in alcohol-induced mood changes and the possible involvement of the ANS. First, alcohol did not amplify ambiance-induced mood, but it improved mood in the unpleasant ambiance as shown by increased scores for feelings of happiness. Second, parameters for ANS activity were influenced by alcohol consumption, but not by ambiance-induced mood, except for some minor changes in skin conductance. Although no effects of pre-drinking mood state were found on ANS activity, ANS parameters were associated to subjective feelings of high or low arousal after alcohol consumption. This indicates that ANS parameters can be used as objective measures of the arousal dimension of mood.

We hypothesized that alcohol would amplify the ambiance-induced mood, but the opposite was found; in a more negative mood state, created by an unpleasant ambiance, moderate alcohol consumption with a meal increased self-reported happiness feelings. In the pleasant ambiance mood was not further improved by alcohol. Alcohol did not affect any other mood scales that were influenced by ambiance. With the exception of happiness, alcohol and ambiance influenced different mood scales.

We successfully induced a positive and negative mood state by creating rooms with either a pleasant or an unpleasant ambiance. In the pleasant ambiance, subjects scored higher on pleasantness and relaxation and lower on depression and tension than in the unpleasant ambiance. This indicates that subjects were indeed in a better mood in the pleasant ambiance than in the unpleasant ambiance. This is in accordance with previous research showing that mood declines when there is no color, and when light is too bright, similar to the conditions of the unpleasant ambiance in our study (36). The mood effects of ambiance were present as long as the subjects were in the rooms, which was almost 3 hours. Other mood induction methods used previously, such as images, music, film clips and personalized recall, generally affect mood for a much shorter period (27, 37-39). Therefore, the mood induction by ambiance used in this study may be more useful for intervention studies in which a longer mood induction is preferred, such as experiments on food intake and eating behavior. Ambiance has been shown previously to influence eating behavior. This is probably caused by mood changes, although mood effects were not evaluated in these studies (40-42).

Moderate alcohol consumption with a meal resulted in biphasic mood effects. During the first

hour after wine consumption subjects reported higher happiness and stimulation and lower calmness and mental alertness than after consumption of alcohol-free wine. During the first hour following alcohol consumption also sedative feelings were increased, but these effects remained elevated until the last measurement. This biphasic pattern differs from studies in which alcohol is consumed without food in the early-late afternoon. Those studies found that sedative effects arose on the descending limb of the BAC curve, while we observed sedative effects directly after consumption (31, 43, 44). However, Addicot et al. (2007) also observed sedative effects before BAC levels started to decline (45). The earlier onset of sedative feelings in the present study might be due to an increase in tiredness caused by the food intake. Indeed, in the alcohol-free condition, fatigue scores were increasing and vigor scores were decreasing after the meal. Lloyd & Rogers (1997) and Markus et al. (2004) also measured the influence of combined alcohol and food intake on mood (18, 19). Lloyd & Rogers (1997) observed no effects of moderate alcohol consumption on elation, energy and tiredness, which is in contrast to our findings. An explanation may be the lower number of subjects in the study of Lloyd & Rogers (1997) (18). Markus et al. (2004) did not find any mood effects of a moderate dose of alcohol (40 g) with an evening meal. However, they measured mood only once, 2 h after consumption (19). Therefore, the study designs of both studies may not have had enough power to detect differences.

Motives to drink a moderate amount of alcohol are mostly to enhance mood or for social reasons, but with heavy alcohol consumption motives as coping with stress or negative mood also occur frequently. Although in the present study mood effects of moderate alcohol consumption were measured, we realize that coping motives in heavy alcohol consumption are related to problem drinking and addiction (1, 2).

The second hypothesis was that alcohol consumption in a positive pre-drinking mood would cause higher heart rate variability and lower skin conductance than in a negative pre-drinking mood. This hypothesis, however, is not confirmed by our results, as we did not find interaction effects of alcohol and ambiance on ANS activity.

Ambiance only had a small influence on ANS activity. Skin conductance was further declined 2.5 h after consumption in the pleasant ambiance compared to the unpleasant ambiance, but heart rate variability parameters were not affected by ambiance. The small influence of ambiance on ANS activity may be due to the fact that varying ambiances resulted in mood changes. In contrast to emotional changes, mood changes are suggested to have no influence on ANS activity, because mood is not related to an object and no action from the body is required (25).

Moderate alcohol consumption influenced ANS activity. Compared to the situation in which no alcohol was consumed, alcohol increased skin conductance within the first hour, which indicates a short-term increase in sympathetic activity. On the other hand, alcohol consumption decreased heart rate variability (RMSSD and HF power), indicating a decrease in parasympathetic tone and vagal withdrawal. As a result, LF:HF ratio and heart rate, indices

of sympathovagal balance, were increased after alcohol consumption.
These results are in accordance with previous research on the effect of alcohol on heart rate variability (10, 20-22). Skin conductance was also increased after alcohol consumption in a study by Levenson et al (1980) (10). However, Stritzke et al (1995) found an attenuated skin conductance response after alcohol consumption when subjects watched either pleasant or unpleasant pictures inducing arousal (46). In contrast to previous studies, in the present study alcohol was combined with food consumption. Autonomic activity plays an important role in energy balance and thermoregulation. Sympathetic stimulation increases energy metabolism and thermogenesis and reduces food intake (47, 48). In the present study, energy metabolism was not measured and food intake was standardized. However, the distinct short-term increase in sympathetic outflow could have caused an increase in energy metabolism and energy expenditure. This is in line with a study by Addicott et al. (2007), which showed that moderate alcohol consumption increases physical activity during rising BACs. Surprisingly, when sympathetic tone was returned to normal values, parasympathetic tone stayed decreased and sympathovagal balance was not restored 2 hours after consumption as shown by elevated levels of the LF:HF ratio an heart rate. However, heart rate was recorded until 2.5 hour after consumption and at this time its levels were recovered. Whether this 2 hours increase in sympathovagal balance is related to long-term effects is not known. Epidemiological studies found inconsistent results on the association between chronic moderate alcohol consumption and heart rate variability (49-51). An acute increase in sympathovagal balance is also seen during physical activity and mental stress (52). Furthermore, previous studies showed that even a meal increased sympathovagal balance for 1 or 2 hours due to a continuous inhibition of parasympathetic activity (53, 54). Therefore, we suggest that the acute increase in sympathovagal balance after combined food and alcohol consumption indicates an activated physiological state of the body, which may be generated to restore homeostasis.

ANS activity was found to be related to the arousal dimension of mood. The increased sympathetic tone was related to increased happiness and stimulation and decreased calmness and fatigue. This indicates that sympathetic activity was related to increased arousal and can be used as an objective measure of the arousal dimension of mood. The reduced parasympathetic tone was related to decreased mental alertness and increased sedation, suggesting this was caused by a central effect of alcohol. These associations are not in line with the idea that mood states, in contrast to emotions, are not related to ANS activity (25). However, Matthews et al. (1990) also found a relation between skin conductance and self-report of the arousal dimension of mood in subjects measured prior to an intelligence test (55). Strengths of the study are the randomized crossover design and the controlled study conditions. However, the study design also has some limitations that warrant consideration. We did not measure the mood effects of a meal separately from the alcohol effects. Therefore we cannot disentangle the alcohol and meal effects on mood. Second, although the alcohol intervention was blinded, the subjects might have noticed the alcohol content of the

beverages. A confounding effect of expectancy in the observed mood effects can therefore not be excluded. Third, mood was measured in a laboratory setting and the physiological measurements may have influenced the mood of the subjects. However, we tried to interfere as little as possible with the physiological measurements. The subjects did not notice much of the autonomic nervous system measurements as they were carrying a small ambulatory device, which all electrodes from the ECG and skin conductance were connected to, so they could move freely in the rooms.

The study was carried out in women and the generalizability of the results to men may not be directly possible because men and women showed different mood effects of a meal in a previous study (56). Furthermore, subjects were eating and drinking alone in this study. Social interaction has been shown to influence mood effects of alcohol and therefore the results may not be generalizable to food and alcohol consumption in a social setting (57, 58).

To conclude, mood inductions and autonomic nervous system parameters may be useful to evaluate mood changes by nutritional interventions. Moderate alcohol consumption elevates happiness in an unpleasant ambiance. However, drinking alcohol during a pleasant mood results in an equally positive mood state. Consuming alcohol with a meal does not result in different mood changes than alcohol consumption alone, although sedative feelings may have an earlier onset.

Acknowledgements

We gratefully acknowledge those involved in the conduct of the study and the data analyses and thank the volunteers for their enthusiastic participation.

Funding

This work was supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption.

References

1. Cooper ML. Motivations for alcohol use among adolescents: Development and validation of a four-factor model. Psychol Assess. 1994;6(2):117.
2. Cooper ML, Frone MR, Russell M, Mudar P. Drinking to regulate positive and negative emotions: A motivational model of alcohol use. J Pers Soc Psychol. 1995;69(5):990.
3. Russell JA. Core affect and the psychological construction of emotion. Psychol Rev. 2003;110(1):145.
4. Tucker JA, Vuchinich RE, Sobell MB. Alcohol's effects on human emotions: A review of the stimulation/depression hypothesis. Int J Addict. 1982;17(1):155-80.
5. King A, de Wit H. Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. Arch Gen Psychiat. 2011;68(4):389-99.
6. Hendler RA, Ramchandani VA, Gilman J, Hommer DW. Stimulant and sedative effects of alcohol. Curr Top Behav Neurosci. 2013;13:489-509.
7. Russell JA, Mehrabian A. The mediating role of emotions in alcohol use. J Stud Alcohol. 1975;36(11):1508-36.
8. Warren G, Raynes A. Mood changes during three conditions of alcohol intake. Q J Stud Alcohol. 1972;33(4):979-89.
9. Sher KJ. Subjective effects of alcohol: The influence of setting and individual differences in alcohol expectancies. J Stud Alcohol. 1985 03;46(0096-882; 0096-882; 2):137-46.
10. Levenson RW, Sher KJ, Grossman LM, Newman J, Newlin DB. Alcohol and stress response dampening: Pharmacological effects, expectancy, and tension reduction. J Abnorm Psychol. 1980;89(4):528-38.
11. McCollam JB, Burish TG, Maisto SA, Sobell MB. Alcohol's effects on physiological arousal and self-reported affect and sensations. J Abnorm Psychol. 1980;89(2):224-33.
12. Gibson EL. Emotional influences on food choice: Sensory, physiological and psychological pathways. Physiol Behav. 2006;89(1):53-61.
13. Wells AS, Read NW. Influences of fat, energy, and time of day on mood and performance. Physiol Behav. 1996;59(6):1069-76.
14. Pasman WJ, Blokdijk VM, Bertina FM, Hopman WPM, Hendriks HFJ. Effect of two breakfasts, different in carbohydrate composition, on hunger and satiety and mood in healthy men. Int J Obes Relat Metab Disord. 2003;27(6):663-8.
15. Boelsma E, Brink EJ, Stafleu A, Hendriks HFJ. Measures of postprandial wellness after single intake of two protein-carbohydrate meals. Appetite. 2010;54(3):456-64.
16. Gentry RT. Effect of food on the pharmacokinetics of alcohol absorption. Alcohol Clin Exp Res. 2000;24(4):403-4.
17. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, et al. Effects of moderate alcohol consumption on the central nervous system. Alcohol Clin Exp Res. 1998;22(5):998-1040.
18. Lloyd HM, Rogers PJ. Mood and cognitive performance improved by a small amount of alcohol given with a lunchtime meal. Behav Pharmacol. 1997;8(2-3):188-95.
19. Markus CR, Sierksma A, Verbeek C, van Rooijen JJM, Patel HJ, Brand AN, et al. Moderate whisky consumption in combination with an evening meal reduces tryptophan availability to the brain but does not influence performance in healthy volunteers. Br J Nutr. 2004;92(06):995-1000.
20. Romanowicz M, Schmidt JE, Bostwick JM, Mrazek DA, Karpyak VM. Changes in heart rate variability associated

with acute alcohol consumption: Current knowledge and implications for practice and research. Alcohol Clin Exp Res. 2011;35(6):1092-105.

21. Spaak J, Tomlinson G, McGowan CL, Soleas GJ, Morris BL, Picton P, et al. Dose-related effects of red wine and alcohol on heart rate variability. Am J Physiol Heart Circ Physiol. 2010;298(6):H2226-31.

22. Spaak J, Merlocco AC, Soleas GJ, Tomlinson G, Morris BL, Picton P, et al. Dose-related effects of red wine and alcohol on hemodynamics, sympathetic nerve activity, and arterial diameter. Am J Physiol Heart Circ Physiol. 2008;294(2):H605-12.

23. Iwase S, Matsukawa T, Ishihara S, Tanaka A, Tanabe K, Danbara A, et al. Effect of oral ethanol intake on muscle sympathetic nerve activity and cardiovascular functions in humans. J Auton Nerv Syst. 1995;54(3):206-14.

24. Stritzke W, Lang A, Patrick C. Beyond stress and arousal: A reconceptualization of alcohol-emotion relations with reference to psychophysiological methods. Psychol Bull. 1996;120(3):376-95.

25. Kreibig SD. Autonomic nervous system activity in emotion: A review. Biol Psychol. 2010;84(3):394-421.

26. Gross JJ, Levenson RW. Emotion elicitation using films. Cogn Emot. 1995;9(1):87-108.

27. Rottenberg J, Ray RD, Gross JJ. Emotion elicitation using films. In: Coan JA, Allen JJB, editors. The handbook of emotion elicitation and assessment. New York: Oxford University Press, Inc; 2007. p. 9-28.

28. Marsh-Richard DM, Hatzis ES, Mathias CW, Venditti N, Dougherty DM. Adaptive visual analog scales (AVAS): A modifiable software program for the creation, administration, and scoring of visual analog scales. Behav Res Methods. 2009;41(1):99-106.

29. Nyenhuis DL, Yamamoto C, Luchetta T, Terrien A, Parmentier A. Adult and geriatric normative data and validation of the profile of mood states. J Clin Psychol. 1999;55(1):79-86.

30. Lane AM, Soos I, Leibinger E, Karsai I, Hamar P. Validity of the brunel mood scale for use with UK, italian and hungarian athletes. In: Lane AM, editor. Mood and human performance: Conceptual, measurement and applied issues. New York: Nova Science Publishers, Inc; 2007. p. 119-30.

31. Martin CS, Earleywine M, Musty RE, Perrine MW, Swift RM. Development and validation of the biphasic alcohol effects scale. Alcohol Clin Exp Res. 1993;17(1):140-6.

32. Rueger SY, McNamara PJ, King AC. Expanding the utility of the biphasic alcohol effects scale (BAES) and initial psychometric support for the brief-BAES (B-BAES). Alcohol Clin Exp Res. 2009;33(5):916-24.

33. Brownley KA, Hurwitz BE, Schneiderman N. Cardiovascular psychophysiology. In: Cacioppo JT, Tassinary LG, Berntson GG, editors. Handbook of Psychophysiology, 2nd ed. Cambridge (UK): Cambridge University Press; 2000. p. 342-67.

34. Goedhart AD, Van Der Sluis S, Houtveen JH, Willemsen G, De Geus EJ. Comparison of time and frequency domain measures of RSA in ambulatory recordings. Psychophysiology. 2007;44(2):203-15.

35. Task Force of the European Society of Cardiology, the North American Society of Pacing and Electrophysioloy. Heart rate variability: Standards of measurement, physiological interpretation, and clinical use. Circulation. 1996;93(5):1043-65.

36. Küller R, Ballal S, Laike T, Mikellides B, Tonello G. The impact of light and colour on psychological mood: A cross-cultural study of indoor work environments. Ergonomics. 2006;49(14):1496-507.

37. Lang PJ, Greenwald MK, Bradley MM, Hamm AO. Looking at pictures: Affective, facial, visceral, and behavioral reactions. Psychophysiology. 1993;30(3):261-73.

38. Bouhuys AL, Bloem GM, Groothuis TGG. Induction of depressed and elated mood by music influences the perception of facial emotional expressions in healthy subjects. J Affect Disord. 1995;33(4):215-26.

39. Philippot P, Schaefer A, Herbette G. Consequences of specific processing of emotional information: Impact of general versus specific autobiographical memory priming on emotion elicitation. Emotion. 2003;3(3):270.

40. Lindman R, Lindfors B, Dahla E, Toivola H. Alcohol and ambience: Social and environmental determinants of intake and mood. Alcohol Alcohol Suppl. 1987;1:385-8.

41. Stroebele N, De Castro JM. Effect of ambience on food intake and food choice. Nutrition. 2004;20(9):821-38.

42. Wansink B. Environmental factors that increase the food intake and consumption volume of unknowing consumers. Annu Rev Nutr. 2004; 2011/07;24(1):455-79.

43. Holdstock L, de Wit H. Individual differences in the biphasic effects of ethanol. Alcohol Clin Exp Res. 1998;22(9):1903-11.

44. King AC, Houle T, de Wit H, Holdstock L, Schuster A. Biphasic alcohol response differs in heavy versus light drinkers. Alcohol Clin Exp Res. 2002;26(6):827-35.

45. Addicott MA, Marsh-Richard DM, Mathias CW, Dougherty DM. The biphasic effects of alcohol: Comparisons of subjective and objective measures of stimulation, sedation, and physical activity. Alcohol Clin Exp Res. 2007;31(11):1883-90.

46. Stritzke WGK, Patrick CJ, Lang AR. Alcohol and human emotion: A multidimensional analysis incorporating startle-probe methodology. J Abnorm Psychol. 1995;104(1):114-22.

47. Bray G. Reciprocal relation of food intake and sympathetic activity: Experimental observations and clinical implications. Int J Obes Relat Metab Disord. 2000;24:S8-17.

48. Messina G, De Luca V, Viggiano A, Ascione A, Iannaccone T, Chieffi S, et al. Autonomic nervous system in the control of energy balance and body weight: Personal contributions. Neurol Res Int. 2013;2013.

49. Janszky I, Ericson M, Blom M, Georgiades A, Magnusson J, Alinagizadeh H, et al. Wine drinking is associated with increased heart rate variability in women with coronary heart disease. Heart. 2005;91(3):314-8.

50. Kupari M, Virolainen J, Koskinen P, Tikkanen MJ. Short-term heart rate variability and factors modifying the risk of coronary artery disease in a population sample. Am J Cardiol. 1993;72(12):897-903.

51. Ryan J, Howes L. Relations between alcohol consumption, heart rate, and heart rate variability in men. Heart. 2002;88(6):641-2.

52. Malliani A, Pagani M, Lombardi F, Cerutti S. Cardiovascular neural regulation explored in the frequency domain. Circulation. 1991;84(2):482-92.

53. Lu C, Zou X, Orr WC, Chen J. Postprandial changes of sympathovagal balance measured by heart rate variability. Dig Dis Sci. 1999;44(4):857-61.

54. Chang C, Ko C, Lien H, Chou M. Varying postprandial abdominovagal and cardiovagal activity in normal subjects. Neurogastroenterol Motil. 2010;22(5):546-e119.

55. Matthews G, Jones DM, Chamberlain AG. Refining the measurement of mood: The UWIST mood adjective checklist. Br J Psychol. 1990;81(1):17-42.

56. Wells AS, Read NW, Uvnas-Moberg K, Alster P. Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones. Physiol Behav. 1997;61(5):679-86.

57. Doty P, de Wit H. Effect of setting on the reinforcing and subjective effects of ethanol in social drinkers. Psychopharmacology (Berl). 1995;118(1):19-27.

58. Kirkpatrick MG, de Wit H. In the company of others: Social factors alter acute alcohol effects. Psychopharmacology (Berl). 2013;230:215-26.

Chapter 3

Effects of mood inductions by meal ambiance and moderate alcohol consumption on endocannabinoids and *N*-acylethanolamines in humans: A randomized crossover trial

Ilse C. Schrieks
Dina Ripken
Annette Stafleu
Renger F. Witkamp
Henk F. J. Hendriks

Accepted for publication in PLoS One

Abstract

Background: The endocannabinoid system is suggested to play a regulatory role in mood. However, the response of circulating endocannabinoids (ECs) to mood changes has never been tested in humans. In the present study, we examined the effects of mood changes induced by ambiance and moderate alcohol consumption on plasma ECs 2-arachidonoylglycerol (2-AG), anandamide (AEA), and some *N*-acylethanolamine (NAE) congeners in humans.

Methods: Healthy women (n=28) participated in a randomized cross-over study. They consumed sparkling white wine (340 mL; 30 g alcohol) or alcohol-free sparkling white wine (340 mL; <2 g alcohol) as part of a standard evening meal in a room with either a pleasant or an unpleasant ambiance.

Results: Plasma concentrations of palmitoylethanolamide (PEA) and stearoylethanolamide (SEA) increased after 30 min in the unpleasant ambiance, while they decreased in the pleasant ambiance. Changes in ECs and their NAE congeners correlated with mood states, such as happiness and fatigue, but in the pleasant ambiance without alcohol only. ECs and their NAE congeners were correlated with serum free fatty acids and cortisol.

Conclusions: This is the first human study to demonstrate that plasma NAEs are responsive to an unpleasant meal ambiance. Furthermore, associations between mood states and ECs and their NAE congeners were observed.

Trial registration: Clinicaltrials.gov NCT01426022

Introduction

The endocannabinoid system (ECS) has been suggested to play a role in the regulation of mood (1, 2). Endocannabinoids (ECs) are endogenous agonists of the cannabinoid receptors CB_1 and CB_2. The CB_1 receptor is located primarily in the brain but has also been identified in peripheral tissues, such as the gastro-intestinal tract and adipose tissue (3). Anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are the two most well-studied and described ECs. AEA belongs to the *N*-acylethanolamines (NAEs), a group of endogenous compounds that is assumed to share the same biosynthesis and degradation pathways. Unlike AEA, most other NAEs are not able to bind CB_1 or CB_2 receptors, but are suggested to be involved in physiological processes via their action on other receptors or via a potentiating (entourage) effect on AEA (4). NAEs are synthesized from the hydrolysis of their corresponding N-acylphosphatidylethanolamines (NAPEs), whereas 2-AG is produced from diacylglycerol after conversion by diacylglycerol lipase (DAGL). NAEs and 2-AG also have different degradation pathways; NAEs are hydrolysed by fatty acid amide hydrolase (FAAH), while 2-AG is degraded by monoacylglycerol lipase (MAGL) (5).

The effects of AEA and 2-AG on anxiety and depression have been demonstrated in animals. Decreased anxiety and depression responses were demonstrated after inhibition of AEA and 2-AG degradation (6-8) or by microinjection of an AEA analogue into the prefrontal cortex of rats (9). Human studies also indicate a role of ECs in mood regulation (10-13). Considerable insight has become available from the use of rimonabant, an inverse CB_1 agonist. The drug was not approved by the FDA and taken from the market in Europe 1 year after its approval because of side-effects, including anxiety and a depressed mood (10). Further support for an involvement of AEA and 2-AG in mood comes from a study of Hill et al. (2009*)* who showed decreased AEA and 2-AG serum levels in individuals with major depression. However, in another study of Hill et al. (2008) individuals with minor depression displayed higher AEA and 2-AG serum levels. These findings suggest that progression of depression might be associated with a reduced ECS activity. Hill and Patel (2013) hypothesized that in healthy individuals the ECS acts as a buffer system that dampens negative emotions to regulate mood, while in individuals with major depression the ECS is hypoactive (11). This is supported by neuroimaging studies showing a reduced amygdala activity in response to aversive emotional stimuli after cannabinoid administration, or in regular marijuana users (12, 13).

ECs have been demonstrated to have a regulatory role in the stress response as well (14-17). Mood is negatively influenced by psychological stress, which increases tension and depression. In human interventions, an increase in circulating 2-AG and AEA was shown in response to psychological stress (18, 19). In the study of Dlugos et al. (2012) also the other NAEs oleoylethanolamide (OEA) and palmitoylethanolamide (PEA) were increased by stress. Therefore, we suggest that NAEs other than AEA may also play a role in mood regulation.

Taken together, these studies suggest that circulating ECs and their NAE congeners are

relevant to measure in relation to mood and psychological stress regulation. Circulating concentrations of ECs have been suggested to be a 'spill-over' of local production in tissues, as ECs are produced on demand and are thought to have mainly local effects (3, 20). In a previous study, we showed that plasma AEA and related NAEs strongly correlate with serum free fatty acid (FFA) levels after food intake, suggesting that changes in NAE and FFA concentrations may be induced via common mechanisms (21).

However, to date, no study in humans has been done in which the response of ECs and their NAE congeners on mood changes were evaluated. Mood can be influenced by ambiance both positively and negatively. Ambiance consists of multiple environmental factors, such as lighting and music, which can each affect mood. For example, soft lighting and listening to preferred music makes people feel more comfortable compared to bright lighting and listening to non-preferred music (22). In addition, mood can be influenced by moderate alcohol consumption. Well-known effects of alcohol on mood are stimulating effects, e.g. feelings of elation and happiness, and increased feelings of relaxation and sleepiness (23-26).

The objective of this study was to examine the effects of mood induction on circulating concentrations of ECs and related NAEs in humans. It was hypothesized that a more negative mood would increase circulating ECs and NAE congeners, whereas a more positive mood would not influence circulating ECs and NAE congeners. Mood was induced by either a positive or negative meal ambiance and by moderate alcohol consumption or no alcohol consumption. Mood was evaluated by a short version of the 'Profile of Mood States' (POMS-SF), a validated and widely used mood questionnaire (27). Plasma concentrations of the ECs 2-AG and AEA and 4 related NAEs were analysed by a validated LC-MS/MS method (28).

Materials and Methods

Ethics statement

The study was conducted at TNO (The Netherlands Organization for Applied Scientific Research) in Zeist, The Netherlands, and was performed according to the International Conference on Harmonisation Guidelines for Good Clinical Practice. The study also complied with the Declaration of Helsinki and was approved by an independent ethics committee (METOPP, Medisch-Ethische Toetsing Onderzoek Patiënten en Proefpersonen, Tilburg, The Netherlands). Written informed consent was obtained from all subjects. The study is registered at ClinicalTrials.gov (NCT): NCT01426022. The protocol for this trial and supporting CONSORT checklist are available as supporting information (S1 Protocol and S1 Checklist) (26).

Subjects

We recruited 28 non-smoking healthy women within the age of 18-45 years from a pool of

volunteers at TNO in Zeist, The Netherlands (Figure 3.1). Eligible subjects did not use any medication, habitually consumed alcohol (3-20 glasses/week), and had no (family) history of alcoholism. During the screening also the use of THC was excluded. We chose women who were taking oral contraceptives, thus expecting to reduce possible effects of the menstrual cycle on mood. They were not tested in the week they were not taking oral contraceptives. The calculated sample size was 24 subjects, where α was 0.05 (two-sided), β was 0.80, the effect size was 1.2 and the within-person SD was 2.0, based on a previous study with Profile of Mood States (POMS) as outcome measure (29). This was calculated for a within-subjects analysis of the differences of means in mood (POMS). Twenty-eight subjects were included to guarantee sufficient power, even in case drop out may occur. Furthermore, there were two backup subjects to replace potential early drop out. Subjects were recruited and enrolled in the trial between September and December 2011 (Figure 3.1).

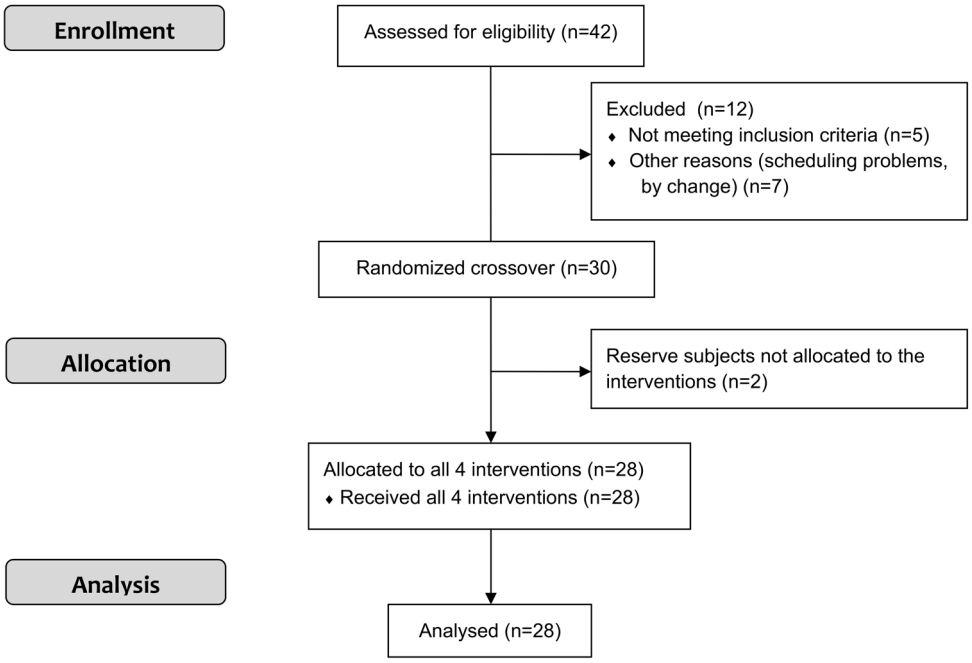


Figure 3.1. Flow chart (CONSORT).
Adapted from Schrieks et al. (2014) (26).

Experimental protocol

The study used a randomized, single-blind, crossover design. Subjects consumed three glasses of sparkling white wine (30 g alcohol) or alcohol-free sparkling white wine with a meal in either a pleasant or an unpleasant ambiance. Each subject participated in all four experimental

conditions, which occurred at least one week apart. Subjects were equally divided in 4 groups with different intervention orders according to a Latin square design. Allocation to intervention order was randomized according to body fat percentage and age by a computer-generated randomization scheme. Randomization and intervention order allocation were performed by statisticians of TNO. Subjects were kept ignorant to the study aim; they were informed that the study aim was to investigate the effect of different meal settings and alcohol on hormones and satiety. In addition, they were informed the alcohol content of the beverages could vary per intervention day. Subjects were blinded to the alcohol intervention.

An overview of the procedures during a study day is shown in Figure 3.2. Subjects were instructed to refrain from drinking alcohol on the preceding evening, to eat their normal breakfast and lunch on standard times, and to refrain from eating and drinking anything except water 2 h before testing. After a baseline blood collection at 16:30 h, subjects relaxed for at least 15 min in a room where soft music was playing. Afterwards, they went to the test rooms with either a pleasant or an unpleasant ambiance. In these rooms, subjects filled out a computer questionnaire on mood before they consumed their first glass of sparkling white wine or alcohol-free white wine at t=0 within 5 min. Immediately after they consumed their wine, the participants were served a meal consisting of a macaroni dish (2004 kJ, Apetito B.V., Denekamp, The Netherlands) with two more glasses of wine. The meal and 2 glasses of wine had to be finished in 15 min. Blood was drawn by venapuncture before the first drink and 30 min and 120 min afterwards. BAC was measured with a breathalyzer (Alcotest 7410, Dräger Nederland, Zoetermeer, The Netherlands) at regular time points. The mood questionnaire was filled out before the first drink and at 20 min, 50 min and 110 min after.

Minor changes were made in the study procedure after the second study day to reduce potential stress from e.g. number of blood collections. These changes were approved by the Medical Ethics Committee METOPP.

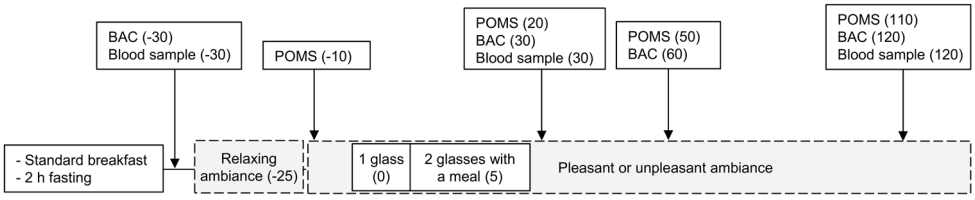


Figure 3.2. Overview of the experimental procedures during a study day.
Time points are indicated within parentheses. BAC, Blood alcohol concentration; POMS, Profile of Mood States questionnaire.

Mood induction by meal ambiance

Rooms with a pleasant or an unpleasant meal ambiance were created by environmental factors as lighting, music, cleanness, decoration and a film scene. In the pleasant meal ambiance room there was colourful decoration, softened lighting, and music. The unpleasant meal ambiance was created by having very bright lighting, no music, a filled dustbin next to the table, no decoration and plastic cutlery and serving dish. These ambiances were enhanced by showing the participants either a happy or sad film scene from the animation films 'Bambi' (Walt Disney, 1942) and 'The Lion King' (Walt Disney, 1994). The scenes were approximately 2.5 min long and were shown during the first glass of wine or alcohol-free wine. The happy scenes were either the scene of 'Bambi on the ice' or the Lion King scene of 'hakuna matata'. The sad scenes were the scene of 'Bambi's mother dying' (30) or the 'Lion Kings father dying' (31). Subjects watched each film scene once. Subjects had dinner individually and stayed in the room all the time, except for blood collection.

Mood induction by alcohol

The mood induction by alcohol consisted of either three glasses of sparkling white wine (30 g alcohol; Prosecco Santa Chiara, Italy) or alcohol-free sparkling white wine (<2 g alcohol; Vita Nova Sparkling Secco, The Netherlands). As alcohol consumption is combined with food consumption, the blood alcohol concentration was expected not to exceed 0.5‰. Therefore, we considered consumption of 30 g alcohol together with a meal as a moderate dose.

Mood questionnaire

Changes in mood states were measured using the short version of the 'Profile of Mood States' (POMS) (27) questionnaire. The questionnaire was computer based and asked participants to answer questions on a five-point interval scale ranging from 'strongly disagree' to 'strongly agree'. The POMS also comprises adjectives for five different subscales for mood. The subscale Anger (range 7-35), Depression (range 8-40), Fatigue (range 6-30) and Tension (range 6-30) refer to a negative mood state, whereas the subscale Vigour (range 5-25) refers to a positive mood state. We added two more positive mood subscales, Happiness (range 4-20) and Calmness (range 4-20) from the Brunel mood scale (32) to make the questionnaire more balanced. Subjects practised the questionnaire once to familiarize.

Biochemical analyses

Blood samples were collected in tubes containing clot activator for serum or in ice-chilled tubes containing EDTA for plasma. (Vacutainer Systems, Becton Dickinson, Plymouth, UK). For the analysis of ECs and related NAEs, phenylmethanesulfonyl fluoride and URB602 (biphenyl-3-ylcarbamic acid, cyclohexyl ester) were added to the blood samples to inactivate the degradation enzymes fatty acid amide hydrolase and monoglycerol lipase immediately after blood collection. In addition, blood samples for plasma were handled at 4°C and centrifuged

within 30 min for 15 min (2000*g*, 4°C) after which they were stored immediately. Serum and plasma samples were stored at -20°C and -80°C respectively, until further analysis. Plasma levels of 2-AG, AEA and the related NAEs, OEA, PEA, stearoylethanolamide (SEA) and docosahexaenoylethanolamide (DHEA) were determined using an LC-MS/MS technique as previously described (28). Plasma levels of ECs and related NAEs were analysed in a subsample of the subjects (n=16) that had a complete dataset. Serum concentrations of FFAs and cortisol were determined using Olympus analytical equipment and reagents.

Data analysis

Statistical analyses were performed using the SAS statistical software package (SAS version 8; SAS Institute, Cary, NC, USA). Data were visually checked on normality and constant variance of residuals with histograms and plots of residuals vs. corresponding predicted values.

Intervention effects were analysed with a mixed analysis of variance model. Because of the crossover design, intervention effects within subjects were compared by including the random factors subject and subject by study day. Alcohol (alcohol vs. alcohol-free), ambiance (pleasant vs. unpleasant ambiance) and time were included as fixed factors. Since the design was a fully crossed design, the two-way interactions between alcohol and ambiance, time and alcohol and time and ambiance, and the three-way interaction between time, alcohol and ambiance were also included as fixed factors in the model. A *post hoc* test with Tukey-Kramer adjustment was used if an intervention effect occurred.

To assess the correlation of mood changes with changes in plasma concentrations of ECs and NAE congeners, Spearman rank correlations were calculated. These correlations were calculated between changes in scores from pre-meal until 110 min after the meal and changes in ECs and NAEs from pre-meal until 120 min after the meal. A Spearman rank correlation was chosen because it is less sensitive to strong outliers than a Pearson correlation.

To assess the correlation of ECs and NAE congeners with FFAs and cortisol, Pearson correlations were calculated for each subject. On these individual correlations a Fisher's *z* transformation was applied, to correct for deviations from the normal distribution and a 95% confidence interval was calculated. We used this method, because the variation in concentrations was high enough to compute individual correlations, which results in a more powerful calculation of correlations than with Spearman rank correlations.

P values <0.05 were considered statistically significant. The measurements on the first intervention day of the first 11 subjects were considered not valid because of logistic problems that occurred, and were therefore excluded from the analyses. Error bars in figures indicate standard errors of the mean.

Results

Subject baseline characteristics

All 28 women completed the study (Figure 3.1). Subjects' characteristics are shown in Table 3.1. During the pre-study screening the Dutch Eating and Behaviour Questionnaire (DEBQ) and State-Trait Anxiety Inventory (STAI) were taken. Subjects had a mean DEBQ restraint score of 2.5 and were therefore interpreted as being non restraint eaters. Their mean score on the STAI trait scale was 33, which is an average score of trait anxiety for women. Highest BAC was observed 30 min after alcohol consumption and was on average (±SD) 0.53 ± 0.09‰.

Table 3.1. Characteristics of 28 women before intervention.

Variable	Mean ± SD	Range
Age (y)	23 ± 5	18 - 43
Body weight (kg)	66 ± 6	58 - 79
BMI (kg/m^2)	22.1 ± 1.7	19.9 - 26.6
Body fat percentage (%)	24.5 ± 5.5	12.8 - 33.7
STAI trait score	2.5 ± 0.7	1.1 - 3.7
DEBQ restrained score	33 ± 6	22 – 48
Alcohol consumption		
3-6 drinks/week	61%	
7-14 drinks/week	36%	
15-21 drinks/week	4%	

Abbreviations: BMI, body mass index; DEBQ, Dutch Eating Behaviour Questionnaire; STAI, State-Trait Anxiety Inventory.

Characteristics of the induced mood changes

The mood induction by ambiance influenced tension and depression scores. Tension scores were overall higher in the unpleasant ambiance than in the pleasant ambiance (main effect of ambiance: P=0.018). Depression scores increased in the unpleasant ambiance until 110 min after baseline (*post hoc* tests 110 min vs. -10 min and 20 min: P=0.010 and P=0.008, respectively). The mood induction by alcohol affected calmness and happiness scores. Calmness scores were lower immediately after alcohol consumption as compared to after consumption of alcohol-free drinks (*post hoc* test at 20 min: P<0.001). Happiness scores were higher 20 min and 50 min after alcohol consumption than after consumption of alcohol-free drinks (*post hoc* tests: P<0.001 and P=0.029, respectively). However, there was also an interaction effect of ambiance and alcohol on happiness scores. The scores were higher in the unpleasant ambiance with alcohol consumption than without alcohol consumption (P<0.001). Happiness scores were not influenced by moderate alcohol consumption in the pleasant ambiance.

Postprandial responses of ECs and NAE congeners

AEA and related NAEs, with the exception of SEA, changed after a meal (main time effect: all $P<0.05$). *Post hoc* tests showed that OEA and DHEA levels increased 30 min after food intake compared to before the meal ($P=0.033$ and $P<0.001$, respectively). Additionally, AEA, OEA and PEA concentrations were decreased 120 min after food intake compared to before the meal ($P<0.001$, $P<0.001$, $P=0.012$, respectively). Furthermore, AEA, OEA and DHEA concentrations were decreased 120 min after food intake compared to 30 min after food intake (all $P<0.001$). Plasma 2-AG concentrations were not influenced by the meal.

Effects of induced mood changes on ECs and NAE congeners

The effects of the interventions on the changes in ECs and NAE congeners from baseline were calculated to adjust for baseline differences. In Figure 3.3 the effect of ambiance on plasma concentrations of ECs and NAE congeners is shown. PEA and SEA concentrations were influenced by ambiance over time (interaction effect of time*ambiance: $P=0.041$ and $P=0.050$, respectively). PEA and SEA levels were increased in the unpleasant ambiance but decreased in the pleasant ambiance 30 min after consumption (*post hoc* tests at 30 min: $P=0.073$ and $P=0.036$). The ambiance mood induction had no effect on 2-AG, AEA or other NAEs. Furthermore, moderate alcohol consumption did not influence plasma concentrations of 2-AG, AEA or related NAEs.

Relation with mood states

Changes in EC and NAE concentrations from baseline until 120 min after the meal correlated with changes in mood states in the pleasant ambiance without alcohol only (Figure 3.4). Happiness scores were negatively correlated with 2-AG and PEA levels ($r_s=-0.52$ and $r_s=-0.60$, respectively). Furthermore, changes in fatigue scores correlated positively with changes in AEA ($r_s=0.53$) and DHEA ($r_s=0.76$), whereas changes in vigour scores correlated negatively with changes in OEA levels ($r_s=-0.66$). Finally, changes in calmness scores correlated positively with SEA levels ($r_s=0.56$).

Relation with free fatty acids and cortisol

The postprandial responses of ECs and NAE congeners correlated positively with the postprandial responses of FFAs in all intervention combinations (Table 3.2). The correlations were not significantly different between the interventions. For SEA, the correlations with FFA change were lower for all interventions as compared with the other NAEs.

The correlations between EC and NAE responses and cortisol responses differed per intervention combination. Plasma concentrations in 2-AG and AEA were positively correlated with serum cortisol concentrations in every intervention combination, except in the unpleasant ambiance with alcohol. OEA and DHEA were also positively correlated with cortisol in the unpleasant ambiance without alcohol (r=0.82 and r=0.60, respectively). SEA showed a strong

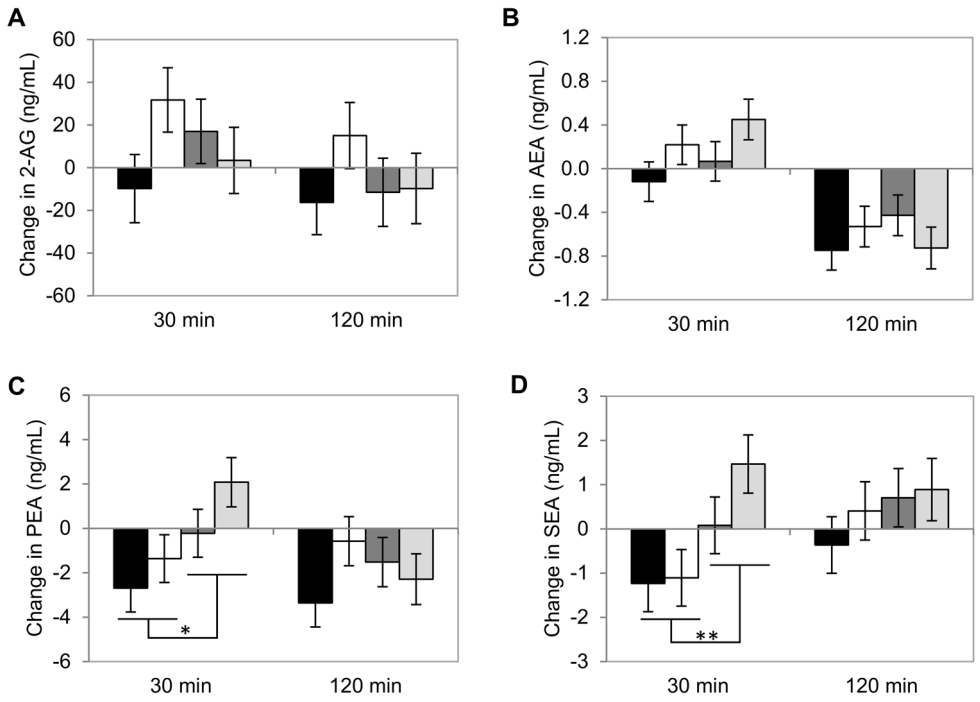


Figure 3.3. Changes in endocannabinoids and *N*-acylethanolamines after mood inductions by ambiance and moderate alcohol consumption[1].

(**A**) 2-arachidonoylglycerol (2-AG), (**B**) anandamide (AEA) and related compounds (**C**) palmitoylethanolamide (PEA) and (**D**) stearoylethanolamide (SEA). Black bars represent pleasant ambiance with alcohol; white bars represent pleasant ambiance without alcohol; dark grey bars represent unpleasant ambiance with alcohol; light grey bars represent unpleasant ambiance without alcohol. PEA and SEA concentrations are increased 30 min after a meal in the unpleasant ambiance, but decreased after a meal in the unpleasant ambiance (**P*=0.073; ** *P*=0.036).

[1] n=16.

negative correlation with cortisol in the unpleasant ambiance with alcohol (r=-0.87).

The effect of meal ambiance and moderate alcohol consumption on the postprandial responses of FFAs and cortisol are shown in Figure 3.5. The FFA response was not influenced by the mood inductions. Cortisol was more decreased 120 min after moderate alcohol consumption as compared to after consumption of alcohol-free drinks (*post hoc* test at 120 min: *P*=0.001).

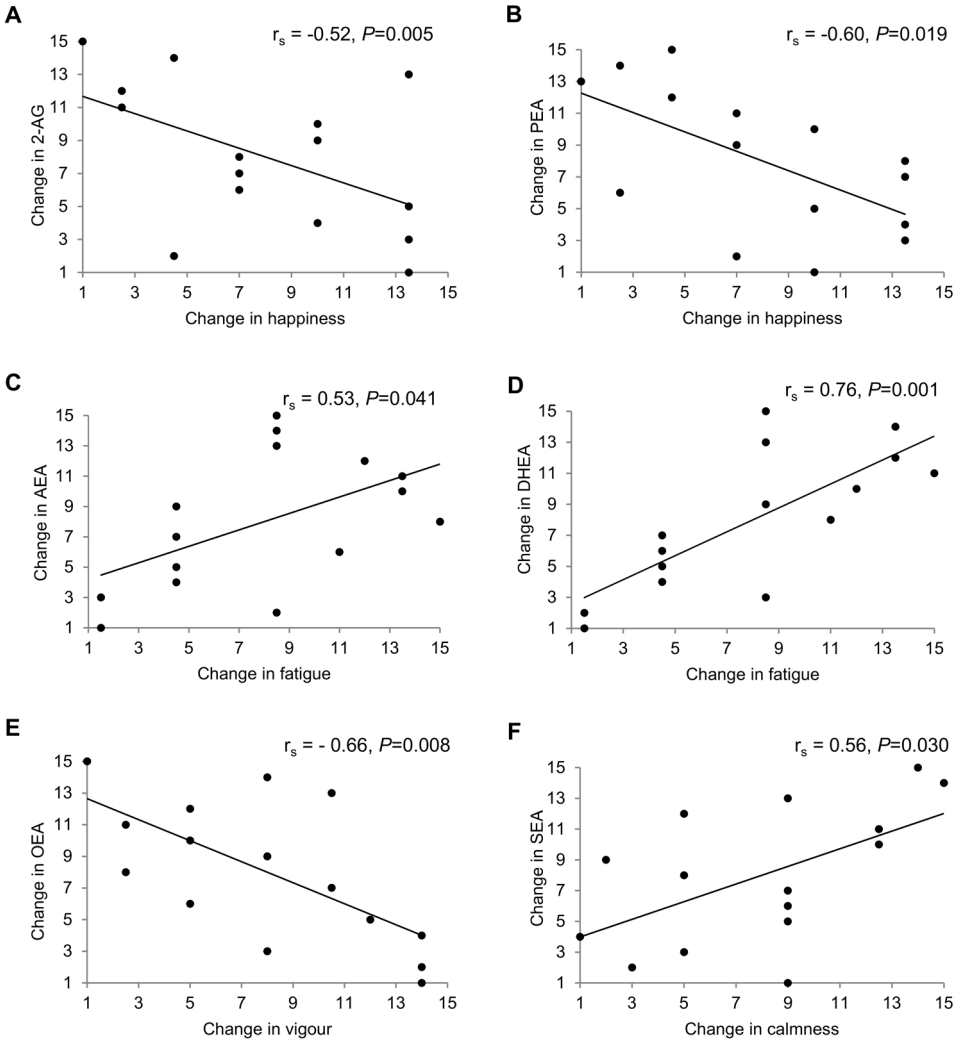


Figure 3.4. Correlations between changes in mood and endocannabinoids and *N*-acylethanolamines in a pleasant ambiance without alcohol[1].

(**A**) 2-arachidonoylglycerol (2-AG), (**B**) anandamide (AEA), (**C**) palmitoylethanolamide (PEA), (**D**) docosahexaenoyl-ethanolamide (DHEA), (**E**) oleoylethanolamide (OEA), and (**F**) stearoylethanolamide (SEA). The figure shows spearman rank correlations with ranked values on both axes of the panels.

[1] n=16.

Table 3.2. Correlations between endocannabinoid and *N*-acylethanolamines responses and FFA and cortisol responses[1].

Variable	Intervention	FFA		Cortisol	
		r	(95% CI)	r	95% CI
2-AG	Pleasant, no alcohol	0.47	(-0.04 to 0.78)	0.62	(0.19 to 0.86)
	Pleasant, alcohol	0.72	(0.34 to 0.89)	0.54	(0.06 to 0.82)
	Unpleasant, no alcohol	0.82	(0.55 to 0.94)	0.86	(0.64 to 0.95)
	Unpleasant, alcohol	0.49	(-0.01 to 0.79)	0.45	(-0.06 to 0.77)
AEA	Pleasant, no alcohol	0.71	(0.34 to 0.89)	0.53	(0.05 to 0.81)
	Pleasant, alcohol	0.73	(0.37 to 0.90)	0.68	(0.27 to 0.88)
	Unpleasant, no alcohol	0.93	(0.82 to 0.98)	0.78	(0.47 to 0.92)
	Unpleasant, alcohol	0.92	(0.77 to 0.97)	0.36	(-0.17 to 0.73)
OEA	Pleasant, no alcohol	0.81	(0.53 to 0.93)	0.38	(-0.14 to 0.74)
	Pleasant, alcohol	0.95	(0.87 to 0.98)	0.79	(0.49 to 0.92)
	Unpleasant, no alcohol	0.95	(0.85 to 0.98)	0.82	(0.54 to 0.93)
	Unpleasant, alcohol	0.91	(0.77 to 0.97)	-0.14	(-0.60 to 0.38)
PEA	Pleasant, no alcohol	0.74	0.39 to 0.91)	-0.38	(-0.74 to 0.14)
	Pleasant, alcohol	0.72	(0.34 to 0.90)	0.55	(0.07 to 0.82)
	Unpleasant, no alcohol	0.44	(-0.07 to 0.77)	0.31	(-0.22 to 0.70)
	Unpleasant, alcohol	0.82	(0.56 to 0.94)	0.62	(0.18 to 0.85)
SEA	Pleasant, no alcohol	0.48	(-0.02 to 0.79)	-0.87	(-0.95 to -0.65)
	Pleasant, alcohol	0.58	(0.12 to 0.84)	0.09	(-0.43 to 0.56)
	Unpleasant, no alcohol	0.47	(-0.03 to 0.79)	0.02	(-0.48 to 0.51)
	Unpleasant, alcohol	-0.23	(-0.65 to 0.30)	-0.51	(-0.80 to -0.01)
DHEA	Pleasant, no alcohol	0.84	(0.58 to 0.94)	0.33	(-0.19 to 0.71)
	Pleasant, alcohol	0.76	(0.42 to 0.91)	0.23	(-0.30 to 0.65)
	Unpleasant, no alcohol	0.88	(0.69 to 0.96)	0.60	(0.15 to 0.85)
	Unpleasant, alcohol	0.80	(0.50 to 0.93)	0.18	(-0.35 to 0.62)

[1] All values are mean [pearson's correlation coefficient (r)] and 95% confidence interval after Fisher's *z* transformation (n=16). Interventions are alcohol (sparkling white wine vs. alcohol-free sparkling white wine) and ambiance (pleasant vs. unpleasant).

Abbreviations: FFA, free fatty acid; NAE, N-acylethanolamine; 2-AG, 2-arachidonoylglycerol; AEA, anandamide; OEA, oleoylethanolamide; PEA, palmitoylethanolamide; SEA, stearoylethanolamide; DHEA, docosahexaenoylethanolamide; DLE, dihomo-γ-linolenoylethanolamide.

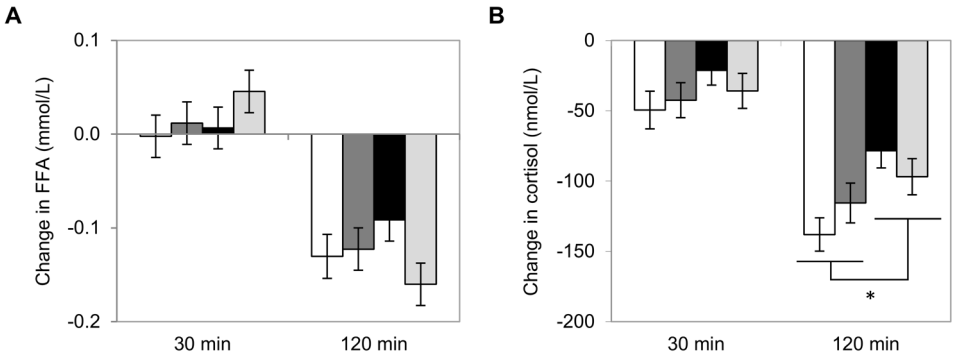


Figure 3.5. Postprandial changes in (A) serum free fatty acid and (B) cortisol after mood inductions[1].
White bars represent pleasant ambiance with alcohol; dark grey bars represent unpleasant ambaince with alcohol; black bars represent pleasant ambiance without alcohol; light grey bars represent unpleasant ambiance without alcohol. Cortisol concentration is more decreased 120 min after a meal with alcohol than without alcohol (*P=0.001).
[1] n=28.

Discussion

The primary outcomes of this study are that 1) PEA and SEA were increased in an unpleasant meal ambiance where tension and depression scores were higher; 2) ECs and NAE congeners correlated with mood states in the pleasant ambiance without alcohol only; 3) 2-AG and AEA were not influenced by the mood inductions but were related to FFA and cortisol concentrations.

This study showed that two NAEs, PEA and SEA, were responsive to the mood induction by meal ambiance. In the unpleasant ambiance, PEA and SEA concentrations were elevated after 30 min, while they were decreased in the pleasant ambiance. Possibly, these NAEs can become elevated in a tension and depression increasing ambiance, as scores for tension and depression were higher in the unpleasant ambiance. This concords with animal studies indicating a regulatory role for ECs and related NAEs in emotion and stress (16, 33). In addition, this would be in line with human studies showing elevated circulating PEA during psychological stress and elevated PEA and SEA in patients with posttraumatic stress disorder (19, 34). Little is known about the role of PEA and SEA in mood, although PEA has been investigated for its anti-inflammatory, neuroprotective and analgesic properties (35-37).

We investigated whether patterns of correlations existed between postprandial mood responses and circulating ECs and their NAE congeners. In the pleasant ambiance without alcohol we found negative correlations with happiness (2-AG and PEA) and vigour (OEA) and positive correlations with fatigue (AEA and DHEA). These findings might suggest that increasing ECs and NAE congeners are related to decreasing mood states as we hypothesized.

However, calmness was positively related to SEA concentrations and no correlations were not found in the other experimental conditions. Therefore, we conclude that we did not observe a consistent pattern of correlations.

The biological relevance of plasma concentrations of ECs and NAE congeners remains speculative because plasma levels are likely to reflect a 'spill over' of 2-AG and NAE synthesis in tissues, such as adipose tissue, liver and perhaps brain. Joosten et al. (2010) observed that circulating FFAs and NAEs are highly correlated. Here, correlations were calculated between NAEs and FFA levels similarly as in Joosten et al. (2010) and similar correlations were found between postprandial responses of FFAs and AEA, OEA and PEA. Therefore, this study confirms that plasma NAE patterns may reflect fatty acids patterns and that their short-term release from *N*-acylphosphatidylethanolamine (NAPE) is triggered by similar mechanisms as are involved in the release of FFA. However, in this study, plasma ECs and related NAEs were also positively related to serum cortisol concentrations, especially in the unpleasant ambiance without alcohol. This finding together with the results from previous human studies where ECs and NAE congeners are shown to be responsive to stress (18, 19, 38), supports a role for plasma ECs and NAE congeners as indicators of stress.

Strengths of the study are the randomized crossover design and the controlled study conditions. Furthermore, the mood induction method by meal ambiance was completely standardized and induced a positive and negative mood for a relative long time of three hours. This was shown by higher depression and tension scores in the unpleasant ambiance and higher happiness scores in the pleasant ambiance. In comparison with the mood induction by ambiance, moderate alcohol consumption influenced different mood states and effects were more acute. We also showed an interaction effect of alcohol and ambiance on happiness, which was not shown before. Moderate alcohol consumption in an unpleasant ambiance increased happiness, but drinking alcohol during a pleasant mood results in an equally positive mood state. This indicates that the mood induction methods used were complementary and interacting, thereby providing the ability to study the response of ECs and NAE congeners on a variety of mood changes.

This study also has some important limitations. The experimental design of this study may not have provided the appropriate conditions to examine the relation between changes in mood and changes in ECs and related NAEs. A further limitation of the study is that ECs and their NAE congeners were measured at three time points and the effects of ambiance on NAEs may have been missed. Additionally, mood was induced by rooms with a pleasant or an unpleasant ambiance. Perhaps, these ambiances did not influence mood to such an extent that correlations between mood scores and plasma ECs and NAE congeners could be observed. Therefore, further research may focus on mood induction by ambiance using more extreme conditions. The role of ECs and NAE congeners may also be better detectable when no food is consumed during mood induction, and consequently exclusively focusing on the association of mood with ECs and NAE congeners.

This study was carried out in women, and generalizability of the results to men may therefore not be directly possible, because men and women showed different mood effects of a meal in a previous study (39).

We conclude that this is the first human study to demonstrate that plasma NAEs are responsive to an unpleasant meal ambiance. Furthermore, associations between mood states and ECs and related NAEs were observed, but not in all intervention combinations. This study provides additional insight in the response of plasma ECs and NAE congeners on mood changing conditions in humans.

The findings are in line with animal and human studies suggesting a role of endocannabinoids in mood and stress regulation. However, for a better translation between animal and human studies, animal studies should also measure circulating levels of ECs and NAE congeners and study their response on mood inductions. This would provide more information on the clinical relevance of changes in circulating ECs and NAEs. For clinical implications on the use of plasma ECs and NAE congeners as diagnostic agents for mood disorders, more research is necessary. Future human studies may focus on mood induction by ambiance using more extreme conditions. Associations may also be better detectable when no food is consumed during mood induction, and consequently exclusively focusing on the association of mood with endocannabinoids and NAEs.

Acknowledgements

We gratefully acknowledge those involved in the conduct of the study and the data analyses and thank the volunteers for their enthusiastic participation.

Funding

This work was supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption.

References

1. Finn DP, Viveros MP, Marco EM. The endocannabinoid system and emotional processing: Pathophysiology and therapeutic potential. J Psychopharmacol. 2012;26(1):3-6.
2. Ruehle S, Rey AA, Remmers F, Lutz B. The endocannabinoid system in anxiety, fear memory and habituation. J Psychopharmacol. 2012;26(1):23-39.
3. Matias I, Bisogno T, Di Marzo V. Endogenous cannabinoids in the brain and peripheral tissues: Regulation of their levels and control of food intake. Int J Obes (Lond). 2006;30:S7-S12.
4. De Petrocellis L, Di Marzo V. An introduction to the endocannabinoid system: From the early to the latest concepts. Best Pract Res Clin Endocrinol Metab. 2009;23(1):1-15.
5. Di Marzo V. Endocannabinoids: synthesis and degradation In: Reviews of Physiology Biochemistry and Pharmacology. Springer; 2008; p. 1-24.
6. Busquets-Garcia A, Puighermanal E, Pastor A, de la Torre R, Maldonado R, Ozaita A. Differential role of anandamide and 2-arachidonoylglycerol in memory and anxiety-like responses. Biol Psychiatry. 2011;70(5):479-86.
7. Sciolino NR, Zhou W, Hohmann AG. Enhancement of endocannabinoid signaling with JZL184, an inhibitor of the 2-arachidonoylglycerol hydrolyzing enzyme monoacylglycerol lipase, produces anxiolytic effects under conditions of high environmental aversiveness in rats. Pharmacol Res. 2011;64(3):226-34.
8. Gobbi G, Bambico FR, Mangieri R, Bortolato M, Campolongo P, Solinas M, Cassano T, Morgese MG, Debonnel G, Duranti A, Tontini A, Tarzia G, Mor M, Trezza V, Goldberg SR, Cuomo V, Piomelli D. Antidepressant-like activity and modulation of brain monoaminergic transmission by blockade of anandamide hydrolysis. Proc Natl Acad Sci U S A. 2005;102(51):18620-5.
9. Rubino T, Realini N, Castiglioni C, Guidali C, Viganó D, Marras E, Petrosino S, Perletti G, Maccarrone M, Di Marzo V, Parolaro D. Role in anxiety behavior of the endocannabinoid system in the prefrontal cortex. Cereb Cortex. 2008;18(6):1292-301.
10. Moreira FA, Grieb M, Lutz B. Central side-effects of therapies based on CB1 cannabinoid receptor agonists and antagonists: Focus on anxiety and depression. Best Pract Res Clin Endocrinol Metab. 2009;23(1):133-44.
11. Hill MN, Patel S. Translational evidence for the involvement of the endocannabinoid system in stress-related psychiatric illnesses. Biol Mood Anxiety Disord. 2013;3(1):19,5380-3-19.
12. Phan KL, Angstadt M, Golden J, Onyewuenyi I, Popovska A, de Wit H. Cannabinoid modulation of amygdala reactivity to social signals of threat in humans. J Neurosci. 2008;28(10):2313-9.
13. Gruber SA, Rogowska J, Yurgelun-Todd DA. Altered affective response in marijuana smokers: An FMRI study. Drug Alcohol Depend. 2009;105(1–2):139-53.
14. Crosby KM, Bains JS. The intricate link between glucocorticoids and endocannabinoids at stress-relevant synapses in the hypothalamus. Neuroscience. 2012;204(0):31-7.
15. Hill MN, Tasker JG. Endocannabinoid signaling, glucocorticoid-mediated negative feedback, and regulation of the hypothalamic-pituitary-adrenal axis. Neuroscience. 2012;204(0):5-16.
16. Hill MN, McEwen BS. Involvement of the endocannabinoid system in the neurobehavioural effects of stress and glucocorticoids. Prog Neuropsychopharmacol Biol Psychiatry. 2010;34(5):791-7.
17. Hill MN. Introduction to the special issue on stress, emotional behavior, and the endocannabinoid system: A decade of research. Neuroscience. 2012;204(0):1-4.

18. Hill MN, Miller GE, Carrier EJ, Gorzalka BB, Hillard CJ. Circulating endocannabinoids and N-acyl ethanolamines are differentially regulated in major depression and following exposure to social stress. Psychoneuroendocrinology. 2009;34(8):1257-62.

19. Dlugos A, Childs E, Stuhr KL, Hillard CJ, de Wit H. Acute stress increases circulating anandamide and other N-acylethanolamines in healthy humans. Neuropsychopharmacology. 2012;37(11):2416-27.

20. Di Marzo V, Verrijken A, Hakkarainen A, Petrosino S, Mertens I, Lundbom N, Piscitelli F, Westerbacka J, Soro-Paavonen A, Matias I, Van Gaal L, Taskinen MR. Role of insulin as a negative regulator of plasma endocannabinoid levels in obese and nonobese subjects. Eur J Endocrinol. 2009;161(5):715-22.

21. Joosten M, Balvers M, Verhoeckx K, Hendriks H, Witkamp R. Plasma anandamide and other N-acylethanolamines are correlated with their corresponding free fatty acid levels under both fasting and non-fasting conditions in women. Nutrition & Metabolism. 2010;7(1):49.

22. Wansink B. Environmental factors that increase the food intake and consumption volume of unknowing consumers. Annu Rev Nutr. 2004;24(1):455-79.

23. Persson LO, Sjöberg L, Svensson E. Mood effects of alcohol. Psychopharmacology (Berl). 1980;68(3):295-9.

24. King A, de Wit H. Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. Arch Gen Psychiat. 2011;68(4):389-99.

25. Hendler RA, Ramchandani VA, Gilman J, Hommer DW. Stimulant and sedative effects of alcohol. Curr Top Behav Neurosci. 2013;13:489-509.

26. Schrieks IC, Stafleu A, Kallen VL, Grootjen M, Witkamp RF, Hendriks HF. The biphasic effects of moderate alcohol consumption with a meal on ambiance-induced mood and autonomic nervous system balance: A randomized crossover trial. PloS one. 2014;9(1):e86199.

27. Nyenhuis DL, Yamamoto C, Luchetta T, Terrien A, Parmentier A. Adult and geriatric normative data and validation of the profile of mood states. J Clin Psychol. 1999;55(1):79-86.

28. Balvers MGJ, Verhoeckx KCM, Witkamp RF. Development and validation of a quantitative method for the determination of 12 endocannabinoids and related compounds in human plasma using liquid chromatography-tandem mass spectrometry. J Chromatogr B Biomed Sci Appl. 2009;877(14-15):1583-90.

29. Pasman WJ, Blokdijk VM, Bertina FM, Hopman WPM, Hendriks HFJ. Effect of two breakfasts, different in carbohydrate composition, on hunger and satiety and mood in healthy men. Int J Obes Relat Metab Disord. 2003;27(6):663-8.

30. Gross JJ, Levenson RW. Emotion elicitation using films. Cogn Emot. 1995;9(1):87-108.

31. Rottenberg J, Ray RD, Gross JJ. Emotion elicitation using films In: Coan JA, Allen JJB, editors. The handbook of emotion elicitation and assessment. New York: Oxford University Press, Inc; 2007; p. 9-28.

32. Lane AM, Soos I, Leibinger E, Karsai I, Hamar P. Validity of the Brunel Mood Scale for use with UK, Italian and Hungarian athletes In: Lane AM, editor. Mood and human performance: Conceptual, measurement and applied issues. New York: Nova Science Publishers, Inc; 2007; p. 119-30.

33. Lutz B. Endocannabinoid signals in the control of emotion. Curr Opin Pharmacol. 2009;9(1):46-52.

34. Hauer D, Schelling G, Gola H, Campolongo P, Morath J, Roozendaal B, Hamuni G, Karabatsiakis A, Atsak P, Vogeser M. Plasma concentrations of endocannabinoids and related primary fatty acid amides in patients with post-traumatic stress disorder. Plos one. 2013;8(5):e62741.

35. Lo Verme J, Fu J, Astarita G, La Rana G, Russo R, Calignano A, Piomelli D. The nuclear receptor peroxisome proliferator-activated receptor-alpha mediates the anti-inflammatory actions of palmitoylethanolamide. Mol

Pharmacol. 2005;67(1):15-9.

36. Keppel Hesselink JM, Kopsky DJ, Witkamp RF. Palmitoylethanolamide (PEA)—'Promiscuous' anti-inflammatory and analgesic molecule at the interface between nutrition and pharma. PharmaNutrition. 2013

37. Ghafouri N, Ghafouri B, Larsson B, Stensson N, Fowler CJ, Gerdle B. Palmitoylethanolamide and stearoylethanolamide levels in the interstitium of the trapezius muscle of women with chronic widespread pain and chronic neck-shoulder pain correlate with pain intensity and sensitivity. Pain. 2013;154(9):1649-58.

38. Choukèr A, Kaufmann I, Kreth S, Hauer D, Feuerecker M, Thieme D, Vogeser M, Thiel M, Schelling G. Motion sickness, stress and the endocannabinoid system. PLoS One. 2010;5(5):e10752.

39. Wells AS, Read NW, Uvnas-Moberg K, Alster P. Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones. Physiol Behav. 1997;61(5):679-86.

Chapter 4

Moderate alcohol consumption stimulates food intake and food reward of savoury foods

Ilse C. Schrieks
Annette Stafleu
Sanne Griffioen-Roose
Cees de Graaf
Renger F. Witkamp
Rianne Boerrigter-Rijneveld
Henk F.J. Hendriks

Appetite. 2015; 89:77-83

Abstract

The aim of this study was to investigate whether food reward plays a role in the stimulating effect of moderate alcohol consumption on subsequent food intake. In addition, we explored the role of oral and gut sensory pathways in alcohol's effect on food reward by modified sham feeding (MSF) or consumption of a preload after alcohol intake.

In a single-blind crossover design 24 healthy men were randomly assigned to either consumption of vodka/orange juice (20 g alcohol) or orange juice only, followed by consumption of cake, MSF of cake or no cake. Food reward was evaluated by actual food intake measured by an ad libitum lunch 45 min after alcohol ingestion and by behavioural indices of wanting and liking of four food categories (high fat, low fat, sweet and savoury).

Moderate alcohol consumption increased food intake during the ad libitum lunch by 11% (+338 kJ, P=0.004). Alcohol specifically increased intake (+127 kJ, P<0.001) and explicit liking (P=0.019) of high-fat savoury foods. Moreover, moderate alcohol consumption increased implicit wanting for savoury (P=0.013) and decreased implicit wanting for sweet (P=0.017) before the meal. Explicit wanting of low-fat savoury foods only was higher after alcohol followed by no cake as compared to after alcohol followed by cake MSF (P=0.009), but not as compared to alcohol followed by cake consumption (P=0.082). Both cake MSF and cake consumption had no overall effect on behavioural indices of food reward.

To conclude, moderate alcohol consumption increased subsequent food intake, specifically of high-fat savoury foods. This effect was related to the higher food reward experienced for savoury foods. The importance of oral and gut sensory signalling in alcohol's effect on food reward remains largely unclear.

Introduction

Consistent evidence shows that alcohol stimulates short-term food intake when it is consumed before or with the meal (1-3). This effect may relate to reduced satiety signalling after alcohol consumption. However, there is only limited evidence in humans to support such a hypothesis (4, 5). Another potential mechanism through which alcohol may stimulate food intake is by increasing the rewarding value of food via its effects on reward systems. Food reward comprises of two components: 'liking' and 'wanting', which can be divided both psychologically and neurologically (6). Psychologically, liking refers to the pleasantness of food and the pleasure derived from tasting the food, and wanting to the intrinsic motivation to eat. Neurologically, liking has been shown to be influenced by opioid, endocannabinoid and GABA neurotransmission, whereas wanting appears to mainly depend on dopaminergic neurotransmission (6-8). Alcohol may stimulate both liking and wanting as it has been shown to enhance opioid release, and stimulate GABA and dopaminergic neurotransmission (9-11). However, previous studies observing an increased food intake showed no influence of alcohol on the pleasantness nor on pleasure of eating either a savoury or a mixed (savoury and sweet) meal (12, 13). The effect of alcohol on food wanting has not been measured previously. Oral nutrient sensing plays an important role in food reward. Orosensory stimulation by food may induce a cephalic phase response but it may also increase the hedonic and rewarding value of food (14, 15). Gut nutrient sensing may also increase the rewarding value of food, although evidence is less strong as compared to oral nutrient sensing (16, 17). Recently, the effects of oral and gut sensory stimulation on brain reward systems were compared in a study performed in pigs, showing that oral and gut stimulation influenced diverse reward regions (18).

To our best knowledge, no studies have been conducted on the effect of moderate alcohol consumption on the satiety or reward response of orally sensed food. A method to study orosensory stimulation is the modified sham feeding (MSF) technique, in which food is smelled, chewed and tasted, but not swallowed (19-21). By the use of MSF after alcohol consumption the role of orosensory stimulation in alcohol's effect on food intake and food reward can be investigated. Typically, the rewarding value of food decreases with food intake, ultimately causing the person to stop eating. Therefore, we predicted that orosensory stimulation only and oral plus gut sensory stimulation would reduce food intake of the next meal, since both conditions will initiate a reward response. The role of oral and gut stimulation could be explored by comparing food intake after alcohol consumption in combination with cake MSF and in combination with cake consumption.

Rewarding food is often highly palatable food, such as sweet and high-fat food, although savoury food, such as pizza, may also be rewarding (22, 23). Finlayson et al. (2012) studied the effect of equi-palatable savoury and sweet drinks on food reward and observed no difference in liking and wanting between the drinks (24). However, exposure to savoury taste has a

stronger modulating effect on subsequent food preferences as compared with exposure to sweet taste (25). In addition, sweet and savoury intake may activate different reward-related brain systems (26). Therefore, we hypothesized that alcohol could differentially influence the rewarding value of specific food categories based on taste or fat content. Previous studies, however, do not show a difference in taste preference after alcohol intake. Studies showing an increased food intake after alcohol consumption mainly used mixed meals and observed no difference in food preferences (13, 27), though, Caton et al. (2004) showed an elevated intake of high-fat savoury food (crisps) after 4 glasses of alcohol.

The primary aim of this study was to investigate if moderate alcohol consumption stimulates subsequent food intake via an increased food reward. Food reward was evaluated by explicit ratings of wanting and liking and an implicit measure of wanting. Second, we investigated the role of oral and gut sensory stimulation in alcohol's effect on food reward. This was evaluated by comparing food reward after only alcohol consumption with food reward after alcohol consumption followed by oral stimulation or followed by both oral and gut stimulation (normal consumption). We hypothesized that alcohol increases food intake via an increased food reward (both explicit and implicit measures of wanting and liking) of high-fat and sweet foods and that alcohol mediates food reward mainly via orosensory pathways. Both oral stimulation and oral plus gut stimulation were predicted to induce a reward response and thereby decrease food intake of the next meal. The combined oral and gut stimulation was expected to have a larger effect.

Method

Participants

Healthy men (n=24, age 25-50 y, BMI 20-25 kg/m^2) participated in the study. The participants were recruited from a pool of volunteers at CHDR (Centre for Human Drug Research) in Leiden, The Netherlands and by advertising in newspapers. Eligible participants did not use any medication, habitually consumed alcohol (5-20 glasses/week, equal to ± 50-200 g alcohol/week (28)) and had no (family) history of alcoholism. Additionally, participants had to like all the food products used in the study. They were excluded if they scored above average (>2.26) on the restraint eating scale of the Dutch Eating Behaviour Questionnaire (DEBQ). The reasons for including only male participants were the stronger association of moderate alcohol consumption with body weight in men than in women and the possible influence of hormonal fluctuations in women on food intake and reward (29, 30).

The study was conducted at CHDR in Leiden, The Netherlands, and was performed according to the International Conference on Harmonisation Guidelines for Good Clinical Practice. The study also complied with the Declaration of Helsinki and was approved by an independent Medical Ethics Committee (The Medical Ethics Committee of the University Medical Centre of Leiden). Written informed consent was obtained from all participants. The study is registered

at ClinicalTrials.gov (NCT): NCT01738906.

Study design

The study used a single-blind randomized crossover design, with the intervention factors alcohol (alcohol vs. alcohol-free) and food exposure (no cake vs. cake MSF vs. cake consumption). Participants came 6 times to CHDR to have all intervention combinations.
Each participant participated in all 6 experimental conditions, which occurred at least 2 days apart. Participants were randomly allocated to one of the 6 groups with different intervention orders according to a 6x6 Williams square design. Randomized allocation was performed by statisticians of CHDR by the use of a computer-generated randomization scheme.

Interventions

Alcohol intervention

The alcohol intervention consisted of either 200 mL vodka orange juice (20 g alcohol) or 200 mL orange juice with 31 g maltodextrin (Nutricia Fantomalt, Nutricia Cuick, Cuijk, The Netherlands) which they had to consume within 5 min. The beverages were matched for calories by adding maltodextrin, a nonsweet carbohydrate, to the orange juice beverage (Table 4.1). The dosage of 20 g alcohol was considered to be moderate for men (28).
Participants were blinded to the alcohol intervention. Beverages were served in a closed cup and at a serving temperature of ca. 5°C. In addition, a little sterilium (an alcohol based disinfection lotion) was placed at the opposite side of the drinking opening of the lid on only the alcohol-free beverage to make it smell like alcohol.

Food exposure intervention

The food exposure intervention consisted of either MSF of 40 g cake or consumption of 40 g cake or a control condition with no cake. The cake was a commercially available plain butter cake (Table 4.1; Albert Heijn, The Netherlands). The MSF procedure consisted of 3 min oral processing of half of the amount of cake administered (part I), two minutes break for data collection, and again 3 min oral processing of the final half of the amount of cake (part II). During MSF the cake was not swallowed, but spit out in a paper cup together with the water used to rinse their mouth. The weight of this cup was measured to calculate MSF production and to assess whether no cake was swallowed and whether the participant successfully performed the procedure. During consumption of the cake, the procedure was similar to the MSF of cake (e.g. equal oral processing time), except that the cake was swallowed and not spit out. In the control condition, participants received no cake and were inactive during this 6 minute period.

Table 4.1. Energy content and nutritional value of the test foods per served quantity[a].

	65 mL Vodka + 135 mL orange juice	175 mL Orange juice + 31 g maltodextrin	40 g Cake
Energy (kJ)	804	800	630
Protein (g)	0.9	1.2	2.2
Carbohydrates (g)	12	45	18
Of which sugars (g)	12	17	11
Fat (g)	-	-	8
Alcohol (g)	20	-	-

[a] Values derived from the package labels.

Experimental procedures

Participants were instructed to refrain from alcohol 24 hours before each test day, to eat their usual breakfast and lunch on standard times on each test day and to refrain from eating and drinking anything except water 3 hours before testing. Compliance to these instructions was checked by nurses before baseline. At baseline (t0) participants received the beverage with either vodka orange juice or orange juice only, and after 20 min the participants received cake for MSF, for consumption or they received no cake. During the day blood and saliva samples were also collected regularly. In between the measurements and interventions participants were inactive. However, after the last questionnaire, participants went to the waiting room where they could watch television or read while they waited for the final blood alcohol concentration measurement.

Food reward

Food reward was evaluated in the following ways: first, actual intake was measured by an *ad libitum* lunch, which was served 45 min after participants started drinking. Second, on regular times behavioural indices of food liking and wanting were measured (Figure 4.1).

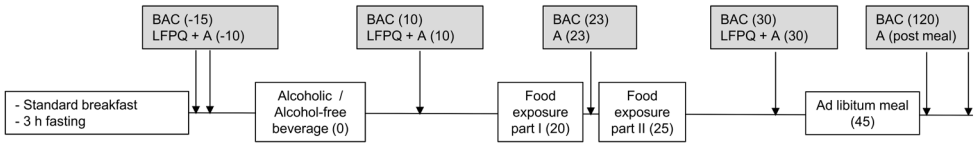


Figure 4.1. Schematic overview of the experimental procedures.

Number in brackets represent the time points (min) at which measurements were performed or food or beverages were administered. BAC, blood alcohol concentration; LFPQ, Leeds Food Preference Questionnaire; A, appetite ratings.

Food intake

Actual intake was measured by an *ad libitum* lunch that was composed of water, bread, diet margarine and several toppings. The exact composition of the lunch is shown in Table 4.2. Participants could choose from 8 toppings, comprising 2 toppings from each of the following food categories: high-fat savoury, low-fat savoury, high-fat sweet, and low-fat sweet. Participants were in separate rooms and had 30 min to eat an *ad libitum* lunch. They could choose and prepare their lunch themselves with the foods supplied. Bread was cut in to quarters, so that cues for amount to consume were lessened.

Behavioural indices of food liking and wanting

The Leeds Food Preference Questionnaire (LFPQ) is a tool to measure various aspects of reward. It is a validated tool developed and extensively described by Finlayson et al. (2007, 2008) (31, 32). We used a Dutch version including 16 photographs of snacks from the food categories high-fat savoury, high-fat sweet, low-fat savoury, and low-fat sweet developed and described by Griffioen-Roose et al. (2010) (25). Explicit wanting and liking was measured on a VAS (100-unit). Participants had to rate their liking (''how pleasant do you find the taste of this food right now?'') and wanting (''how much do you want to eat this food right now?'') for the presented snack products. In addition, implicit wanting was measured by a task where two snack products were shown and participants had to select their most wanted food (''select the food which you most want to eat right now'') as quickly and accurately as possible. During this task the reaction time (RT) was measured and transformed to a standardized 'd-score' (D-RT) according to the algorithm of Greenwald et al. (2003) (33). Smaller D-RT indicates higher implicit wanting for that food category in relation to the other categories.

Appetite

Appetite was evaluated with visual analogue scales (range 0-100) for hunger, desire to eat, prospective food consumption and fullness. Appetite ratings were measured on regular times (Figure 4.1).

Blood alcohol concentration

Blood alcohol concentration (BAC) was measured repeatedly by a breath analyser (Alco-Sensor IV, Honac Nederland B.V., Apeldoorn, The Netherlands). An overview of the measurement time points and food and alcohol administration are shown in Figure 4.1. Participants were not allowed to leave premises until their BAC was below 0.20‰ w/v.

Table 4.2. Energy content and nutritional value[a] of the products offered during the ad libitum lunch (per 100 g).

	Serving[b]	Energy (kJ)	Protein (g)	CHO (g)	Fat (g)
General					
Wholemeal bread	15 slices	930	10	38	2
Diet margarine	1 jar	1200	1	3	30
Water	500 mL	0	0	0	0
High-fat savoury					
Paté	1 piece	1610	8	4	38
Salami	10 slices	1632	17	2	35
Average		1621	13	3	37
Low-fat savoury					
Lean ham	10 slices	397	16	3	2
Smoked beef	10 slices	502	24	1	3
Average		450	20	2	2
High-fat sweet					
Hazelnut spread	1 jar	2273	6	57	32
Coconut slices	6 slices	1730	3	67	14
Average		2002	4	62	23
Low-fat sweet					
Jam	1 jar	1033	0	59	0
Apple syrup	1 jar	1161	1	66	0
Average		1097	1	63	0
Total averages					
High-fat		1811	8	33	30
Low-fat		773	10	32	1
Savoury		1035	16	2	19
Sweet		1549	3	62	11

[a] Values derived from the package labels. CHO, carbohydrate.

[b] Equal servings were supplied to the participants and new jars were provided on every occasion.

Data analysis

Intervention effects on energy intake and choice of the food products from the different categories were analysed within participants with a mixed model analysis of variance including

the fixed factors alcohol (alcohol vs. alcohol-free), food exposure (no cake vs. cake MSF vs. cake consumption), time, and the interaction between alcohol and food exposure. The factors participant and participant by period were added to the model as random factors.
Intervention effects on food reward and appetite before the meal were analysed within participants with a mixed model analysis of variance including the fixed factors alcohol and food exposure, and the 2-way interaction between alcohol and food exposure. The factors participant and participant by period were added to the model as random factors. The interaction effect compared the alcohol effect on food reward and appetite between the food exposure conditions, namely no cake, cake MSF and cake consumption.
Post hoc comparisons were made using Tukey-Kramer adjustment, to correct for multiple testing. Results were considered significantly different at a *P*-value of <0.05. All analyses were performed using SAS version 8 (SAS Institute, Cary, NC, USA) and all data are presented as mean with standard error unless otherwise specified.

Results

Participants

Participants had an average age of 32 ± 0.8 y (median 27.5, range 25-50) and a BMI of 23 ± 0.1 kg/m^2. Their fat percentage, measured by bio-impedance (InBody 720, Biospace Inc, Cerritos, CA, USA), was 14 ± 0.4% and their DEBQ restrained score was 1.63 ± 0.03. The average alcohol consumption was 11 ± 0.3 (range 6-20) glasses per week. Highest mean BAC was measured at 10 min after alcohol ingestion and was on average 0.52‰ w/v.
One participant dropped out for study unrelated reasons; data of this participant's first 3 study days were included in the analysis. One participant was excluded from the analysis because of non-compliance.
Five participants may have swallowed a small portion of the cake during the MSF condition, as the cake weight was lower after MSF than before; data of these occasions were excluded from the analysis. Finally, 130 occasions (of the original 144 occasions) were included in the analysis. The average increase in cake weight after MSF was 13.4 ± 1.2 g (33%).

Energy intake during lunch

Energy intake during lunch is shown in Table 4.3 for all experimental conditions.
Alcohol consumption increased subsequent energy intake during lunch by 11% as compared to no alcohol intake (*P*=0.004), regardless of the food exposure after the beverage.
Cake MSF before lunch did not decrease lunch intake as compared to the control condition without cake. Cake consumption before lunch decreased energy intake during lunch as compared to control (*P*=0.011), regardless of alcohol consumption. Energy intake during lunch was not different after cake MSF and cake consumption.
A trend was observed for an interaction effect of alcohol and food exposure on energy intake

during the lunch (*P*=0.094). Since this was a non-significant trend, *post hoc* comparisons were not conducted. However, for comparability with previous studies we analysed the difference in food intake after alcohol consumption compared to no alcohol consumption in the control condition without cake. Alcohol consumption increased food intake by 19% in the control condition (*P*=0.031).

Table 4.3. Energy intake during lunch (MJ)[a].

	Mean ± SE		Mean ± SE	Mean difference ± SE	*P*-value
Alcohol effect					
Alcohol-free	3.02 ± 0.21	Alcohol	3.36 ± 0.21	-0.34 ± 0.11	0.004
Food exposure effect					0.015
MSF	3.20 ± 0.22	Control	3.39 ± 0.21	-0.19 ± 0.14	0.365
Consumption	2.99 ± 0.21	MSF	3.20 ± 0.22	-0.21 ± 0.14	0.294
Consumption	2.99 ± 0.21	Control	3.39 ± 0.21	-0.40 ± 0.14	0.011
Interaction effect					0.094
Alcohol-free, control	3.10 ± 0.23				
Alcohol-free, MSF	3.21 ± 0.24				
Alcohol-free, consumption	2.77 ± 0.23				
Alcohol, control	3.68 ± 0.23				
Alcohol, MSF	3.19 ± 0.24				
Alcohol, consumption	3.21 ± 0.24				

[a] Least squared means and differences of least squared means are shown as calculated from the mixed model with Tukey-Kramer adjustment for *post hoc* comparisons.

Energy intake from food categories

The influence of alcohol on energy intake from food categories is shown in Figure 4.2A. Overall, participants consumed 24% more high-fat savoury lunch toppings after alcohol consumption as compared to no alcohol consumption (+127 kJ, *P*<0.001). A trend was observed for a higher intake of low-fat savoury toppings after alcohol vs. alcohol-free (+21 kJ, *P*=0.076). Also, more bread was consumed in the alcohol condition (+124 kJ, *P*=0.004). Together, these elevated intakes of bread and savoury toppings accounted for 35% and 52%, respectively, of the total increase in lunch intake by alcohol.

In Figure 4.2B the influence of the food exposure intervention on the energy intake from different food categories is shown. The intake of high-fat sweet toppings was 31% lower when cake was consumed as compared to when no cake was received (*P*=0.009). Food exposure did not influence the intake of other food categories.

The effect of alcohol on food intake of different food categories was not influenced by the

exposure to cake or not.

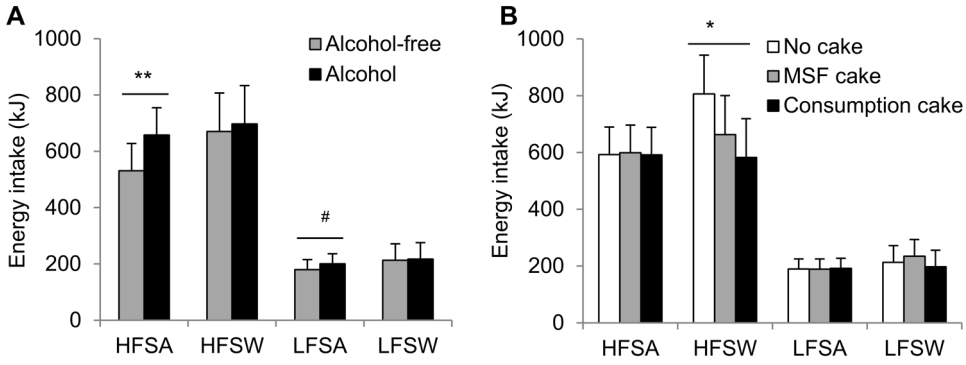


Figure 4.2. The effect of (A) alcohol and (B) food exposure on energy intake of high-fat and low-fat savoury and sweet lunch toppings during the ad libitum lunch.

HFSA, high-fat savoury; HFSW, high-fat sweet; LFSA, low-fat savoury; LFSW, low-fat sweet. ***P*<0.001 for alcohol vs. alcohol-free; #*P*=0.076 trend for alcohol vs. alcohol-free; **P*=0.009 cake consumption vs. control.

Behavioural indices of wanting and liking

The effect of alcohol and food exposure on the wanting and liking of food categories before the meal (30 min after baseline) was studied. Results on implicit wanting are shown in Figure 4.3.

Explicit liking of savoury food was higher after alcohol consumption than after consumption of alcohol-free drinks (high-fat savoury *P*=0.019; low-fat savoury *P*=0.055). Explicit liking of sweet food was not influenced by alcohol. After alcohol consumption implicit wanting was higher for savoury (lower D-RT, *P*=0.013) and lower for sweet (higher D-RT, *P*=0.017) in the alcohol condition as compared to the alcohol-free condition (Figure 4.3).

Food exposure to any of the cake conditions had no overall effect on wanting nor on liking of savoury and sweet food. However, there was an interaction effect of alcohol and food exposure on wanting of low-fat savoury food (*P*=0.018). This was caused by a higher wanting of low-fat savoury food in the condition where alcohol was consumed without cake as compared to the alcohol-free condition without cake consumption (*P*=0.015) and as compared to the alcohol condition with cake MSF (*P*=0.009).

Rated appetite

Overall, hunger and desire to eat ratings were lower during alcohol consumption followed by cake MSF as compared to alcohol consumption only (*P* =0.04, *P*=0.003, respectively).

The different food exposures influenced the time course of hunger (*P*=0.019), desire to eat (*P*=0.002) and fullness (*P*=0.011) ratings. *Post hoc* comparisons showed that desire to eat was lower during cake consumption (in between food exposure part I and II) as compared to

the control condition. Additionally, hunger and desire to eat ratings increased from 10 min after the drink until the meal when no cake was consumed (*P*=0.007 and *P*=0.009, respectively). This rise in hunger and desire to eat was not observed after cake consumption or cake MSF. Fullness ratings were higher during and after cake consumption compared to baseline measures (both *P*<0.001).

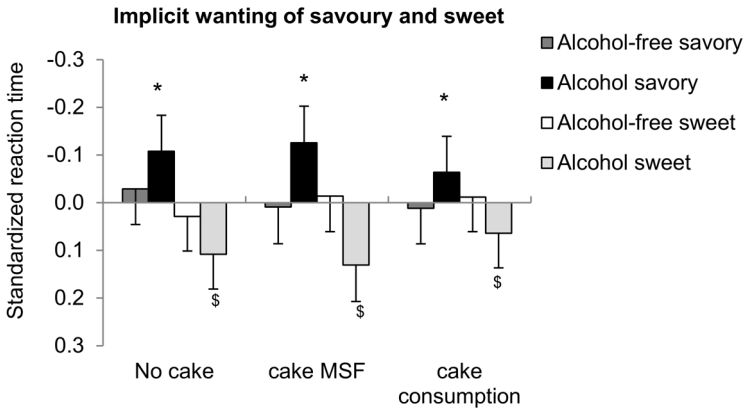


Figure 4.3. Implicit wanting of savoury and sweet food before the meal in the conditions with or without alcohol and after no cake, cake modified sham feeding (MSF) or cake consumption.
* *P*=0.013 alcohol vs. alcohol-free. $ *P*=0.017 alcohol vs. alcohol-free.

Discussion

The role of food reward in the stimulatory effect of moderate alcohol consumption on subsequent food intake was studied. We showed that alcohol increased subsequent food intake, especially of high-fat savoury food, which concurred with an increased food reward for savoury foods. We also explored if moderate alcohol consumption increases food reward via oral or gut sensory pathways, by the use of cake MSF or cake consumption. However, this study showed no modulation of alcohol's influence on food reward by oral stimulation only (cake MSF) or oral and gut stimulation (cake consumption).

The finding that consumption of a moderate amount (20 g) of alcohol stimulated subsequent food intake confirms findings from previous studies. In the present study, a 19% increased energy intake was observed after 20 g alcohol consumption in the control condition, which is comparable to observed increases of 26-30% in energy intake in previous studies using dosages of 15-30 g alcohol (3, 12, 34). This is the first study, however, that measured the effect of moderate alcohol consumption on food intake in combination with behavioural indices of reward, liking and wanting. Liking and wanting of different food categories was measured in order to discriminate reward of sweet and savoury foods and of high-fat and low-fat foods.

Moderate alcohol consumption increased the intake of savoury food, and liking and wanting of savoury food as compared to no alcohol consumption. More specifically, alcohol consumption increased the intake and explicit liking of high-fat savoury food and increased the explicit wanting of low-fat savoury food. Also implicit wanting of savoury food was increased after alcohol consumption. These effects were not shown by previous studies, although Caton et al. (2004) showed an increased intake of crisps after 4 glasses of alcohol as compared to 1 glass of alcohol, suggesting a higher preference of high-fat savoury by alcohol consumption (13, 27). Yeomans and Phillips (2002) showed that alcohol acutely increased the saltiness perception and intake of a savoury test meal, which suggests that altered taste perception may play a role (35). The higher savoury food intake and reward after alcohol consumption could also be a learned effect, because in Western societies alcohol consumption is often combined with the consumption of savoury foods.

To study the role of oral and gut sensory signalling in alcohol's effect on food intake and reward, we compared food intake after alcohol consumption in combination with cake MSF and in combination with cake consumption. Oral stimulation by MSF has been shown to induce a cephalic phase response via oral sensory signalling, which in turn activates different physiological processes, such as a satiation and a reward response (14, 21). In our study, cake consumption reduced subsequent food intake compared to control. After cake MSF, subsequent food intake was not reduced. Moreover, only in the cake consumption condition, a decreased intake of high-fat sweet food was observed. This reduction in high-fat sweet after cake consumption may have been due to sensory specific satiation, since the cake was high-fat sweet too. Furthermore, we observed an increased appetite over time in the control condition, but not in the MSF and consumption condition, suggesting a reduced appetite by MSF and consumption relative to the control. However, fullness was only increased over time by consumption of cake but not by cake MSF. In contrast to our hypotheses, no main effects of cake MSF and consumption were found on wanting and liking of the food categories. These results suggest that the reduction of overall food intake during lunch and high-fat sweet food after cake consumption appear more related to a reduction in general appetite feelings than to a reduced food reward for specific food categories. Other studies have shown an effect of MSF on subsequent food intake and sensory specific satiation (21, 36, 37). However, similar to our findings, they also found a non-significant decrease in appetite after MSF. It could be that the sham feeding time was too short or the volume was too small to influence subsequent food intake and reward (21). It should be noted that the MSF procedure may have been experienced as unpleasant for some of the participants. It is possible that the combination with alcohol consumption made the experience even more unpleasant, resulting in reduced appetite. However, none of the participants reported feeling nauseous after alcohol consumption and MSF.

We observed one interaction effect between alcohol and food exposure: wanting of low-fat savoury was lower after cake MSF compared to control when preceded by alcohol

consumption. Since this was the only interaction effect observed, the role of oral and gut sensory stimulation in alcohol's appetizing effect remains unclear. Therefore, further research on the role of oral and gut sensory signalling in alcohol's effect on food reward is needed.

A strength of this study is the randomized crossover design. Therefore, effects could be studied within participants and without influence of between-person variance, which may be large for behavioural indices of reward. Moreover, this is the first study that measured the effect of alcohol on both intake and food reward of different food categories. Therefore, the increased intake of savoury foods during the lunch could be linked to the increased explicit liking and wanting and implicit wanting of savoury food measured by the LFPQ. This consistency between the outcomes also strengthens our findings.

However, the study design also had limitations that warrant consideration. First, blinding of the alcohol treatment might not have been complete due to physiological and behavioural effects of alcohol. Most participants guessed correctly whether the beverage contained alcohol. Therefore, learned associations may have influenced the increased consumption of savoury food with alcohol. However, Yeomans et al. (2010) showed that when alcohol is provided in a non-alcohol context, such as orange juice, learned associations with increased food intake have a lower impact than when it is provided in an alcohol-context (38). Yet, it would be interesting to study this effect on savoury in cultures where alcohol consumption is not associated with savoury food intake. Second, the type and taste of alcohol used in this study may have influenced the rewarding value and intake of savoury food. In the present study vodka orange juice was used as alcoholic beverage, which has a sweet and bitter taste. It could be hypothesized that effects on savoury intake and reward would have been different with other alcoholic beverages. Third, the beverages could not be fully matched on taste and smell, although we tried to make them as equal as possible. The vodka orange juice had a more bitter taste than the orange juice due to the alcohol content. This may have influenced subsequent food choice and food reward of the different food categories and may also have decreased the effectiveness of the blinding. Also, the palatability of the beverages has not been measured. Therefore, a difference in palatability between the alcoholic and non-alcohol drink may also have influenced subsequent food reward of the different food categories. Fourth, the study design was single-blind. The experimenter was not blinded to the treatment and may therefore have influenced the participant. A final limitation is the strong association between the measurement of explicit liking and wanting indices by the LFPQ, also shown previously (25). This strong association made it difficult to differentiate these two components of reward.

To conclude, moderate alcohol consumption increased subsequent food intake, specifically of high-fat savoury foods. This effect was related to the higher food reward experienced for savoury foods. The importance of oral and gut sensory signalling in alcohol's effect on food reward remains largely unclear.

Acknowledgements

We gratefully acknowledge the Centre for Human Drug Research for the successful collaboration on the conduct of the study, specifically P. A. M. Peeters, and J. M. A. van Gerven. We thank the volunteers for their enthusiastic participation. We also thank E. D. Schoen, C. M. de Jong-Rubingh and S. Bijlsma from TNO for their statistical advice.

Funding

This work was supported both by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO (Grant EZ1503). Their joint aim is to independently study the health effects of moderate alcohol consumption. The funding sources had no role in the study design, in conducting, analysing, or interpreting the data or in the decision to submit the article for publication.

References

1. Yeomans MR. Effects of alcohol on food and energy intake in human subjects: Evidence for passive and active over-consumption of energy. British Journal of Nutrition. 2004;92:S31-4.
2. Yeomans MR. Alcohol, appetite and energy balance: Is alcohol intake a risk factor for obesity? Physiology & Behavior. 2010;100(1):82-9.
3. Westerterp-Plantenga MS, Verwegen CR. The appetizing effect of an aperitif in overweight and normal-weight humans. Am J Clin Nutr. 1999;69(2):205-12.
4. 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(1):91-100.
5. Röjdmark S, Calissendorff J, Brismar K. Alcohol ingestion decreases both diurnal and nocturnal secretion of leptin in healthy individuals. Clin Endocrinol. 2001;55(5):639-47.
6. Berridge KC. 'Liking' and 'wanting' food rewards: Brain substrates and roles in eating disorders. Physiology & Behavior. 2009;97(5):537-50.
7. Berridge KC. Food reward: Brain substrates of wanting and liking. Neuroscience & Biobehavioral Reviews. 1996;20(1):1-25.
8. Cooper SJ. Palatability-dependent appetite and benzodiazepines: New directions from the pharmacology of $GABA_A$ receptor subtypes. Appetite. 2005 4;44(2):133-50.
9. Kumar S, Porcu P, Werner D, Matthews D, az-Granados J, Helfand R, et al. The role of GABA(A) receptors in the acute and chronic effects of ethanol: A decade of progress. Psychopharmacology. 2009;205(4):529-64.
10. Oswald LM, Wand GS. Opioids and alcoholism. Physiology & Behavior. 2004;81(2):339-58.
11. Melis M, Diana M, Enrico P, Marinelli M, Brodie MS. Ethanol and acetaldehyde action on central dopamine systems: Mechanisms, modulation, and relationship to stress. Alcohol. 2009;43(7):531-9.
12. Yeomans MR, Hails NJ, Nesic JS. Alcohol and the appetizer effect. Behav Pharmacol. 1999;10(2):151-61.
13. Caton SJ, Marks JE, Hetherington MM. Pleasure and alcohol: Manipulating pleasantness and the acute effects of alcohol on food intake. Physiology & Behavior. 2005;84(3):371-7.
14. Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. Central nervous system control of food intake and body weight. Nature. 2006;443(7109):289-95.
15. Berthoud HR. Vagal and hormonal gut-brain communication: From satiation to satisfaction. Neurogastroenterology & Motility. 2008;20:64-72.
16. Spetter MS, de Graaf C, Mars M, Viergever MA, Smeets PAM. The sum of its Parts—Effects of gastric distention, nutrient content and sensory stimulation on brain activation. PloS one. 2014;9(3):e90872.
17. Sclafani A, Ackroff K. Role of gut nutrient sensing in stimulating appetite and conditioning food preferences. Am J Physiol Regul Integr Comp Physiol. 2012;302(10):R1119-33.
18. Clouard C, Meunier-Salaün M, Meurice P, Malbert C, Val-Laillet D. Combined compared to dissociated oral and intestinal sucrose stimuli induce different brain hedonic processes. Frontiers in psychology. 2014;5:851.
19. Teff KL, Engelman K. Oral sensory stimulation improves glucose tolerance in humans: Effects on insulin, C-peptide, and glucagon. Am J Physiol Regul Integr Comp Physiol. 1996;39(6):R1371.

20. Joosten MM, de Graaf C, Rietman A, Witkamp RF, Hendriks HFJ. Short-term oral exposure to white wine transiently lowers serum free fatty acids. Appetite. 2010;55(1):124-9.

21. Wijlens AGM, Erkner A, Alexander E, Mars M, Smeets PAM, Graaf C. Effects of oral and gastric stimulation on appetite and energy intake. Obesity. 2012;20(11):2226-32.

22. Tetley AC, Brunstrom JM, Griffiths PL. The role of sensitivity to reward and impulsivity in food-cue reactivity. Eating Behav. 2010;11(3):138-43.

23. Egecioglu E, Skibicka K, Hansson C, varez-Crespo M, Friberg P, Jerlhag E, et al. Hedonic and incentive signals for body weight control. Reviews in Endocrine & Metabolic Disorders. 2011;12(3):141-51.

24. Finlayson G, Bordes I, Griffioen-Roose S, de Graaf C, Blundell JE. Susceptibility to overeating affects the impact of savory or sweet drinks on satiation, reward, and food intake in nonobese women. J Nutr;142(1):125-30.

25. Griffioen-Roose S, Finlayson G, Mars M, Blundell JE, de Graaf C. Measuring food reward and the transfer effect of sensory specific satiety. Appetite. 2010;55(3):648-55.

26. Spetter MS, de Graaf C, Viergever MA, Smeets PAM. Anterior cingulate taste activation predicts ad libitum intake of sweet and savory drinks in healthy, normal-weight men. J Nutr. 2012;142(4):795-802.

27. Caton SJ, Ball M, Ahern A, Hetherington MM. Dose-dependent effects of alcohol on appetite and food intake. Physiology & Behavior. 2004;81(1):51-8.

28. Kalant H, Poikolainen K. Moderate drinking: Concepts, definitions and public health significance. In: MacDonald IE, editor. Health issues related to alcohol consumption. Philadelophia, PA: ILSI Europe, Blackwell Science; 1999. p. 1-27.

29. Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: A systematic review. Nutr Rev. 2011;69(8):419-31.

30. Bryant M, Truesdale K, Dye L. Modest changes in dietary intake across the menstrual cycle: Implications for food intake research. Br J Nutr. 2006;96(05):888-94.

31. Finlayson G, King N, Blundell JE. Is it possible to dissociate 'liking' and 'wanting' for foods in humans? A novel experimental procedure. Physiology & Behavior. 2007;90(1):36-42.

32. Finlayson G, King N, Blundell JE. The role of implicit wanting in relation to explicit liking and wanting for food: Implications for appetite control. Appetite. 2008;50(1):120-7.

33. Greenwald AG, Nosek BA, Banaji MR. Understanding and using the implicit association test: I. an improved scoring algorithm. J Pers Soc Psychol. 2003;85(2):197.

34. Caton SJ, Bate L, Hetherington MM. Acute effects of an alcoholic drink on food intake: Aperitif versus co-ingestion. Physiology & Behavior. 2007;90(2):368-75.

35. Yeomans MR, Phillips MF. Failure to reduce short-term appetite following alcohol is independent of beliefs about the presence of alcohol. Nutr Neurosci. 2002;5(2):131-9.

36. Nolan LJ, Hetherington MM. The effects of sham feeding-induced sensory specific satiation and food variety on subsequent food intake in humans. Appetite. 2009;52(3):720-5.

37. Smeets AJPG, Westerterp-Plantenga MS. Oral exposure and sensory-specific satiety. Physiol Behav. 2006;89(2):281-6.

38. Yeomans MR. Short term effects of alcohol on appetite in humans. effects of context and restrained eating. Appetite. 2010;55(3):565-73.

Chapter 5

Moderate alcohol consumption after a mental stressor attenuates the stress response

Ilse C. Schrieks
Michel M. Joosten
Ineke A.A. Klöpping-Ketelaars
Renger F. Witkamp
Henk F.J. Hendriks

Submitted

Abstract

Aims: Alcohol is often consumed to reduce tension and improve mood when exposed to stressful situations. Previous studies showed that moderate alcohol consumption may reduce stress when alcohol is consumed prior to a stressor, but data on the effect of alcohol consumption after a mental stressor is limited. Therefore, our objective was to study whether moderate alcohol consumption immediately after a mental stressor attenuates the stress response.

Methods: Twenty-four healthy men (age 21-40 y, BMI 18-27 kg/m2) participated in a placebo-controlled trial. They randomly consumed 2 cans (660 mL, ~26 g alcohol) of beer or alcohol-free beer immediately after a mental stressor (Stroop task and Trier Social Stress Test). Physiological and immunological stress response was measured by monitoring heart rate and repeated measures of the hypothalamic-pituitary-adrenal axis (HPA-axis), white blood cells and a set of cytokines.

Results: After a mental stressor, cortisol and adrenocorticotropic hormone (ACTH) concentrations were 100% and 176% more reduced at 60 min (P=0.012 and P=0.001, respectively) and 92% and 60% more reduced at 90 min (P<0.001 and P=0.056, respectively) after beer consumption as compared to alcohol-free beer consumption. Heart rate and dehydroepiandrosterone (DHEA) were not influenced by alcohol consumption. Plasma IL-8 concentrations remained lower during the stress recovery period after beer consumption than after alcohol-free beer consumption (P<0.001).

Conclusions: Consumption of a moderate dose of alcohol after a mental stressor may facilitate stress response recovery as reflected by decreasing plasma ACTH and cortisol.

Introduction

Humans have to respond adequately to physiological and behavioral challenges occurring in a dynamically changing environment in order to survive. Physiological reactions to stress generally comprise changes in neuroendocrine, hormonal and immune functions (1).
Several studies have shown that alcohol consumption shortly before a mental stressor blunts the stress response (2-4). Such a stress-response dampening effect of alcohol has first been proposed by Levenson et al. (1980) (5). They showed in male students that alcohol consumption prior to a stressor (either speech or electric shock) reduced anxiety and heart rate during the stress response as compared to the placebo condition. Similarly, a reduced hypothalamic-pituitary-adrenal axis (HPA axis) activity, evaluated as adrenocorticotropic hormone (ACTH) and cortisol has been reported (2, 3). However, an increased activity of the HPA axis by alcohol consumption has also been reported (6). This difference in hormonal responses may be related to the dosage used in the studies. Although there is some evidence for a stress-response dampening effect of alcohol, when consumed before a stressor, data on the effect of alcohol during stress response recovery is scarce. This is surprising, because alcohol is frequently used to reduce the stress after a working day or stressful event. Childs et al. (2011) compared the influence of an intravenous alcohol infusion immediately or 30 min after a mental stressor on the stress response (7). They showed that infusion of alcohol immediately after the stressor blocked the cortisol response. Another study by de Wit et al. (2003) showed no effect of moderate alcohol consumption on cortisol levels immediately after mental stress. However, they measured cortisol only once after the stressor, possibly leaving an effect of alcohol consumption on cortisol recovery undetected (8). Moderate alcohol consumption may also shortly induce arousal and increase heart rate (9). This might interfere with the stress-response dampening effects of alcohol when consumed after the stressor.
The neuroendocrine and immune systems are highly interrelated (10). The acute immune response after a mental stressor has been well described. A meta-analysis has shown robust effects, such as increased levels of circulating IL-6 and IL-1β, and marginal effects on CRP (11). Additionally, Kimura et al. (2007) have documented changes in the number circulating T and B cells and natural killer cells following acute mental stress (12). Moderate alcohol consumption has been suggested to have immuno-modulatory and anti-inflammatory effects (13). For example, Mandrekar et al. (2006) observed an attenuated monocyte inflammatory response and an augmented response of anti-inflammatory cytokine IL-10 after alcohol consumption (14). However, the influence of moderate alcohol consumption on the immune response to a mental stressor has not been investigated.
Therefore, our primary aim was to investigate whether moderate alcohol consumption immediately after a mental stressor attenuates the stress response. Our secondary aim was to investigate whether the stress-induced immune response was also affected by moderate alcohol consumption.

Materials and methods

Participants

Twenty-four healthy men (age 21-40 years, BMI 18-27 kg/m^2) participated in the study. The participants were recruited from a pool of volunteers at TNO (The Netherlands Organization for Applied Scientific Research) in Zeist. Eligible participants did not use any medication, habitually consumed alcohol (5-27 standard units/week), were non-smokers and had no (family) history of alcoholism. The study was performed according to the International Conference on Harmonisation Guidelines for Good Clinical Practice. The study also complied with the Declaration of Helsinki and was approved by an independent Medical Ethics Committee (METOPP, Tilburg, The Netherlands). Written informed consent was obtained from all participants.

Experimental protocol

The study was originally set up as a randomized, open-label, crossover design. Participants consumed either 2 cans of beer (660 mL, ~26 g alcohol) or alcohol-free beer (<0.5 g alcohol) in a randomized order shortly after they performed a mental stress test. Because of a strongly attenuated cortisol and ACTH stress response after the second mental stress test, indicating a strong learning effect, we decided to use data from the first mental stress test only.

Participants were instructed to refrain from eating or drinking anything except water 2.5 hours before testing (participants arrived at 11:30 h). The mental stress test was performed after initial blood samples were taken and heart rate had been recorded. Immediately after the stress test, 2 cans of beer (either beer or alcohol-free beer) were consumed within 30 min. Participants were instructed to drink the first can during the first 15 min, and the second can during the remaining 15 min. During the following 3 hours subjects were kept in a fasted state. During this period blood samples were obtained at regular intervals and heart rate was continuously monitored (Table 5.1).

Table 5.1. Overview of experimental protocol.

	-45 min	-30 min stressor	0 min 1st can	15 min 2nd can	30 min	60 min	90 min	150 min	210 min
Heart rate [a]		x	x			x			
HPA-axis response [b]	x	x	x	x	x	x	x		
Immune response [c]	x	x	x		x		x	x	x

[a] heart rate was continuously monitored and afterwards the average heart rate was calculated at baseline (-45 min till -30 min), during the stress period (-20 min till 0 min), and during the stress recovery period (10 min till 210 min). These time points will be referred to as t=-30 min, t=0 min and t=60 min, respectively.

[b] measurement of adrenocorticotropic hormone (ACTH), cortisol and dehydroepiandrosterone (DHEA)

[c] measurement of IL-1β, IL-6, IL-8, TNF-α, number of white blood cells

HPA-axis, hypothalamic-pituitary-adrenal axis.

Mental stress test

The mental stress test comprised of two stress protocols: the Stroop task and the Trier Social Stress Test (TSST). These tests were performed in approximately 30 min. Both the Stroop task and the TSST have been shown to induce a physiological stress-response (15).

The Stroop task is a computerized color-word interference task, involving the successive presentation of target color words printed in an incongruous color. Participants had to press the computer key that corresponded to the name of the color in which the word was printed.

The TSST is a standardized laboratory stress test that was performed following the procedure of Kirschbaum et al. (1993) (16). The TSST consists of two tasks: a mental arithmetic task (10 min) and a public speaking exercise (10 min). In the mental arithmetic task participants had to serially subtract the number 13 from 1022 as fast and accurate as possible. In case of miscalculation they had to start over. For the public speaking exercise, participants got 5 minutes to prepare a talk about their personal characteristics, e.g. their strengths and weaknesses. Afterwards, they went to a room where they had 5 min to present their talk in front two actors, who criticized their talk non-verbally.

Physiological measures

Blood samples were collected at 7 time points for the measurement of ACTH, cortisol and dehydroepiandrosterone (DHEA) and at 8 time points for the measurement of cytokines and total and differential white blood cell count (i.e. leucocytes, lymphocytes, neutrophils, monocytes, eosinophils and basophils) (Table 5.1). Blood was obtained from the antecubital vein of the forearm and collected in pre-chilled tubes containing EDTA for plasma (Vacutainer Systems, Becton Dickinson, Plymouth, UK). After the tubes had been centrifuged, the plasma samples were stored at -80°C until assayed.

Plasma ACTH concentrations were measured by an enzyme-linked immunosorbent assay (ELISA) (AlpcoDiagnostics, Salem, NH). The intra-assay coefficient of variation was 6.7%. Plasma cortisol concentrations were determined using Olympus analytical equipment and reagents. Plasma DHEA concentrations were measured with an ELISA (Enzo Life Sciences, Lausen, Switzerland) with an intra-assay coefficient of variation of 4.8%.

The plasma concentration of the cytokines IL-1β, IL-6, IL-8, TNF-α were analyzed with a multi-spot assay (Meso Scale Diagnostics, Rockville, MD). For total and differential white blood cell count, blood was collected at room temperature in tubes containing EDTA. After collection, the tubes were stored at room temperature and analysed within 6 h using an Advia 120 hematology system.

Data analysis

Repeated measures of changes in physiological responses (ACTH, cortisol, DHEA, heart rate, total white blood cells and differential white blood cells) were compared between beer and alcohol-free beer consumers, using mixed model analyses. Time, treatment (beer vs. alcohol-

free beer) and the interaction of time and treatment were added as fixed factors to the model. Post hoc comparisons were made using Tukey-Kramer adjustment, to correct for multiple testing. Statistical analyses were performed using the SAS statistical software package (SAS version 8; SAS Institute, Cary, NC, USA). Error bars in figures express standard errors of the mean. Results were considered significantly different at a *P*-value of <0.05.

Results

Subjects

The baseline characteristics are shown in Table 5.2. Participants in the beer group and the alcohol-free beer group did not differ in their baseline values of stress hormones, heart rate and immune function. Although age, body weight and BMI were slightly higher in the alcohol-free beer group, the difference did not reach significance.

Table 5.2. Baseline characteristics of 24 men[a]

	Beer (n=12)	Alcohol-free beer (n=12)	*P*-value
Age (years)	24.8 (4.6)	27.4 (6.2)	0.245
Body weight (kg)	78.6 (7.2)	81.1 (11.3)	0.533
BMI (kg/m^2)	22.2 (1.3)	23.4 (2.0)	0.091
Heart rate (beats/min)	73.9 (8.7)	73.3 (10.9)	0.872
Cortisol (nmol/L)	353 (124)	343 (102)	0.832
Log ACTH (pg/mL)	2.68 (0.57)	2.51 (0.39)	0.402
DHEA (ng/mL)	4.37 (3.01)	4.66 (4.57)	0.870
Leucocytes (giga/L)	6.85 (1.53)	6.71 (1.19)	0.802
- Lymphocytes (%)	35.03 (9.36)	33.20 (6.18)	0.578
- Neutrophils (%)	56.63 (9.83)	57.85 (5.46)	0.710
- Monocytes (%)	5.53 (1.38)	5.18 (0.90)	0.481
- Eosinophils (%)	2.33 (0.98)	3.21 (2.96)	0.348
- Basophils (%)	0.49 (0.12)	0.53 (0.20)	0.625
IL-1β (pg/ml)	0.59 (0.41)	0.37 (0.14)	0.105
IL-6 (pg/ml)	4.25 (2.34)	3.62 (1.44)	0.438
IL-8 (pg/ml)	2.81 (1.08)	3.35 (1.15)	0.247
TNF-α (pg/ml)	2.38 (0.74)	2.34 (0.69)	0.878
STAI trait score	31.3 (6.1)	30.3 (5.9)	0.689

[a] All characteristics are mean (SD) and were measured on the treatment day, except for age, body weight, BMI and STAI that were measured during the screening.

Physiological response to the mental stress test

Physiological responses to the mental stress test are shown in Figure 5.1 for the alcohol-free condition. Cortisol increased immediately after the stress test (+28%, *P*=0.044) and decreased again at 90 min (*P*=0.002). A similar pattern was observed for ACTH and DHEA, although the rise and fall in ACTH and DHEA did not reach significance. ACTH tended to increase immediately after the stressor (+13%, P=0.070) and decreased again at 90 min (*P*<0.001). DHEA did not increase (*P*=0.71), but showed a significant decrease from 0 min to 90 min (*P*=0.013). Heart rate increased by the mental stress test from 73.3 to 89.1 beats/min (*P*=0.024).

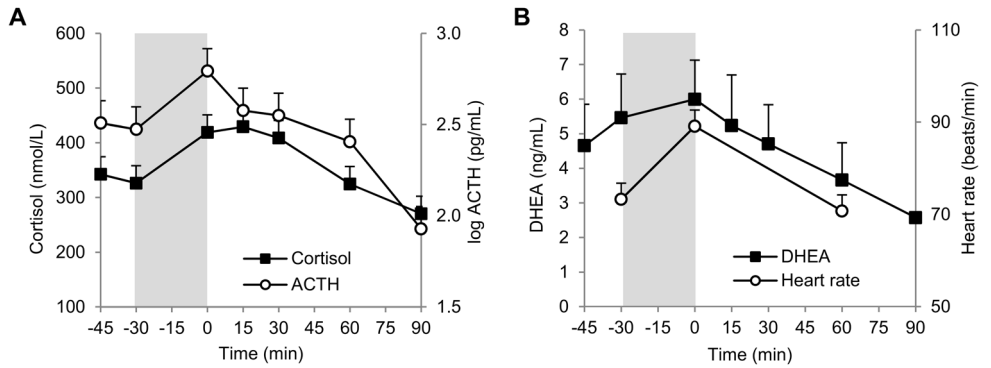


Figure 5.1. The response in cortisol, ACTH (A), DHEA and heart rate (B) before and after the mental stress test in the alcohol-free beer condition.

The grey area represents the period of mental stress.

Stress response recovery

Recovery of cortisol and ACTH after the mental stress test is shown in Figure 5.2. Overall, plasma cortisol and ACTH were more decreased during the stress recovery period after beer consumption than after alcohol-free beer consumption (*P*<0.001). However, there was an alcohol x time interaction effect. Plasma cortisol concentrations decreased 100% and 90% more at 60 and 90 min after beer consumption as compared to after alcohol-free beer consumption (*P*=0.012 and *P*<0.001, respectively). A similar pattern was observed for ACTH; concentrations decreased 176% more at 60 min after beer consumption vs. alcohol-free beer consumption (*P*=0.001) and tended to decrease more after beer consumption at 90 min after the stress test (*P*=0.056). Heart rate and DHEA were not influenced by alcohol consumption (*P*=0.659 and *P*=0.882, respectively).

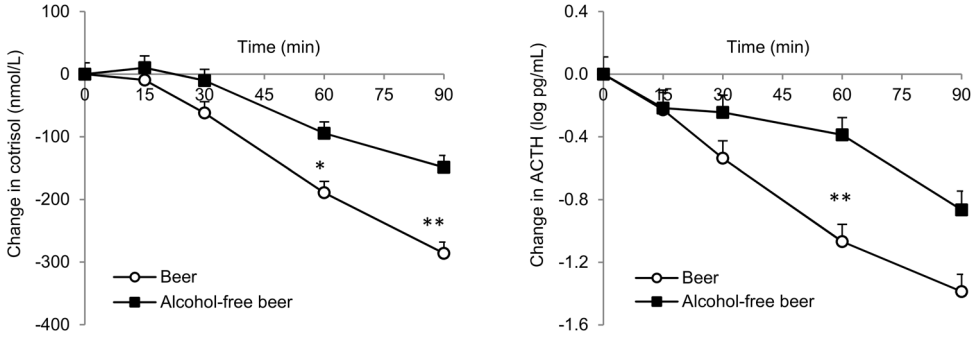


Figure 5.2. Changes in cortisol and ACTH response after consumption of beer (n=12) or alcohol-free beer (n=12) after a mental stress test.

* *P*<0.05 beer vs. alcohol-free beer; ** *P*<0.01 beer vs. alcohol-free beer.

Immune response

The influence of beer or alcohol-free beer consumption on the immune response is shown in Figure 5.3. IL-8 response was overall lower when beer was consumed than when alcohol-free beer was consumed after the mental stressor (*P*<0.001). No influence of alcohol consumption was observed on the change in cytokines IL-1β, IL-6, and TNF-α. Of the white blood cells, only the percentage of monocytes were affected by alcohol. Monocytes were more decreased at 150 min after beer consumption (*P*<0.001) but not after alcohol-free beer consumption *(P*=0.81). This resulted in a lower monocyte level after beer vs. alcohol-free beer consumption (*P*=0.009).

Discussion

The study showed that moderate alcohol consumption immediately after a mental stressor increases the decline of plasma cortisol and ACTH concentrations during stress recovery. Additionally, moderate alcohol consumption decreased plasma concentrations of IL-8 during stress recovery.

The finding of a more pronounced decrease in HPA-axis hormones, cortisol and ACTH, is in accordance with previous studies showing a reduced HPA-axis response when alcohol was consumed before stress or was intravenously administered immediately after stress induction (2, 3, 7). De Wit et al. (2003) observed no difference in cortisol concentration after stress when this was followed by moderate alcohol consumption or placebo. However, in this study cortisol was measured at 10 min after the stressor only (8). In the present study, the difference in cortisol and ACTH response was not observed until 60 min after alcohol consumption. This is in agreement with a previous study in which we observed a reduction in

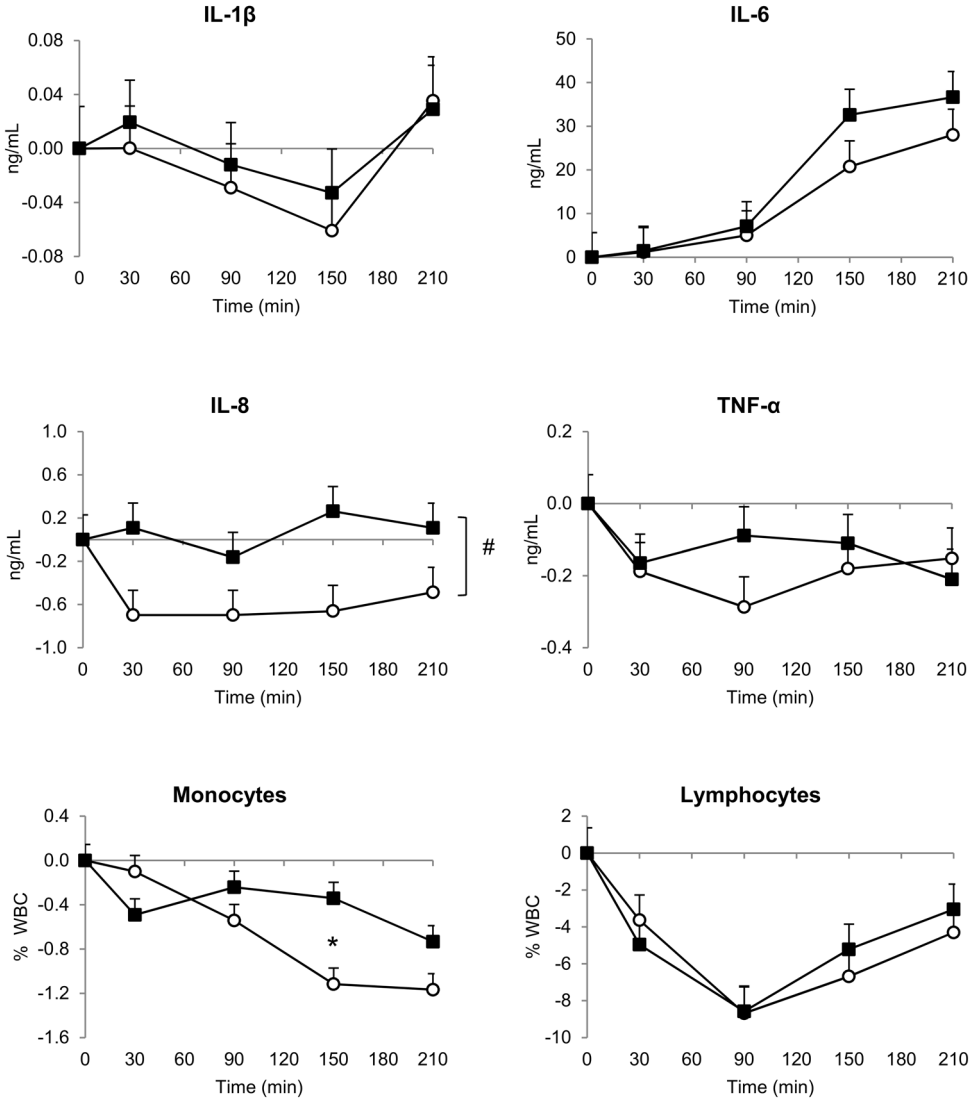


Figure 5.3. Changes in selected cytokines, monocytes and lymphocytes after a psychological stressor during beer consumption (-○-) (n=12) or alcohol-free beer consumption (-■-) (n=12).

[#] IL-8 concentrations were overall lower after beer vs. alcohol-free beer consumption (P<0.001). * Percentage monocytes were lower at 150 min after beer consumption vs. alcohol-free beer consumption (P=0.009).

cortisol 2 h after alcohol consumption (unpublished data). To our knowledge, this is the first study measuring the effect of alcohol on the DHEA response to mental stress. DHEA is, like cortisol, secreted by the adrenal cortex in response to ACTH. It is a hormone with anabolic

properties and has been related to anti-inflammatory and anti-glucocorticoid effects (17). Sierksma et al. (2004) showed that 6 weeks of moderate alcohol (beer) consumption resulted in 16.5% higher DHEA sulphate concentrations compared to alcohol-free beer consumption (18). Previous studies showed an increase in DHEA in response to acute stress (19, 20). In our study, however, DHEA was not significantly increased by stress, but showed a reduction during stress recovery. We did not observe any indication for even a slight effect of alcohol on DHEA. We observed a large inter-individual variation, however, which was also observed by Lennartsson et al. (2012) (19).

In contrast to the studies of Levenson et al. (1980) and Sher et al. (1986), we did not observe an effect of alcohol consumption on heart rate (5, 21). This might be due the limited time frames at which average heart rate was calculated in our study (i.e. baseline, stress, and recovery period). However, Sher et al. (2007) did also not observe an effect of alcohol on heart rate response during stress, while they analyzed heart rate during a larger number of time frames around the mental stressor (4).

We also observed small effects of alcohol consumption on the immune response during the stress recovery period. However, the interpretation of some of the cytokines, especially IL-1β, was difficult due to values measured below the detection limit. We observed a lower IL-8 concentration throughout the stress recovery period and a lower percentage of monocytes 150 min after alcohol consumption than after consumption of alcohol-free drinks. Both IL-8 and monocytes have an important function in the pathology of atherosclerosis. The chemokine IL-8 has been shown to trigger the firm adhesion of monocytes to vascular endothelium (22). In addition, IL-8 has been suggested to stimulate plaque formation via its angiogenic properties (23). This would be in line with previous studies showing the beneficial effects of alcohol on vascular health (24-26). However, other cytokines and white blood cells, which are also important immune factors in atherosclerosis development (27), were not affected by alcohol consumption in the current study.

The current study is limited by several factors. First, it was not possible to have a within-subjects design because of the large learning effect which resulted in an attenuated stress response on the second mental stress test. This limited our power to detect differences, because of the inter-individual differences in the effect of alcohol on stress-response dampening (21). Nevertheless, we observed effects of alcohol on the stress response and some factors of the immune system. Second, the effects of alcohol on the physiological stress response could not be compared to perceived stress responses as we did not included subjective measures on anxiety or tension. Therefore, the interpretation that alcohol reduces feelings of stress cannot be made. Third, the study was an open-label study, and therefore expectancy effects may have influenced the results (3). This should be taken into account when interpreting the results. Finally, the response of ACTH and cortisol may not have been measured long enough to measure the complete period during which alcohol influences these hormones, because the effect of alcohol was still increasing at the last measurement at 90 min after consumption.

This study was performed in young men, who were not very anxious before they started the stress test as indicated by STAI state scores. Anxiety before the stress test may have influenced both the cortisol response to stress as well as the effects of alcohol on stress (20, 28). Therefore, the effects are not directly generalizable to more anxious men and women.
In conclusion, consumption of a moderate dose of alcohol after a mental stressor may facilitate a reduction in stress by decreasing ACTH and cortisol. Additionally, the lower levels of monocytes and IL-8 after alcohol consumption, suggest a modest attenuation of the immune response during the stress recovery period by alcohol consumption. The high incidence of stress-related disorders underlines the relevance of further exploring the influence of moderate alcohol consumption on mental stress and stress disorders. The influence of alcohol consumption on the stress-induced immune response needs further research and may include nuclear factor-κB (NF-κB) and anti-inflammatory cytokine IL-10. In previous studies we showed that NF-κB may play a pivotal role in the effect of alcohol consumption on immune function (29, 30), and Mandrekar et al. (2006) showed an increased IL-10 response after alcohol consumption (14). Additionally, the influence of long-term moderate alcohol consumption on stress-response dampening and stress habituation to repeated stress would improve our understanding of the influence of alcohol on stress-related disorders.

Acknowledgements

We acknowledge all those involved in the conduct of the study and the volunteers for their enthusiastic participation.

Funding

This work was supported both by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO (Grant EZ1503). Their joint aim is to independently study the health effects of moderate alcohol consumption.

References

1. Glaser R, Kiecolt-Glaser JK. Stress-induced immune dysfunction: Implications for health. Nature Reviews Immunology. 2005;5(3):243-51.
2. Dai X, Thavundayil J, Gianoulakis C. Response of the hypothalamic-pituitary-adrenal axis to stress in the absence and presence of ethanol in subjects at high and low risk of alcoholism. Neuropsychopharmacology. 2002;27(3):442-52.
3. Balodis IM, Wynne-Edwards KE, Olmstead MC. The stress–response-dampening effects of placebo. Horm Behav. 2011;59(4):465-72.
4. Sher KJ, Bartholow BD, Peuser K, Erickson DJ, Wood MD. Stress-response-dampening effects of alcohol: Attention as a mediator and moderator. J Abnorm Psychol. 2007;116(2):362-77.
5. Levenson RW, Sher KJ, Grossman LM, Newman J, Newlin DB. Alcohol and stress response dampening: Pharmacological effects, expectancy, and tension reduction. J Abnorm Psychol. 1980;89(4):528-38.
6. Schuckit MA, Gold E, Risch C. Plasma cortisol levels following ethanol in sons of alcoholics and controls. Arch Gen Psychiatry. 1987;44(11):942-5.
7. Childs E, O'Connor S, de Wit H. Bidirectional interactions between acute psychosocial stress and acute intravenous alcohol in healthy men. Alcoholism: Clinical and Experimental Research. 2011;35(10):1794-803.
8. de Wit H, Söderpalm AH, Nikolayev L, Young E. Effects of acute social stress on alcohol consumption in healthy subjects. Alcoholism: Clinical and Experimental Research. 2003;27(8):1270-7.
9. Schrieks IC, Stafleu A, Kallen VL, Grootjen M, Witkamp RF, Hendriks HF. The biphasic effects of moderate alcohol consumption with a meal on ambiance-induced mood and autonomic nervous system balance: A randomized crossover trial. PloS one. 2014;9(1):e86199.
10. Cohen S, Hamrick NM, Rodriguez MS, Feldman PJ, Rabin BS, Manuck SB. The stability of and intercorrelations among cardiovascular, immune, endocrine, and psychological reactivity. Annals of Behavioral Medicine. 2000;22(3):171-9.
11. Steptoe A, Hamer M, Chida Y. The effects of acute psychological stress on circulating inflammatory factors in humans: A review and meta-analysis. Brain Behav Immun. 2007;21(7):901-12.
12. Kimura K, Ohira H, Isowa T, Matsunaga M, Murashima S. Regulation of lymphocytes redistribution via autonomic nervous activity during stochastic learning. Brain Behav Immun. 2007;21(7):921-34.
13. Romeo J, Warnberg J, Nova E, Diaz LE, Gomez-Martinez S, Marcos A. Moderate alcohol consumption and the immune system: A review. Br J Nutr. 2007;98 Suppl 1:S111-5.
14. Mandrekar P, Catalano D, White B, Szabo G. Moderate alcohol intake in humans attenuates monocyte inflammatory responses: Inhibition of nuclear regulatory factor kappa B and induction of interleukin 10. Alcoholism: Clinical and Experimental Research. 2006;30(1):135-9.
15. Kirschbaum C, Ebrecht M, Hellhammer D. Similar cortisol responses to the TSST and to a modified stroop test-two laboratory stress protocols for studies of intervention-induced changes in HPA responsiveness? Psychosom Med. 2001;63(1):161.
16. Kirschbaum C, Pirke KM, Hellhammer DH. The 'trier social stress test'--a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology. 1993;28(1-2):76-81.
17. Maninger N, Wolkowitz OM, Reus VI, Epel ES, Mellon SH. Neurobiological and neuropsychiatric effects of dehydroepiandrosterone (DHEA) and DHEA sulfate (DHEAS). Frontiers in Neuroendocrinology. 2009;30:65-91.

18. Sierksma A, Sarkola T, Eriksson C, Gaag MS, Grobbee DE, Hendriks HF. 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. Alcoholism: Clinical and Experimental Research. 2004;28(5):780-5.

19. Lennartsson A, Kushnir MM, Bergquist J, Jonsdottir IH. DHEA and DHEA-S response to acute psychosocial stress in healthy men and women. Biol Psychol. 2012;90(2):143-9.

20. Shirotsuki K, Izawa S, Sugaya N, Yamada KC, Ogawa N, Ouchi Y, Nagano Y, Nomura S. Salivary cortisol and DHEA reactivity to psychosocial stress in socially anxious males. International Journal of Psychophysiology. 2009;72(2):198-203.

21. Sher KJ, Walitzer KS. Individual differences in the stress-response-dampening effect of alcohol: A dose-response study. J Abnorm Psychol. 1986;95(2):159.

22. Gerszten RE, Garcia-Zepeda EA, Lim Y, Yoshida M, Ding HA, Gimbrone MA, Luster AD, Luscinskas FW, Rosenzweig A. MCP-1 and IL-8 trigger firm adhesion of monocytes to vascular endothelium under flow conditions. Nature. 1999;398(6729):718-23.

23. Simonini A, Moscucci M, Muller DW, Bates ER, Pagani FD, Burdick MD, Strieter RM. IL-8 is an angiogenic factor in human coronary atherectomy tissue. Circulation. 2000;101(13):1519-26.

24. Sierksma A, Gaag MS, Kluft C, Hendriks HF. Effect of moderate alcohol consumption on fibrinogen levels in healthy volunteers is discordant with effects on C-Reactive protein. Ann N Y Acad Sci. 2001;936(1):630-3.

25. Sierksma A, Lebrun CE, van der Schouw YT, Grobbee DE, Lamberts SW, Hendriks HF, Bots ML. Alcohol consumption in relation to aortic stiffness and aortic wave reflections: A cross-sectional study in healthy postmenopausal women. Arterioscler Thromb Vasc Biol. 2004;24(2):342-8.

26. Sierksma A, Muller M, van der Schouw, Yvonne T, Grobbee DE, Hendriks HF, Bots ML. Alcohol consumption and arterial stiffness in men. J Hypertens. 2004;22(2):357-62.

27. Tracy RP. Epidemiological evidence for inflammation in cardiovascular disease. Thromb Haemost. 1999;82(2):826-31.

28. Zack M, Poulos CX, Aramakis VB, Khamba BK, MacLeod CM. Effects of Drink-Stress sequence and gender on alcohol stress response dampening in high and low anxiety sensitive drinkers. Alcoholism: Clinical and Experimental Research. 2007;31(3):411-22.

29. Schrieks IC, van den Berg R, Sierksma A, Beulens JW, Vaes WH, Hendriks HF. Effect of red wine consumption on biomarkers of oxidative stress. Alcohol Alcohol. 2013;48(2):153-9.

30. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HF. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. Br J Nutr. 2012;108(04):620-7.

Chapter 6

Effect of red wine consumption on biomarkers of oxidative stress

Ilse C. Schrieks
Robin van den Berg
Aafje Sierksma
Joline W.J. Beulens
Wouter H.J. Vaes
Henk F. J. Hendriks

Alcohol and Alcoholism. 2013; 48(2):153-159

Abstract

Aims: To evaluate the effect of acute and chronic consumption of red wine or de-alcoholized red wine with a similar antioxidant capacity on plasma total antioxidant capacity (TEAC), nuclear factor-κB (NF-κB) activity and F2-isoprostanes (8-iso-$PGF_{2\alpha}$) in healthy men.

Methods: Nineteen healthy men with an increased waist circumference (≥94 cm) and a BMI above 25 kg/m^2 participated in a randomized, controlled cross-over design trial. They daily consumed 450 mL of red wine (4 drinks; 41.4 gram alcohol) or 450 mL de-alcoholized red wine during dinner for 4 weeks each. On the last day of every treatment period, blood was collected before and 1 h after a standardized dinner with red wine or de-alcoholized red wine and also 24 hour urine was collected.

Results: Absolute TEAC levels were higher 1 h after dinner with red wine compared with dinner with de-alcoholized red wine (1.3 mmol versus 1.1 mmol Trolox equivalents/L; *P*=0.03). Consumption of dinner together with de-alcoholized red wine acutely stimulated NF-κB activity in peripheral blood mononuclear cells (0.4 to 0.7 HeLa equivalents/2.5 µg protein; *P*=0.006), whereas this increase was completely suppressed when the dinner was combined with red wine. A chronic increase in urinary 8-iso-$PGF_{2\alpha}$ after 4 weeks of red wine consumption compared with de-alcoholized red wine consumption (157 pg/mg creatinine and 141 pg/mg creatinine, respectively, *P*=0.006) was also observed.

Conclusions: Consumption of a moderate dose of red wine can acutely increase plasma TEAC and suppress NF-κB activation induced by a meal. Controversially, 4 weeks of red wine consumption compared with de-alcoholized red wine consumption increases the oxidative lipid damage marker 8-iso-$PGF_{2\alpha}$.

Introduction

Epidemiological studies have provided abundant evidence that moderate alcohol consumption is associated with a lower risk for cardiovascular disease (CVD) (1-3). Atherosclerosis, the underlying cause of CVD, is a process in which lipoproteins, fibrinolytic and inflammatory factors are involved.

The formation of reactive oxygen species (ROS) and an enhanced oxidative stress status is associated with CVD and CVD risk factors, such as obesity, diabetes type II and smoking (4, 5). It has been shown that red wine consumption can increase plasma antioxidant capacity (6-9) demonstrated that this was probably caused by the polyphenolic content of the wine. However, Arendt et al. (2005) found no increase in antioxidant capacity after red wine consumption, while the polyphenolic plasma content was increased (10). It has been suggested that ethanol in wine is capable of increasing plasma antioxidant capacity in an indirect way, because the absorption of polyphenols is insufficient to explain the total increase of antioxidant capacity (8, 11).

Nuclear factor-κB (NF-κB) is an oxidative stress related transcription factor involved in the regulation of inflammatory responses. Oxidative stress, for example induced by cigarette smoke (12) activates NF-κB whereas antioxidants may inhibit this activation (13-15). In vitro studies have shown that exposure to moderate doses of alcohol can inhibit NF-κB activation in human monocytes (16-18). Furthermore, Joosten et al. (2011) demonstrated that four weeks of moderate alcohol consumption resulted in a decreased NF-κB gene expression compared to abstention (19). This suggests that down-regulation of NF-κB may be a mechanism involved in the alcohol-induced suppression of inflammatory processes.

Excessive intake of alcohol, however, is associated with increased inflammation (20-22).

To our knowledge, only two human intervention studies have examined the effect of alcohol consumption on NF-κB activation. Dhindsa et al. (2004) showed that consumption of 300 kcal from alcohol did not have acute effects on NF-κB activation, while 300 kcal from glucose increased NF-κB activation (23). In a study from Blanco-Colio et al. (2000) it was demonstrated that the antioxidants in red wine rather than moderate alcohol consumption prevented NF-κB activation induced by a fat-enriched breakfast, however an alcohol free control condition was not included (24). In contrast, previous studies (25-27) showed that alcohol consumption may increase oxidative damage markers (F2-isoprostanes), while red wine consumption may protect against low-density lipoprotein lipid oxidation (28). Therefore, we performed a human intervention study to investigate the effect of moderate alcohol consumption on NF-κB activity and other biomarkers of oxidative stress. In a randomized crossover design healthy men consumed a moderate dose of red wine or de-alcoholized red wine with similar antioxidant capacity.

Subjects and Methods

Subjects

The study was conducted at TNO (a Dutch acronym for the Netherlands Organization for Applied Scientific Research), in Zeist, The Netherlands. TNO is an independent research organization which collaborates with universities and companies, and has the facilities to carry out clinical studies. The study was performed according to the ICH Guideline for Good Clinical Practice, complied with the Declaration of Helsinki and approved by the independent Medical Ethics Committee of TNO (authorization no 01/22). Nineteen subjects, all non-smoking, were recruited from the pool of volunteers of TNO. The volunteers received complete information about the study by verbal briefing and in writing and subsequently signed for informed consent. A questionnaire (self-report) was used for information on alcohol intake, medical history and (family) history of alcoholism. Subjects were considered healthy, based on the values of the pre-study laboratory tests (haematology, clinical chemistry and safety parameters), their medical history, and the physical examination. Subjects fulfilled the following inclusion criteria: consumption between 10 and 28 alcohol-containing beverages weekly, waist circumference ≥94 cm and no (family) history of alcoholism, diabetes mellitus type 2 or CVD. Subjects were selected for having an increased waist circumference as this represents the growing group of obese subjects in the western world. Obesity has been associated with an increased state of oxidative stress (Vincent et al., 2007), therefore we hypothesize that moderate alcohol consumption improves oxidative stress in this population.

Study design

The subjects entered a randomized crossover trial consisting of 2 periods of 4 weeks in which they consumed red wine or de-alcoholized red wine.

The red wine and the de-alcoholized red wine were specially bottled for this study (Carl Jung GmbH, Rüdesheim am Rhein, Germany). The red wine had an alcohol content of 11.5 vol%. The de-alcoholized wine (alcohol content of 0.13 vol%) was made from exactly the same base wine, however it was sweetened with sugar (4%). The alcohol was extracted from the wine by vacuum distillation at low temperature (<30°C) to maintain the taste and characteristics of the wine.

Half of the subjects were randomly allocated to the treatment order red wine followed by de-alcoholized red wine. The other half of the subjects consumed de-alcoholized red wine first followed by red wine. In this way, any bias due to the beverage order and a possible drift of variables over time was eliminated.

Subjects were instructed to drink 450 mL (4 glasses) of red wine (41.4 g alcohol) or de-alcoholized red wine (control) with dinner, representing the habitual pattern of alcohol intake in The Netherlands. 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, the subjects completed a questionnaire detailing beverage intake, dietary habits, exercise performed, medications taken and illnesses incurred. The questionnaires were routinely reviewed by the medical investigator and all problems identified were discussed with the subjects during the next visit or by telephone call. In addition, compliance was checked by counting the number of bottles returned and measuring the left-over. Body weight was determined halfway and at the end of each treatment period, with the subjects wearing indoor clothing, without shoes, wallet and keys.
On the last day of every treatment period subjects collected 24 h urine and had a standardized dinner together with red wine or de-alcoholized red wine at TNO. They were requested not to eat or drink anything starting at 3 h before this standard dinner, which consisted of approximately 19% energy from protein, 31% energy from fat, and 50% energy from carbohydrate.

Analysis of total antioxidant capacity and flavonoid content of the beverages
The antioxidant capacity was measured using the Trolox equivalent antioxidant capacity (TEAC) assay as described by Van den Berg et al. (1999) (29). This method was used to relate total antioxidant capacity to oxidative stress markers (NF-κB, F2-isoprostanes) by all compounds present with an antioxidant potential. Briefly, the beverages were diluted using ethanol, added to an 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonate) (ABTS) radical solution and the decrease in radical concentration was monitored spectrophotometrically at 734 nm.
Quantitative flavonoid analysis of the major flavonoids myricitin, quercetin and isoramnetin was performed using high-performance liquid chromatography (HPLC) equipped with photodiode array detection according to a method described by Hertog et al. (1992) (30). Sample preparation to analyze for free flavonoids consisted of diluting the sample with ethanol (50%, V/V) containing 1% ascorbate, and subsequent filtration, after which the filtrate was analyzed directly. Glycosilated flavonoids were analyzed as their corresponding deglycosilated analogs. For this, samples were treated as described above. The resulting filtrate was diluted 5 fold with water and cleaned using a C18 SPE cartridge. After elution of the glycosilated flavonoids with 50/50 water/methanol, the eluate was hydrolyzed for 2 h at 1.5 M HCl under reflux conditions. The resulting hydrolysate was analyzed using an Alliance 2690 HPLC system, equipped with a Hypersil BDS column (4.6 x 250 mm) and photodiode array detection. Compounds were quantified at their compound-specific absorption maxima.

Biological sample collection and analysis
In vitro studies have shown that ethanol can affect NF-κB as early as after 1 h (16, 18) and therefore blood was collected before and 1 h after the standardized dinner at TNO on the last day of each treatment period. The following morning a fasting blood sample was collected to determine serum HDL-cholesterol and plasma adiponectin levels. Staff members who

conducted the laboratory analysis were blind to the treatment assignments.

Isolation of peripheral blood mononuclear cells

For determination of NF-κB activity, peripheral blood mononuclear cells (PBMCs) were isolated immediately after blood collection. Heparinized blood (20 mL) was transferred into LeucoSep tubes (Greiner-Bio One GmbH, Frickenhausen, Germany) containing Ficoll Paque (Amersham Biosciences, Piscataway, New Jersey, USA) and diluted with a balanced salt solution. Samples were centrifuged for 30 min at 800 x g at room temperature and subsequently PBMCs were collected, washed twice and divided over 2 aliquots containing approximately 1 x 10^7 cells. These samples were stored at -80°C until preparation of nuclear extracts.

Preparation of nuclear extracts

Nuclear extracts were prepared using a nuclear extraction kit (Active Motif Europe, Rixensart, Belgium) by using approximately 1 x 10^7 PBMCs. Briefly, PBMCs were washed with ice-cold phosphate-buffered saline containing phosphatase inhibitors. Cytoplasmic fractions were collected by incubating the cells in a hypotonic buffer for 15 min at 4°C. After addition of detergent, cell suspensions were thoroughly mixed, centrifuged for 30 seconds at 14,000 x g and 4°C, supernatants were transferred into pre-cooled tubes. Finally, nuclear fractions were collected by addition of complete lysis buffer, incubated for 30 min on ice and centrifuged for 10 min at 14,000 g and 4ºC. The supernatants (nuclear fractions) were transferred into pre-cooled tubes and stored at -80ºC until analysis.

NF-κB activity

For detection and quantification NF-κB activation a TransAM™ NF-κB p65 Kit (Active Motif Europe) containing a 96-well plate to which oligonucleotides with NF-κB consensus binding sites has been immobilized was used. Nuclear extracts were added to the 96-well plate and analysed using an antibody directed against the NF-κB p65 subunit from the NF-κB complex which is bound to the oligonucleotides. Addition of a secondary antibody conjugated to horseradish peroxidase provided a colorimetric readout that was quantified spectrophotometrically at 450 nm.

Plasma total antioxidant capacity

For determination of plasma total antioxidant capacity, blood was collected in tubes containing ethylenediaminetetraacetic acid. Blood was centrifuged for 10 min at 2000*g* and 4°C. Plasma samples were stored at -80°C until analysis. Plasma total antioxidant capacity as measured by the TEAC assay was determined according to the method described by Van den Berg et al. (1999) (29). Briefly, plasma samples were deproteinized by adding an equal volume of ethanol. After centrifugation the supernatant was added to an ABTS radical solution. The decrease in radical concentration was monitored and related to the decrease obtained with

Trolox.

Serum HDL-cholesterol and plasma adiponectin

Serum HDL-cholesterol levels were determined using fasting blood samples which were collected in tubes containing cloth activator. Between 15 and 30 min after collection blood was centrifuged for 15 min at 2000*g* and 4°C. Serum samples were stored at −80°C until analysis. HDL-cholesterol was determined enzymatically with a commercially available kit (Roche Diagnostics, Mannheim, Germany).

For plasma adiponectin determination, blood was collected in tubes containing citrate-theophylline-adenosine-dipyridamole and centrifuged for 15 min at 2000*g* and 4°C. Plasma samples were stored at −80°C until analysis. Fasting plasma adiponectin concentrations were determined using a validated sandwich enzyme-linked immunosorbent assay employing an adiponectin-specific antibody (ANOC9108), as described by Arita et al. (1999) (31).

Urinary F2-isoprostane (8-iso $PGF_{2\alpha}$) analysis

Twenty-four hours urine samples were stored in 10 mL aliquots containing 100 µg butylated hydroxytoluene at −80°C until analysis. Measurement of 8-iso $PGF_{2\alpha}$ was performed using gas chromatography mass spectrometry using negative chemical ionization using a method described by Morrow et al. (1999) with some minor modifications (32).

Statistical analysis

Statistical analyses were performed with SAS statistical software package (SAS/STAT Version 8.02, SAS Institute, Cary, NC, USA). Differences in the characteristics (TEAC and flavonoid concentrations) of the wines were assessed by an independent sample *t*-test. Treatment effects on F2-isoprostanes and fasting clinical parameters at the end of each treatment period were assessed by the mixed model procedure with period as random term and treatment and treatment order as fixed terms. Treatment effects on NF-κB activity and TEAC before and 1 h after dinner were assessed by the mixed model procedure that included a random term for period and fixed terms for treatment (red wine and de-alcoholized red wine) and moment (before and 1 h after dinner) and an interaction term of treatment and moment. Treatment order was added to the model as fixed factor to correct for possible carry-over effects.

Model terms were considered significant at $P \leq 0.05$. Data are presented as means and SEs.

Results

The characteristics of the 19 subjects are provided in Table 6.1. The total antioxidant capacity and the major glycosylated flavonoids concentrations were similar for red wine and de-alcoholized red wine (Table 6.2).

Table 6.1 Characteristics of the subjects at baseline[a].

	Mean (range)
Age (y)	55 (35-68)
Height (cm)	180.8 (169.2-196.0)
Weight (kg)	95.5 (82.5-154.5)
BMI (kg/m^2)	29.2 (25.0-45.2)
Waist circumference (cm)	109.5 (95.0-151.0)
Hip circumference (cm)	106.6 (99.5-137.0)
Waist-hip ratio	1.03 (0.93-1.12)
Hemoglobin (mmol/L)	9.4 (8.4-10.2)
Triacylglycerol (mmol/L)	1.84 (1.12-4.07)
Total cholesterol (mmol/L)	5.90 (4.54-7.77)
HDL cholesterol (mmol/L)	1.28 (0.97-1.69)
Low-density lipoprotein (LDL) cholesterol (mmol/L)	3.77 (2.23-5.23)
Aspartate aminotransferase (ASAT) (U/L)	25 (12-43)
Alanine aminotransferase (ALAT) (U/L)	34 (9-74)
γ-glutamyl transpeptidase (GGT) (U/L)	38 (11-79)
Glucose (mmol/L)	5.7 (4.7-6.4)
Insulin (mU/L)	10.9 (4.7-19.3)

[a] n=19.

Table 6.2. Flavonoids content and antioxidant capacity of red wine and de-alcoholized red wine.

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

[a] mmol Trolox equivalents/L.

Compliance with the beverage intake was good, as judged from the daily questionnaire and the return of the empty bottles. Another indication that the subjects were compliant with the beverage intake was their fasting serum HDL-cholesterol which increased approximately 8.5% after 4 weeks red wine consumption compared with de-alcoholized red wine consumption (1.15 mmol/L and 1.06 mmol/L, respectively; $P<0.01$). Additionally, fasting adiponectin levels was approximately 8.6% higher after 4 weeks red wine consumption compared to de-alcoholized red wine consumption (6.3 µg/mL and 5.8 µg/mL, respectively; $P=0.02$). The effects of 4 weeks red wine or de-alcoholized red wine consumption on clinical fasting parameters and body weight are shown in Table 6.3. Average body weight did not differ between red wine and de-alcoholized red wine treatments, suggesting that the dietary habits and exercise patterns did not materially change.

Plasma TEAC before dinner did not differ after four weeks daily consumption of red wine or de-alcoholized red wine ($P=0.87$) (Figure 6.1). Postprandial TEAC was influenced differently by red wine and de-alcoholized red wine consumption. Plasma TEAC was 17% higher 1 h after dinner with red wine compared with the plasma TEAC 1 h after dinner with de-alcoholized red wine ($P=0.03$) (Figure 6.1).

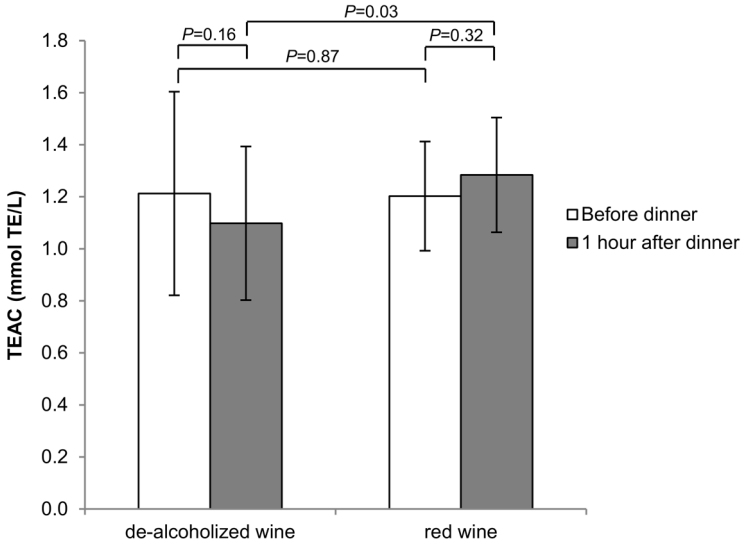


Figure 6.1. Plasma total antioxidant capacity (TEAC) was increased 1 h after dinner with red wine compared with the plasma TEAC 1 h after dinner with de-alcoholized red wine ($P=0.03$).

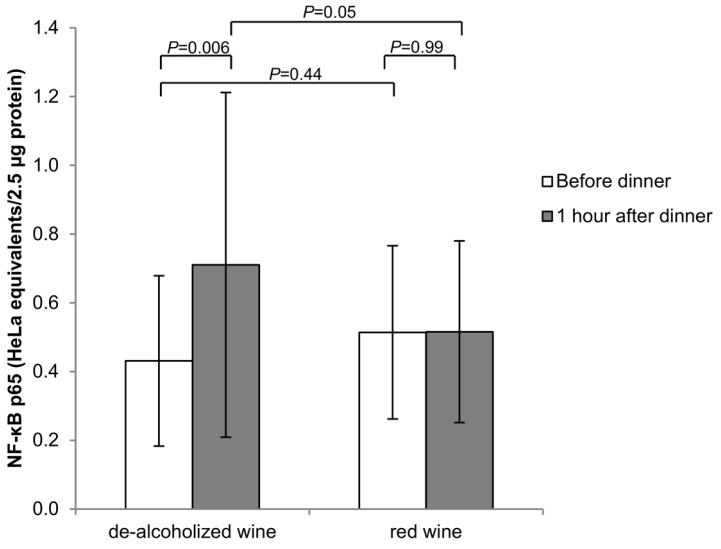


Figure 6.2. Intake of dinner with de-alcoholized red wine significantly increased NF-κB activity (*P*=0.006) while this effect was absent (*P*=0.99) when dinner was consumed with red wine. NF-κB activity was higher 1 h after dinner with de-alcoholized red compared with NF-κB activity 1 h after a dinner with red wine (*P*=0.05).

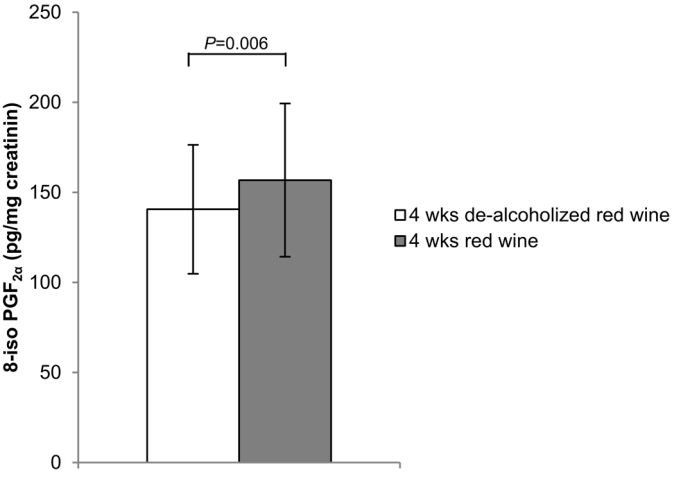


Figure 6.3. Four weeks red wine consumption compared with de-alcoholized red wine consumption significantly increased the oxidative damage marker isoprostane (8-iso-$PGF_{2\alpha}$; *P*=0.006).

Four weeks daily consumption of red wine did not influence the basal NF-κB activity differently than daily consumption de-alcoholized red wine (*P*=0.44), although NF-κB activity before dinner was on average 19% higher after 4 weeks red wine consumption. Postprandial NF-κB activity was influenced differently by dinner with de-alcoholized red wine compared to dinner with red wine. Intake of dinner with de-alcoholized red wine increased NF-κB activity with 65% after 1h (*P*=0.006) while this effect was absent (+0.3%, *P*=0.99) when dinner was consumed with red wine. This resulted in an absolute difference in NF-κB activity 1 h after dinner between the red wine and the de-alcoholized red wine condition, with the first being 38% higher (*P*=0.05) (Figure 6.2).

Four weeks red wine consumption compared with de-alcoholized red wine consumption significantly increased the oxidative damage marker isoprostanes (8-iso-$PGF_{2\alpha}$; 157 pg/mg creatinine and 141 pg/mg creatinine, respectively, *P*=0.006, Figure 6.3).

Table 6.3. Fasting blood parameters (mean ± SE) at the end of each treatment period

	De-alcoholized red wine	Red wine	*P*-value
Body weight (kg)	95.3 ± 4.1	95.8 ± 4.1	0.06
Glucose (mmol/L)	5.44 ± 0.12	5.55 ± 0.12	0.12
Insulin (mU/L)	10.60 ± 1.21	9.95 ± 0.86	0.44
Triacylglycerol (mmol/L)	1.66 ± 0.10	1.63 ± 0.11	0.78
Total cholesterol (mmol/L)	5.59 ± 0.19	5.76 ± 0.19	0.08
HDL cholesterol (mmol/L)	1.06 ± 0.04	1.15 ± 0.04	<0.01
LDL cholesterol (mmol/L)	3.76 ± 0.17	3.86 ± 0.16	0.30
Adiponectin (µg/mL)	5.8 ± 0.4	6.3 ± 0.6	0.02
Leukocytes (10^9/L)	5.6 ± 0.2	5.5 ± 0.2	0.79
Platelets (10^9/L	205 ± 9	200 ± 9	0.24
Creatinine (µmol/L)	77.7 ± 1.9	78.9 ± 2.1	0.30
Alkaline phosphatase (U/L)	64.7 ± 3.8	64.4 ± 4.1	0.72
ASAT (U/L)	22.4 ± 1.2	23.3 ± 1.2	0.36
ALAT (U/L)	25.2 ± 2.6	24.6 ± 2.3	0.54
GGT (U/L)	27.4 ± 2.3	32.2 ± 3.0	<0.01

Discussion

The results of our study show that red wine or de-alcoholized red wine consumption with dinner differently affected biomarkers of oxidative stress.

Plasma total antioxidant capacity increased approximately 17% 1 h after red wine consumption

compared with de-alcoholized red wine consumption, despite similar total antioxidant capacity of both beverages. Since plasma total antioxidant capacity reflects the absorption of all compounds with antioxidant capacity this may suggest that alcohol improves the short-term uptake of these compounds.

These results suggest that consumption of a dinner decreases plasma total antioxidant capacity. Post-prandial oxidative stress has been described to occur after ingestion of high-fat or high-carbohydrate meals (33). Hyperlipidemia and hyperglycemia are both associated with an increased ROS production. A meal high in oxidized and oxidizable lipids can increase plasma levels of lipid hydroperoxides, which is associated with a higher susceptibility to low-density lipoprotein oxidation (34). High plasma glucose levels may induce labile nonenzymatic glycation and increase the intracellular NADH/NAD^+ ratio, which are both accompanied with ROS production (35).

We observed no effect on plasma antioxidant capacity before dinner between the red wine and de-alcoholized red wine period. This indicates that long-term alcohol consumption does not affect plasma antioxidant capacity.

The results of our study show that the intake of a dinner with de-alcoholized red wine acutely (1 h) stimulated NF-κB activation. Dinner with red wine attenuated this food-induced NF-κB activation. Both red wine and de-alcoholized red wine had similar total antioxidant capacity, suggesting that the food-induced NF-κB activation is suppressed by alcohol itself or that alcohol possibly facilitates the action of antioxidants present in red wine. The last may be supported by the increased plasma antioxidant capacity measured 1h after wine consumption compared with de-alcoholized wine consumption.

Since NF-κB plays an important role in the coordinated expression of inflammatory genes, the prevention of diet-induced NF-κB activation suggests a mechanism for cellular regulation of the anti-inflammatory effects of moderate alcohol consumption. This is in agreement with the findings of Joosten et al. (2011) indicating a central role for transcription factor NF-κB in altered gene expression profiles in immune response after four weeks moderate alcohol consumption (19).

The prevention of diet-induced NF-κB activation after moderate alcohol consumption is in agreement with in vitro observations (16-18), but does not correspond with findings of (24). In their intervention study an increased NF-κB activity was observed after a fat-enriched breakfast with vodka, whereas a fat-enriched breakfast with red wine prevented NF-κB activation. The authors concluded that not alcohol but antioxidants in red wine caused this effect. Unfortunately they did not include an alcohol-free control condition. A combined effect of alcohol and compounds with antioxidant capacity can therefore not be excluded. The acute reduction of postprandial oxidation of a meal by antioxidants and wine has been very well described (34, 36, 37). These observations are in line with our study showing the acute suppression of NF-κB induced by the dinner.

The prevention of food-induced NF-κB activation with moderate red wine consumption was

acute and transient, because NF-κB activity did not differ before dinner between the red wine and the de-alcoholized red wine period. Similarly, Van den Berg et al. (2001) did not observe a chronic effect on NF-κB activation in smokers after 3 weeks high antioxidant intake (38). These two studies suggest that the effect of nutritional compounds on NF-κB activation in healthy subjects can only be measured acutely after activation of NF-κB. The induced NF-κB activation after consumption of a meal or single foods was also shown in previous studies (39-41).

In our study sugar was added to the de-alcoholized red wine to improve taste and to compensate for caloric loss due to the de-alcoholization process (4%). The de-alcoholized red wine still had a lower caloric content than the red wine, while the sugar content was somewhat higher (18 grams, equal to 72 kcal per 450 ml). The study of Dhindsa et al. (2004) reported an increased NF-κB activity 1 h after intake of 300 kcal from glucose compared to intake of 300 kcal from alcohol (23). However, the amount of sugar used by Dhindsa et al. (2004) was 4 times higher than the amount of sugar added to the de-alcoholized red wine in our study. Therefore, we do not expect that the difference in NF-κB activation between the two conditions caused by alcohol may be confounded by the difference in sugar content.

Coinciding with the acute suppressive effect on NF-κB activity there was an increase in urinary excretion of 8-iso-$PGF_{2\alpha}$ after 4 weeks daily consumption of 450 mL red wine consumption compared to the de-alcoholized red wine period. F2-isoprostanes are oxidative products of arachidonic acid and are regarded as a reliable and specific measure of in vivo lipid peroxidation. Our results show that (4 weeks) consumption of 450 mL (41.4 g alcohol) of red wine chronically increases the lipid oxidation marker F2-isoprostanes in comparison with de-alcoholized red wine. This effect is independent of the acute dinner effects and the acute suppression of postprandial oxidative stress by alcohol consumption. Chronic increases in F2-isoprostanes can therefore only be attributed to the difference in alcohol content of the beverages. This is in line with the observations of Caccetta et al. (2001) and Hartman et al. (2005) (25, 26). They showed that chronic consumption of alcohol compared with no alcohol resulted in increased F2-isoprostane levels. Beulens et al. (2008) also reported increased urinary F2-isoprostane levels after chronic alcohol consumption, although this did not reach significance (P=0.09) (27). Rifici et al. (2002) showed that polyphenols in red wine did decrease lipoprotein oxidation in vitro, while alcohol did not (42). Wine polyphenols have also been shown to reduce the release of nitric oxide and to scavenge ROS in vitro (43). However, the absorption of polyphenols from wine is very low and after entering plasma, they are quickly metabolized and excreted. The effect of plasma polyphenols after wine consumption on oxidative capacity is therefore negligible when compared to endogenous antioxidants (44). More research is required to establish whether a chronic increase in isoprostanes caused by oxidative stress induced-lipid peroxidation is physiologically relevant. Additional research is also needed to establish whether other nutritional compounds can attenuate NF-κB activation. The relation between NF-κB and inflammation should be further investigated as

well as the effect on other transcription and inflammation related factors, in order to explain the physiological relevance of both contradictory effects.
In conclusion, red wine consumption can acutely increase plasma total antioxidant capacity and suppress NF-κB activation induced by a meal. However, chronic red wine consumption compared to de-alcoholized red wine consumption may increase the oxidative lipid damage marker 8-iso-$PGF_{2\alpha}$.

Acknowledgements

We acknowledge all those involved in the conduct of the study and thank the volunteers for their enthusiastic participation. Carl Jung GmbH (Rudesheim am Rhein, Germany) supplied the red wine and the de-alcoholized red wine which were specially bottled for this study.

Funding

This work was supported both by the Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption.

References

1. Colditz GA, Branch LG, Lipnick RJ, Willett WC, Rosner B, Posner B, Hennekens CH. Moderate alcohol and decreased cardiovascular mortality in an elderly cohort. Am Heart J. 1985;109(4):886-9.
2. Stampfer MJ, Colditz GA, Willett WC, Speizer FE, Hennekens CH. A prospective study of moderate alcohol consumption and the risk of coronary disease and stroke in women. N Engl J Med. 1988;319(5):267-73.
3. Grobbee D, Rimm E, Keil U, Renaud S. Alcohol and the cardiovascular system. Health issues related to alcohol consumption. 1999;2:125-79.
4. Vincent HK, Innes KE, Vincent KR. Oxidative stress and potential interventions to reduce oxidative stress in overweight and obesity. Diabetes, Obesity and Metabolism. 2007;9(6):813-39.
5. Lakshmi SV, Padmaja G, Kuppusamy P, Kutala VK. Oxidative stress in cardiovascular disease. Indian J Biochem Biophys. 2009;46(6):421-40.
6. Micallef M, Lexis L, Lewandowski P. Red wine consumption increases antioxidant status and decreases oxidative stress in the circulation of both young and old humans. Nutr J. 2007;6:27.
7. Covas MI, Gambert P, Fitó M, de lT. Wine and oxidative stress: Up-to-date evidence of the effects of moderate wine consumption on oxidative damage in humans. Atherosclerosis. 2010;208(2):297-304.
8. Boban M, Modun D. Uric acid and antioxidant effects of wine. Croat Med J. 2010;51(1):16-22.
9. Serafini M, Maiani G, Ferro-Luzzi A. Alcohol-free red wine enhances plasma antioxidant capacity in humans. J Nutr. 1998;128(6):1003-7.
10. Arendt BM, Ellinger S, Kekic K, Geus L, Fimmers R, Spengler U, Muller W, Goerlich R. Single and repeated moderate consumption of native or dealcoholized red wine show different effects on antioxidant parameters in blood and DNA strand breaks in peripheral leukocytes in healthy volunteers: A randomized controlled trial (ISRCTN68505294). Nutr J. 2005;4:33.
11. Duthie GG, Pedersen MW, Gardner PT, Morrice PC, Jenkinson AM, McPhail DB, Steele GM. The effect of whisky and wine consumption on total phenol content and antioxidant capacity of plasma from healthy volunteers. Eur J Clin Nutr. 1998;52(10):733-6.
12. van den Berg R, Haenen GR, van den Berg H, Bast A. Nuclear factor-κB activation is higher in peripheral blood mononuclear cells of male smokers. Environ Toxicol Pharmacol. 2001;9(4):147-51.
13. Blackwell TS, Blackwell TR, Holden EP, Christman BW, Christman JW. In vivo antioxidant treatment suppresses nuclear factor-kappa B activation and neutrophilic lung inflammation. J Immunol. 1996;157(4):1630-7.
14. Sen CK, Packer L. Antioxidant and redox regulation of gene transcription. FASEB J. 1996;10(7):709-20.
15. van den Berg R, Haenen G, Van den Berg H, Bast A. Transcription factor NF-κB as a potential biomarker for oxidative stress. Br J Nutr. 2001;86(S1):S121-7.
16. Mandrekar P, Catalano D, Szabo G. Alcohol-Induced regulation of nuclear regulatory Factor-kB in human monocytes. Alcoholism: Clinical and Experimental Research. 1997;21(6):988-94.
17. Mandrekar P, Catalano D, Szabo G. Inhibition of lipopolysaccharide-mediated NF-kB activation by ethanol in human monocytes. Int Immunol. 1999;11(11):1781-90.
18. Mandrekar P, Dolganiuc A, Bellerose G, Kodys K, Romics L, Nizamani R, Szabo G. Acute alcohol inhibits the induction of nuclear regulatory factor κB activation through CD14/Toll-Like receptor 4, Interleukin-1, and tumor necrosis factor receptors: A common mechanism independent of inhibitory κBα degradation? Alcoholism: Clinical and Experimental Research. 2002;26(11):1609-14.

19. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HFJ. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. British Journal of Nutrition. 2011:1-8.

20. Romeo J, Warnberg J, Nova E, Diaz LE, Gomez-Martinez S, Marcos A. Moderate alcohol consumption and the immune system: A review. Br J Nutr. 2007;98 Suppl 1:S111-5.

21. Goral J, Karavitis J, Kovacs EJ. Exposure-dependent effects of ethanol on the innate immune system. Alcohol. 2008;42(4):237-47.

22. Szabo G, Mandrekar P. A recent perspective on alcohol, immunity, and host defense. Alcoholism: Clinical and Experimental Research. 2009;33(2):220-32.

23. Dhindsa S, Tripathy D, Mohanty P, Ghanim H, Syed T, Aljada A, Dandona P. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kB in mononuclear cells. Metabolism. 2004;53(3):330-4.

24. Blanco-Colio LM, Valderrama M, Alvarez-Sala LA, Bustos C, Ortego M, Hernandez-Presa MA, Cancelas P, Gomez-Gerique J, Millan J, Egido J. Red wine intake prevents nuclear factor-kappaB activation in peripheral blood mononuclear cells of healthy volunteers during postprandial lipemia. Circulation. 2000;102(9):1020-6.

25. Caccetta RA, Burke V, Mori TA, Beilin LJ, Puddey IB, Croft KD. Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. Free Radical Biology and Medicine. 2001;30(6):636-42.

26. Hartman T, Baer D, Graham L, Stone W, Gunter E, Parker C, Albert P, Dorgan J, Clevidence B, Campbell W. Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. Eur J Clin Nutr. 2005;59(2):161-8.

27. Beulens JW, van den Berg R, Kok FJ, Helander A, Vermunt SH, Hendriks HF. Moderate alcohol consumption and lipoprotein-associated phospholipase A2 activity. Nutrition, Metabolism and Cardiovascular Diseases. 2008;18(8):539-44.

28. Serafini M, Laranjinha JAN, Almeida LM, Maiani G. Inhibition of human LDL lipid peroxidation by phenol-rich beverages and their impact on plasma total antioxidant capacity in humans. The Journal of Nutritional Biochemistry. 2000;11(11):585-90.

29. van den Berg R, Haenen GR, 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 Chem. 1999;66(4):511-7.

30. Hertog MG, Hollman PC, Venema DP. Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. J Agric Food Chem. 1992;40(9):1591-8.

31. Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K. Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. Biochem Biophys Res Commun. 1999;257:79-83.

32. Morrow JD, Zackert WE, Yang JP, Kurhts EH, Callewaert D, Dworski R, Kanai K, Taber D, Moore K, Oates JA. Quantification of the major urinary metabolite of 15-F 2t-isoprostane (8-iso-PGF 2α) by a stable isotope dilution mass spectrometric assay. Anal Biochem. 1999;269(2):326-31.

33. Gregersen S, Samocha-Bonet D, Heilbronn LK, Campbell LV. Inflammatory and oxidative stress responses to high-carbohydrate and high-fat meals in healthy humans. J Nutr Metab. 2012;2012:238056.

34. Sies H, Stahl W, Sevanian A. Nutritional, dietary and postprandial oxidative stress. J Nutr. 2005;135(5):969-72.

35. Ceriello A, Bortolotti N, Motz E, Pieri C, Marra M, Tonutti L, Lizzio S, Feletto F, Catone B, Taboga C. Meal-induced oxidative stress and low-density lipoprotein oxidation in diabetes: The possible role of hyperglycemia. Metabolism. 1999;48(12):1503-8.

36. Natella F, Ghiselli A, Guidi A, Ursini F, Scaccini C. Red wine mitigates the postprandial increase of LDL susceptibility to oxidation. Free Radical Biology and Medicine. 2001;30(9):1036-44.

37. Natella F, Belelli F, Gentili V, Ursini F, Scaccini C. Grape seed proanthocyanidins prevent plasma postprandial oxidative stress in humans. J Agric Food Chem. 2002;50(26):7720-5.

38. van den Berg R, van Vliet T, Broekmans WM, Cnubben NH, Vaes WH, Roza L, Haenen GR, Bast A, van den Berg H. A vegetable/fruit concentrate with high antioxidant capacity has no effect on biomarkers of antioxidant status in male smokers. J Nutr. 2001;131(6):1714-22.

39. Bellido C, Lopez-Miranda J, Blanco-Colio LM, Perez-Martinez P, Muriana FJ, Martin-Ventura JL, Marin C, Gomez P, Fuentes F, Egido J, Perez-Jimenez F. Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor kappaB in peripheral blood mononuclear cells from healthy men. Am J Clin Nutr. 2004;80(6):1487-91.

40. Patel C, Ghanim H, Ravishankar S, Sia CL, Viswanathan P, Mohanty P, Dandona P. Prolonged reactive oxygen species generation and nuclear factor-kB activation after a high-fat, high-carbohydrate meal in the obese. J Clin Endocrinol Metab. 2007;92(11):4476-9.

41. Dickinson S, Hancock DP, Petocz P, Ceriello A, Brand-Miller J. High-glycemic index carbohydrate increases nuclear factor-kappaB activation in mononuclear cells of young, lean healthy subjects. Am J Clin Nutr. 2008;87(5):1188-93.

42. Rifici VA, Schneider SH, Khachadurian AK. Lipoprotein oxidation mediated by J774 murine macrophages is inhibited by individual red wine polyphenols but not by ethanol. J Nutr. 2002;132(9):2532-7.

43. Cíz M, Pavelkova M, Gallova L, Kralova J, Kubala L, Lojek A. The influence of wine polyphenols on reactive oxygen and nitrogen species production by murine macrophages RAW 264.7. Physiol Res. 2008;57(3):393-402.

44. Huisman A, van dW, Rabelink TJ, van Faassen EE. Wine polyphenols and ethanol do not significantly scavenge superoxide nor affect endothelial nitric oxide production. The Journal of Nutritional Biochemistry. 2004;15(7):426-32.

Chapter 7

Effect of moderate alcohol consumption on fetuin-A levels in men and women: Post-hoc analyses of three open-label randomized crossover trials

Michel M. Joosten
Ilse C. Schrieks
Henk F. J. Hendriks

Diabetology & Metabolic Syndrome. 2014; 6:24

Abstract

Background: Fetuin-A, a liver-derived glycoprotein that impairs insulin-signalling, has emerged as a biomarker for diabetes risk. Although moderate alcohol consumption has been inversely associated with fetuin-A, data from clinical trials are lacking. Thus, we evaluated whether moderate alcohol consumption decreases circulating levels of fetuin-A.

Methods: We analyzed data of three separate open-label, randomized, crossover trials: 1) 36 postmenopausal women consuming 250 mL white wine (25 g alcohol) or white grape juice daily for 6 weeks, 2) 24 premenopausal women consuming 660 mL beer (26 g alcohol) or alcohol-free beer daily for 3 weeks, and 3) 24 young men consuming 100 mL vodka (30 g alcohol) orange juice or only orange juice daily for 4 weeks. After each treatment period fasting blood samples were collected.

Results: Circulating fetuin-A concentrations decreased in men after vodka consumption (Mean ± SEM: 441 ± 11 to 426 ± 11 μg/mL, *P*=0.02), but not in women after wine (448 ± 17 to 437 ± 17 μg/mL, *P*=0.16) or beer consumption (498 ± 15 to 492 ± 15 μg/mL, *P*=0.48) compared to levels after each corresponding alcohol-free treatment. Post-hoc power analyses indicated that the statistical power to detect a similar effect as observed in men was 30% among the postmenopausal women and 31% among the premenopausal women.

Conclusions: In these randomized crossover trials, moderate alcohol consumption decreased fetuin-A in men but not in women. This sex-specific effect may be explained by the relatively short intervention periods or the low statistical power in the trials among women.

Trials registration: ClinicalTrials.gov ID no's: NCT00285909, NCT00524550, NCT00918918.

Introduction

Fetuin-A (α-Heremans-Schmid glycoprotein) is an abundant hepatokine that impairs insulin signalling by inhibiting tyrosine kinase activity (1, 2). Several prospective studies have reported positive associations between circulating fetuin-A and type 2 diabetes risk and, concomitantly, observed inverse relations between alcohol consumption and fetuin-A (3-5). More importantly, a recent case-control study suggested that fetuin-A may partially explain the reduced risk of type 2 diabetes (6) that has consistently been observed with moderate alcohol consumption (7-9). However, the cross-sectional and observational nature of these alcohol-fetuin-A associations may raise concern about potential confounding. Thus, to comprehensively investigate the effect of moderate alcohol consumption on fetuin-A levels, we performed post-hoc analyses of three randomized crossover interventions with different alcohol-containing beverages in men and women.

Materials and methods

The rationale of the three trials was to study the effect of moderate alcohol consumption on markers of insulin sensitivity and/or inflammation. Each trial is registered at ClinicalTrials.gov: NCT00285909, NCT00524550, and NCT00918918. Independent medical ethics committees approved the research protocols (The Medical Ethics Committee of the University Medical Centre Utrecht; Utrecht, the Netherlands [NCT00285909] and METOPP; Tilburg, the Netherlands [NCT00524550, and NCT00918918]) and all participants gave written informed consent. Eligible subjects were apparently healthy, were habitual alcohol consumers, refrained from smoking, and had no family history of alcoholism. The design of each individual intervention has been described in more detail elsewhere (10-12). In short, the three studies were open-label, randomized, crossover intervention trials and were all conducted at TNO (a Dutch acronym for Netherlands Organisation of Applied Scientific Research) in Zeist, the Netherlands. The trials consisted of 1) 36 postmenopausal women consuming 250 mL white wine (25 g alcohol; Chardonnay; Jean d'Alibert, Rieux, France) or whitegrape juice (Albert Heijn, Zaandam, the Netherlands) daily for 6 weeks between March and June 2006, 2) 24 premenopausal women consuming 660 mL beer (26 g alcohol) or alcohol-free beer daily (both Amstel, Amsterdam, the Netherlands) for 3 weeks between August and November 2007, and 3) 24 young men consuming 100 mL vodka (30 g alcohol; Smirnoff, Diageo, London, UK) and 200 mL orange juice (Appelsientje, Riedel, Ede, The Netherlands) or only orange juice daily for 4 weeks between August and November 2009. Postmenopausal women had an absence of menses for at least two years. Premenopausal women used phase I or II oral contraceptives. Allocation to treatment order (alcohol-containing vs. alcohol-free period) was randomized according to age and body mass index (BMI). After each treatment period, fasting blood samples were obtained. Plasma samples were stored at −80°C (beer and vodka

trials) and serum samples at −20°C (wine trial) until analysis. Fetuin-A concentrations were determined by a sandwich enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Minneapolis, MN) with a mean intra-assay coefficient of variation of 6.8%.
Data were analyzed using SAS statistical software (version 8.2; SAS Institute, Cary, NC, USA). Variables were compared between treatments with a mixed analysis of variation (ANOVA) model that included terms for treatment, period and the interaction between period and treatment (indicating possible carryover effects). Correlation coefficients were computed according to Spearman rank order to assess associations between intervention- induced changes in fetuin-A and other biochemical variables. Data are presented as mean ± standard error of the mean (SEM). All tests were two-sided. Statistical significance was defined as $P<0.05$.

Results

All subjects completed both arms of their intervention. No notable adverse effects were reported. Age and BMI were 56.5 ± 4.2 y and 25.4 ± 3.3 kg/m^2 in postmenopausal women, 23.9 ± 4.3 y and 22.2 ± 1.6 kg/m^2 in the premenopausal women, and 25.5 ± 4.3 y and 22.2 ± 1.6 kg/m^2 in the men, respectively. Indicators of compliance were the increased high-density lipoprotein (HDL)-cholesterol and adiponectin levels after each of the three alcohol consumption periods compared with after the alcohol-free consumption periods (Table 7.1).
No carry-over effects were found in fetuin-A, indicating that a possible effect on fetuin-A levels due to a treatment given in the first time period of the crossover trial did not persist into the second period and influence the effect of the second treatment. Fetuin-A levels decreased in men after vodka juice consumption (441 ± 11 to 426 ± 11 µg/mL, $P=0.02$) but not significantly in postmenopausal women after wine (448 ± 17 to 437 ± 17 µg/ mL, $P=0.16$) or in premenopausal women after beer consumption (498 ± 15 to 492 ± 15 µg/mL, $P=0.48$) (Figure 7.1) as compared to levels after each corresponding alcohol- free beverage consumption.
No correlations were observed between alcohol-induced changes in fetuin-A and corresponding changes in the homeostasis model assessment of insulin resistance (HOMA-IR) ($\rho=0.01$, $P=0.95$; $\rho=0.25$, $P=0.26$; $\rho=0.20$, $P=0.24$) or changes in adiponectin ($\rho=0.22$, $P=0.31$; $\rho=0.17$, $P=0.44$; $\rho=0.25$, $P=0.15$) among young men, pre- or postmenopausal women, respectively. Changes in HOMA-IR and adiponectin were also not correlated among men ($\rho=0.14$, $P=0.51$), premenopausal women ($\rho=0.01$, $P=0.96$), or postmenopausal women ($\rho=0.27$, $P=0.11$). Also, no consistent correlations were observed between alcohol-induced changes in fetuin-A and analogous changes in fasting blood lipids including HDL-cholesterol and free fatty acids (FFA), or liver function parameters across the three trials.

Table 7.1. Biochemical markers of 36 postmenopausal women, 24 premenopausal women and 24 young men sampled after an overnight fast after 6, 3 and 4-week treatment periods, respectively, of consuming alcohol-free or alcohol-containing beverages.

	Postmenopausal women			Premenopausal women			Young men		
	White grape juice	White wine	*P* value	Alcohol-free beer	Beer	*P* value	Orange juice	Vodka and orange juice	*P* value
Fetuin-A (µg/mL)	448 ± 17	437 ± 17	0.16	498 ± 15	492 ± 15	0.48	441 ± 11	426 ± 11	0.02
Adiponectin (µg/mL)	12.0 ± 0.7	13.1 ± 0.7	<0.001	6.8 ± 0.4	7.2 ± 0.4	0.01	10.5 ± 1.0	11.8 ± 1.0	0.005
Insulin (pmol/L)	46.5 ± 3.4	40.0 ± 3.4	0.90	45.7 ± 4.0	46.1 ± 4.0	0.90	59.8 ± 8.5	55.7 ± 8.6	0.53
Glucose (mmol/L)	5.4 ± 0.11	5.4 ± 0.11	0.36	4.8 ± 0.11	4.8 ± 0.11	0.36	5.3 ± 0.10	5.3 ± 0.10	0.76
HOMA-IR	1.64 ± 0.13	1.42 ± 0.13	0.02	1.41 ± 0.11	1.43 ± 0.11	0.81	1.98 ± 0.30	1.87 ± 0.30	0.61
HDL cholesterol (mmol/L)	1.57 ± 0.07	1.68 ± 0.07	<0.001	1.52 ± 0.07	1.62 ± 0.07	0.008	1.12 ± 0.05	1.22 ± 0.05	0.009
LDL cholesterol (mmol/L)	3.84 ± 0.12	3.51 ± 0.12	<0.001	2.40 ± 0.07	2.37 ± 0.07	0.77	2.63 ± 0.17	2.70 ± 0.17	0.55
Triglycerides (mmol/L)	1.18 ± 0.08	1.04 ± 0.08	<0.001	1.27 ± 0.08	1.25 ± 0.08	0.61	1.23 ± 0.14	1.33 ± 0.14	0.30
Free fatty acids (mmol/L)	0.43 ± 0.04	0.44 ± 0.04	0.67	0.34 ± 0.03	0.29 ± 0.03	0.26	0.42 ± 0.03	0.35 ± 0.03	0.07
Alanine aminotransferase (U/L)	13.8 ± 2.5	17.4 ± 2.5	0.29	10.8 ± 1.8	10.0 ± 1.8	0.21	15.2 ± 1.1	15.9 ± 1.1	0.49
Alkaline phosphates (U/L)	72.7 ± 2.9	73.6 ± 2.9	0.73	56.9 ± 6.5	57.8 ± 6.5	0.68	65.1 ± 4.3	65.8 ± 4.3	0.70
Aspartate aminotransferase (U/L)	20.9 ± 2.0	24.8 ± 2.0	0.13	17.8 ± 2.0	17.8 ± 2.0	0.95	21.0 ± 1.0	20.6 ± 1.0	0.62
γ-Glutamyltransferase (U/L)	18.4 ± 5.2	27.5 ± 5.2	0.21	16.5 ± 2.2	18.5 ± 2.2	0.01	19.8 ± 2.4	24.3 ± 2.4	0.003

Data are presented as means ± SEM. P-values are obtained from a mixed-model ANOVA. Abbreviations: HOMA-IR homeostasis model assessment of insulin resistance; HDL high-density lipoprotein; LDL low-density lipoprotein.

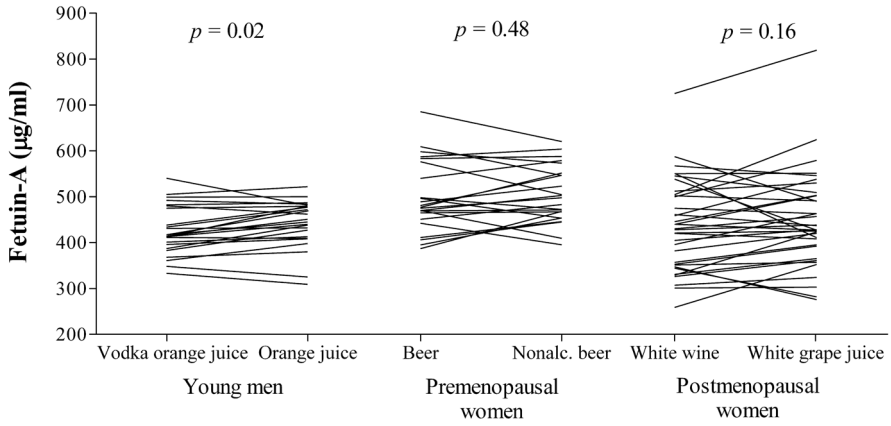


Figure 7.1. Individual changes of circulating fetuin-A levels at the end of the alcohol or alcohol-free treatment periods after an overnight fast for three open-label randomized crossover trials.

Conclusions

In post-hoc analyses of three separate open-label randomized crossover intervention studies, we found that moderate alcohol consumption reduced fetuin-A levels in men but not in women. This decrease was apparent after four weeks of moderate vodka consumption. No consistent correlations between intervention-induced changes in fetuin-A and other biochemical markers were observed across the three studies.

To our knowledge, these are the first intervention studies investigating the effect of different alcohol-containing beverages on circulating fetuin-A. The lowered fetuin-A levels in men after moderate alcohol consumption partially confirm cross-sectional observations in several epidemiological studies (3-6, 13, 14) and may provide some physiological support for the protective effect of moderate alcohol consumption on the risk of developing type 2 diabetes (6, 8) besides adiponectin (15). Furthermore, these findings extend prior evidence of short-term clinical trials that noted favourable changes in selected biological markers associated with diabetes and cardiovascular risk after moderate alcohol consumption (16). The underlying physiological explanation how alcohol consumption may lower fetuin-A is not clear. Also, the sex-specific alcohol-fetuin-A effect was unexpected, particularly since all women were either on oral contraceptives or postmenopausal, which limits potential influences of hormonal fluctuations or menstrual cycles. The null finding in our trials among pre- and postmenopausal women do not seem to correspond with a previous observational study among 1331 middle-

aged and older US female nurses, where moderate alcohol consumption was inversely associated with plasma fetuin-A even after adjustment for several lifestyle variables, demographic information, and medical history (6). Perhaps this discrepancy can be explained by the low statistical power in the two trials among women. Post-hoc power analyses indicated that the power to detect a similar effect as observed in men was only 30% among the postmenopausal women and 31% among the premenopausal women. Circulating fetuin-A was strongly and negatively associated with the insulin-sensitizing adipokine adiponectin in humans (17) and treatment of human adipocytes with fetuin-A repressed ADIPOQ mRNA levels (17). Furthermore, given the prior associations between fetuin-A and insulin resistance (18) and insulin sensitivity (19), we hypothesized that reductions in fetuin-A may play a role in the increased adiponectin levels or improved insulin sensitivity after alcohol consumption (10). Therefore, we analyzed correlations between intervention-induced changes in fetuin-A and adiponectin levels and other markers of insulin sensitivity, such as HOMA-IR. We, however, did not find such inverse correlations despite the fact that moderate alcohol consumption increased both ADIPOQ expression (10) and corresponding circulating adiponectin levels (10-12), suggesting that fetuin-A and adiponectin levels may be independently affected by alcohol. Also, it is important to note that the HOMA-IR index is a weak estimate of insulin resistance, particularly in a small study. The absence of a correlation between alcohol-induced changes in fetuin-A and HOMA-IR may partially be explained by the relatively low FFA levels of the studied participants. In a study among 347 healthy subjects at increased risk of type 2 diabetes, fetuin-A was only inversely associated with insulin sensitivity among individuals with high FFA levels (~ >0.65 mmol/l) (20).

Strengths of the study are the randomized crossover design (considered the ‘gold standard’ for evidence-based research), the assessment of compliance markers (i.e. HDL-cholesterol and adiponectin) to the study treatments, the inclusion of both sexes, and the broad range of biochemical variables. Some limitations warrant consideration. The trials consisted of alcohol-administration periods of 3 to 6 weeks and were performed among fairly insulin-sensitive subjects. Maybe more profound effects on fetuin-A levels would have been observed if the interventions lasted longer and/or were executed in subjects with glucose levels in the (pre) diabetic range. For example, three months of moderate alcohol consumption decreased fasting glucose levels among subjects with impaired glucose metabolism (21) and fetuin-A levels were particularly associated with an increased diabetes risk among subjects with higher fasting glucose (3, 5). Regardless, the duration of the present interventions were long enough to detect alcohol-induced changes in other biochemical markers such as adiponectin and HDL-cholesterol. Also, the association between moderate alcohol consumption and lower risk of type 2 diabetes mellitus is not limited to subjects with impaired glucose metabolism but also exists for subjects already at low risk for diabetes on the basis of multiple combined low-risk lifestyle behaviours (22). Nevertheless, the subjects studied were rather lean (mean BMI values 22-26), had no fatty liver (low liver enzyme levels) and were rather insulin sensitive (low

HOMA-IR). Also, all premenopausal women used oestrogen-containing oral contraceptives, which may explain their somewhat higher fetuin-A levels given the positive associations between oestrogen and fetuin-A (23, 24). Thus, the data are not representative for a typical at-risk population for metabolic diseases. Second, the daily amounts of alcohol consumed by women (~25 g alcohol) were higher than what is considered 'moderate' according to most guidelines (i.e. max. ~15 g alcohol). However, the nadir of the alcohol-diabetes association for women appeared to be at 24 g of alcohol/day in a meta-analysis of 20 prospective studies (8) while alcohol consumption became harmful above 50 g/day (and above 60 g/day for men). Third, post-hoc power analyses showed that there was low statistical power in the two trials among women to detect a similar effect as observed in the trial among men. Fourth, although unlikely since vodka is basically an ethanol-water mixture, we cannot fully exclude a potential beverage specific effect. Finally, the alcohol-induced reductions in fetuin-A were comparable to associations reported in epidemiological studies (3, 5, 6), but were relatively small as compared to alcohol's effect on HDL-cholesterol and adiponectin. It is possible that the findings, including the sex differences, were due to chance.

In conclusion, the results of these three randomized clinical trials with different alcohol-containing beverages demonstrated that short-term moderate alcohol consumption decreases fetuin-A levels in men but not in women. Further research is needed to determine whether long-term moderate alcohol consumption de creases fetuin-A levels. If so, these findings may add to the current knowledge of possible metabolic benefits of moderate alcohol consumption.

Acknowledgement

This work was supported by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption. No potential conflicts relevant to this article were reported.

References

1. Goustin A, Abou-Samra AB. The “thrifty” gene encoding ahsg/fetuin-A meets the insulin receptor: Insights into the mechanism of insulin resistance. Cell Signal. 2011;23(6):980-90.
2. Stefan N, Häring H. The role of hepatokines in metabolism. Nature Reviews Endocrinology. 2013;9(3):144-52.
3. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, Schulze MB. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes. 2008;57(10):2762-7.
4. Ix JH, Biggs ML, Mukamal KJ, Kizer JR, Zieman SJ, Siscovick DS, Mozzaffarian D, Jensen MK, Nelson L, Ruderman N, Djousse L. Association of fetuin-a with incident diabetes mellitus in community-living older adults: The cardiovascular health study. Circulation. 2012;125(19):2316-22.
5. Laughlin GA, Barrett-Connor E, Cummins KM, Daniels LB, Wassel CL, Ix JH. Sex-specific association of fetuin-A with type 2 diabetes in older community-dwelling adults: The rancho bernardo study. Diabetes Care. 2013;36(7):1994-2000.
6. Ley SH, Sun Q, Jimenez MC, Rexrode KM, Manson JE, Jensen MK, Rimm EB, Hu FB. Association between alcohol consumption and plasma fetuin-A and its contribution to incident type 2 diabetes in women. Diabetologia. 2014;57(1):93-101.
7. 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(3):719-25.
8. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, Rehm J. Alcohol as a risk factor for type 2 diabetes: A systematic review and meta-analysis. Diabetes Care. 2009;32(11):2123-32.
9. oosten MM, Chiuve SE, Mukamal KJ, Hu FB, Hendriks HFJ, Rimm EB. Changes in alcohol consumption and subsequent risk of type 2 diabetes in men. Diabetes. 2011;60(1):74-9.
10. Joosten M, Beulens J, Kersten S, Hendriks H. Moderate alcohol consumption increases insulin sensitivity and ADIPOQ expression in postmenopausal women: A randomised, crossover trial. Diabetologia. 2008;51(8):1375-81.
11. Joosten MM, Witkamp RF, Hendriks HF. Alterations in total and high–molecular-weight adiponectin after 3 weeks of moderate alcohol consumption in premenopausal women. Metab Clin Exp. 2011;60(8):1058-63.
12. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HF. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. Br J Nutr. 2012;108(04):620-7.
13. Weikert C, Stefan N, Schulze MB, Pischon T, Berger K, Joost HG, Haring HU, Boeing H, Fritsche A. Plasma fetuin-a levels and the risk of myocardial infarction and ischemic stroke. Circulation. 2008;118(24):2555-62.
14. Jensen MK, Bartz TM, Mukamal KJ, Djousse L, Kizer JR, Tracy RP, Zieman SJ, Rimm EB, Siscovick DS, Shlipak M, Ix JH. Fetuin-A, type 2 diabetes, and risk of cardiovascular disease in older adults: The cardiovascular health study. Diabetes Care. 2013;36(5):1222-8.
15. Beulens JW, Rimm EB, Hu FB, Hendriks HF, Mukamal KJ. Alcohol consumption, mediating biomarkers, and risk of type 2 diabetes among middle-aged women. Diabetes Care. 2008;31(10):2050-5.
16. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: Systematic review and meta-analysis of interventional studies. BMJ. 2011;342
17. Hennige AM, Staiger H, Wicke C, Machicao F, Fritsche A, Häring H, Stefan N. Fetuin-A induces cytokine

expression and suppresses adiponectin production. PLoS One. 2008;3(3):e1765.

18. Stefan N, Hennige AM, Staiger H, Machann J, Schick F, Krober SM, Machicao F, Fritsche A, Haring HU. Alpha2-heremans-schmid glycoprotein/fetuin-A is associated with insulin resistance and fat accumulation in the liver in humans. Diabetes Care. 2006;29(4):853-7.

19. Mori K, Emoto M, Yokoyama H, Araki T, Teramura M, Koyama H, Shoji T, Inaba M, Nishizawa Y. Association of serum fetuin-A with insulin resistance in type 2 diabetic and nondiabetic subjects. Diabetes Care. 2006;29(2):468.

20. Stefan N, Häring H. Circulating fetuin-A and free fatty acids interact to predict insulin resistance in humans. Nat Med. 2013;19(4):394-5.

21. Shai I, Wainstein J, Harman-Boehm I, Raz I, Fraser D, Rudich A, Stampfer MJ. Glycemic effects of moderate alcohol intake among patients with type 2 diabetes: A multicenter, randomized, clinical intervention trial. Diabetes Care. 2007;30(12):3011-6.

22. Joosten MM, Grobbee DE, van der ADL, Verschuren WM, Hendriks HF, Beulens JW. Combined effect of alcohol consumption and lifestyle behaviors on risk of type 2 diabetes. Am J Clin Nutr. 2010;91(6):1777-83.

23. Laughlin GA, Cummins KM, Wassel CL, Daniels LB, Ix JH. The association of fetuin-A with cardiovascular disease mortality in older community-dwelling adults: The rancho bernardo study. J Am Coll Cardiol. 2012;59(19):1688-96.

24. Rasul S, Ilhan A, Reiter MH, Todoric J, Farhan S, Esterbauer H, Kautzky-Willer A. Levels of fetuin-A relate to the levels of bone turnover biomarkers in male and female patients with type 2 diabetes. Clin Endocrinol (Oxf). 2012;76(4):499-505.

Chapter 8

The effect of alcohol consumption on insulin sensitivity and glycemic status: A systematic review and meta-analysis of intervention studies

Ilse C. Schrieks
Annelijn L.J. Heil
Henk F. J. Hendriks
Kenneth J. Mukamal
Joline W.J. Beulens

Diabetes Care. 2015; 38(4):723-732

Abstract

Objective: Moderate alcohol consumption is associated with a reduced risk of type 2 diabetes. This reduced risk might be explained by improved insulin sensitivity or improved glycemic status, but results of intervention studies on this relation are inconsistent. The purpose of this study was to conduct a systematic review and meta-analysis of intervention studies investigating the effect of alcohol consumption on insulin sensitivity and glycemic status.

Research design and methods: PubMed and Embase were searched until August 2014. Intervention studies on the effect of alcohol consumption on biological markers of insulin sensitivity or glycemic status of at least 2 weeks duration were included. Investigators extracted data on study characteristics, outcome measures and methodological quality.

Results: We included 14 intervention studies in a meta-analysis of 6 glycemic endpoints. Alcohol consumption did not influence estimated insulin sensitivity (standardized mean difference [SMD] 0.08 [-0.09 to 0.24]) or fasting glucose (SMD 0.07 [-0.11 to 0.24]) but reduced HbA_{1c} (SMD -0.62 [-1.01 to -0.23]) and fasting insulin concentrations (SMD -0.19 [-0.35 to -0.02]) compared with the control condition. Alcohol consumption among women reduced fasting insulin (SMD -0.23 [-0.41 to -0.04]) and tended to improve insulin sensitivity (SMD 0.16 [-0.04 to 0.37]) but not among men. Results were similar after excluding studies with high alcohol dosages (>40 g/day) and were not influenced by dosage and duration of the intervention.

Conclusions: Although the studies had small sample sizes and were of short duration, the current evidence suggests that moderate alcohol consumption may decrease fasting insulin and HbA_{1c} concentrations among nondiabetics. Alcohol consumption might improve insulin sensitivity among women but did not do so overall.

Introduction

Moderate alcohol consumption, compared to abstaining and heavy drinking, is related to a reduced risk of type 2 diabetes (1, 2). Although the risk is reduced with moderate alcohol consumption in both men and women, the association may differ for men and women. In a meta-analysis, consumption of 24 g alcohol/day reduced the risk of type 2 diabetes by 40% among women, while consumption of 22 g alcohol/day reduced the risk by 13% among men (1).

The association of alcohol consumption with type 2 diabetes may be explained by increased insulin sensitivity, anti-inflammatory effects, or effects of adiponectin (3). Several intervention studies have examined the effect of moderate alcohol consumption on these potential underlying pathways. Recently, a meta-analysis of intervention studies by Brien et al. (2011) showed that alcohol consumption significantly increased adiponectin levels but did not affect inflammatory factors (4). Unfortunately, the effect of alcohol consumption on insulin sensitivity has not been summarized quantitatively. A review of cross-sectional studies by Hulthe and Fagerberg (2005) suggested a positive association between moderate alcohol consumption and insulin sensitivity (5), although the three intervention studies included in that review did not show an effect (6-8). Several other intervention studies also reported inconsistent results (9, 10). Consequently, consensus is lacking about the effect of moderate alcohol consumption on insulin sensitivity. Therefore, we aimed to conduct a systematic review and meta-analysis of intervention studies investigating the effect of alcohol consumption on insulin sensitivity and other relevant glycemic measures.

Research design and methods

This study was performed according to the PRISMA (Preferred Reporting Items for Systematic Reviews) Statement guidelines for the reporting of systematic reviews and meta-analysis of intervention studies. The PRISMA checklist and the protocol for this study are provided in the Supplementary Data.

Data sources and searches

A literature search was conducted in PubMed MEDLINE and Embase for relevant intervention studies published up to August 2014. A prespecified search-string including search terms on alcohol, consumption, and glycemic measures was used for PubMed and Embase (Supplementary Data). References and related citations of articles were screened to identify other relevant papers. The exposure of interest was (moderate) alcohol consumption and the primary outcome measure insulin sensitivity. All estimates of insulin sensitivity were included. This includes indices from direct measures (e.g., hyperinsulinemic euglycemic glucose clamp [HEGC]), and indirect measures of insulin sensitivity (e.g. the frequently sampled intravenous

glucose tolerance test [FSIVGTT] and oral glucose tolerance test [OGTT]). HOMA of insulin resistance (HOMA-IR) was also included, which is based on fasting insulin and glucose levels and therefore primarily reflects hepatic insulin resistance (11). Other relevant outcome measures that were taken into account were fasting insulin, fasting glucose and hemoglobin A_{1c} (HbA_{1c}). HbA_{1c} reflects average plasma glucose levels over the past 8-12 weeks and is therefore used as a measure of glycemic status (12).

Study selection

Relevant studies were selected by two researchers (A.L.J.H., J.W.J.B.) during a multi-phase process based on the following inclusion criteria: trials with an alcohol intervention, relevant outcome measures as previously described, intervention period of at least 2 weeks, and written in English or Dutch. We excluded studies including individuals with (a history of) alcoholism or heavy drinkers (individuals consuming ≥60 g alcohol for at least 1 day per week) and animal studies. No publication date or status restrictions were imposed. In the first phase, titles of all retrieved studies were screened to select papers with a relevant subject; the abstracts of these articles were judged on relevance in the next phase. If judged relevant, the full text was studied in the third phase to determine whether the article was eligible for inclusion. When discrepancies occurred about the inclusion of a particular article, a third author (K.J.M. or I.C.S.) was consulted.

Data extraction and quality assessment

From the included studies, sample size, participant characteristics, inclusion and exclusion criteria, study design, duration of intervention and specific outcome measures were extracted on a prespecified form. Detailed information about the alcohol intervention (e.g. dosage, type, frequency, duration) was described. If a study did not report the grams of alcohol per unit, this was calculated based on the amount in millimeters given to the subjects and the alcohol volume of the beverage (g alcohol = (mL X %v/v) X 0.8, where %v/v is the percentage of alcohol volume per total volume). Authors were contacted if further information was required (13-15).

To assess the quality of the studies, aspects such as randomization procedures, compliance with the intervention, and dropout rates were extracted. Randomization and the inclusion of an alcohol-free control group were regarded as the most important criteria to decide whether a study had sufficient quality. If these criteria were not met, the studies were excluded from further meta-analyses. Because randomization of crossover studies may be less important than randomization of parallel studies, we also conducted a sensitivity analysis including nonrandomized crossover studies. Because blinding of participants to the alcohol intervention is of uncertain effectiveness, this criterion was not regarded as essential for inclusion. To assess the quality of the included studies, the 5-point Jadad scale was used (16).

Data synthesis and analysis

The mean and SD of the outcome variables at the end of the alcohol intervention period and control period were extracted from the articles. If SEs were reported, we used the equation: SD = SE X square root of number of subjects. The mean effects of the different studies measuring insulin sensitivity index (ISI), HOMA-IR, insulin, glucose, or HbA_{1c} were pooled in a meta-analysis and shown in a forest plot. To combine the studies measuring ISI and HOMA-IR in one meta-analysis, the inverted HOMA-IR (1/HOMA-IR) was calculated using the delta method.

Heterogeneity between studies was investigated through calculation of the χ^2 and I^2 statistics. If the χ^2 and I^2 showed no evidence for heterogeneity (I^2<30%) (17), analyses were conducted using the inverse variance fixed-effects model for pooling the studies. Otherwise, the DerSimonian and Laird random-effects model was used. The mean outcomes for insulin, glucose and insulin sensitivity were assessed using different methods and needed to be standardized. Therefore, the Cohen's *d* was used to calculate the standardized mean difference (SMD), which is the mean difference between the intervention and control group divided by the pooled SD.

In sensitivity analyses, the effect of moderate alcohol consumption on the reported outcomes was determined by excluding studies with high alcohol dosages (>40 g/day). Furthermore, if more than one intervention arm was tested in a study, we combined the outcomes (17). Additionally, analyses were performed excluding studies potentially causing heterogeneity to determine their effect on the results.

In a meta-regression, the influences of alcohol dosage and duration of the intervention on the results were tested. The influence of type of alcoholic beverage was not assessed due to too few studies to stratify by alcoholic beverage. Because only two studies used the gold standard HEGC to estimate insulin sensitivity (11), we tested with a meta-regression whether the effect of alcohol on insulin sensitivity differed between these studies.

Because the association of alcohol consumption with type 2 diabetes differs for men and women, we conducted sex-stratified analyses. Effect modification by sex was tested in a meta-regression for insulin sensitivity.

Potential publication bias was examined by visual inspection of the funnel plot and by the Egger's and Begg's statistical tests. In case evidence for publication bias was found, we used the trim and fill method by Duval and Tweedie to calculate a pooled SMD based on filled data to adjust for publication bias (18). The level of significance was set at P<0.05. Analyses were performed with the STATA meta-procedure (STATA 10.0).

Results

In total, 4,991 titles were found through database searching and 24 through additional methods (Supplementary Figure 8.1). After screening of titles and abstracts, 46 papers

remained eligible and the full-text assessed. Finally, 22 articles met criteria for inclusion in the qualitative synthesis.

Study characteristics

Descriptive data of the included studies are summarized in Table 8.1. Of the 22 studies, 15 used a crossover design and 7 a parallel design. The intervention duration of the studies ranged from 2 to 12 weeks, with an average duration of 5.6 weeks for ISI, 4.2 weeks for HOMA-IR, 7.2 weeks for insulin, 5.9 weeks for glucose, and 4.3 weeks for HbA_{1c}. Two studies did not use an alcohol-free control (14, 19). The dosage of alcohol varied from 10 to 70 g/day of which one study used >40 g/day (20). ISI was measured by six studies, of which two used the gold standard HEGC (10, 21) and four used indirect measures of insulin sensitivity (based on OGTT, FSIVGTT or fasting levels) (8, 9, 22, 23) . HOMA-IR was measured by four studies (15, 24-26). Seven studies were performed by the same institute (10, 21-26). They were treated as independent because they included different subjects.

Quality assessment

The results of the quality assessment are shown in Supplementary Table 8.1. Of the 22 studies included in the qualitative synthesis, 4 did not report the measurement of compliance to the intervention (9, 14, 20, 27). Blinding of the researcher was not reported or not conducted in any of the studies. Drop-out rates were described in 18 studies. The studies scored between 1 and 3 points on the Jadad scale (range 0–5). Of the 22 studies, 2 were excluded from the meta-analysis because they did not include an alcohol-free control group (14, 19) and 4 were excluded because they did not have a randomized design (13, 28-30). Because only two studies included subjects with type 2 diabetes, these studies were excluded as well (31, 32). One study included both healthy and type 2 diabetes subjects and from this study only data from healthy subjects was included (15). Overall, 14 studies were included in the meta-analysis (Table 8.1 and Supplementary Table 8.1).

Meta-analysis

The number of included studies in the analysis was 7 for ISI, 5 for HOMA-IR, 9 for insulin, 10 for glucose and 3 for HbA_{1c}. The forest plots on insulin sensitivity and glycemic status are shown in Figure 8.1–8.3.

Pooled analysis showed no difference in ISI after a period of alcohol consumption compared to no alcohol consumption (SMD 0.06, [-0.13 to 0.26]; *P*=0.53, test for heterogeneity *P*=0.76, I^2=0%. For HOMA-IR, both the χ^2 (P<0.01) and I^2 statistic (I^2= 97%) demonstrated heterogeneity. In a random-effects model, the pooled SMD was 0.35 [-0.90 to 1.59], indicating no effect of alcohol consumption on HOMA-IR (*P*=0.59). Similar results were observed when studies measuring ISI and HOMA-IR were combined (SMD -0.12, [-0.61 to 0.39], *P*=0.65). A random-effects model was used because heterogeneity was present (*P*<0.01, I^2=91%). The funnel

plot indicated that the results of the intervention arms (i.e. red wine, gin) of Chiva-Blanch et al. (2013) were largely responsible for this heterogeneity. Exclusion of this study resulted in an SMD of 0.08 [-0.09 to 0.24] (*P*=0.35), with no evidence of heterogeneity (*P*=0.90, I^2=0%). Sex-stratified analysis showed different effects in men and women (P_{sex}=0.018) (Figure 8.1). Alcohol consumption tended to increase insulin sensitivity in women (SMD 0.16 [-0.04 to 0.37], *P*=0.12), but not in men (SMD -0.30 [-1.23 to 0.64], *P*=0.54). In men, heterogeneity was present (*P*<0.01, I^2=95%) and exclusion of the study of Chiva-Blanch et al. resulted in a pooled SMD of -0.07 [-0.34 to 0.20], *P*=0.61. However, after exclusion of Chiva-Blanch et al., the pooled SMDs in men and women were no longer significantly different (*P*=0.18).

Fasting insulin concentrations were lower after alcohol consumption compared to abstaining, as shown by a pooled SMD of -0.19 [-0.35 to -0.02], (P=0.03), test for heterogeneity *P*=0.92, I^2=0%. Sex-stratified analysis showed that alcohol consumption decreased insulin concentrations in women (SMD -0.23, [-0.41 to -0.04], P=0.02). Only two studies measured insulin concentrations in men, showing a decrease in insulin levels (SMD -0.13 [-0.62 to 0.36], *P*=0.59 (Figure 8.2A).

For fasting glucose concentrations, the pooled SMD was 0.07 [-0.11 to 0.24], indicating no effect of alcohol consumption on glucose concentration among individuals without diabetes (*P*=0.45; $P_{heterogeneity}$=0.94, I^2=0%). Similar results were observed when men and women were analyzed separately (Figure 8.2B). In women the SMD was 0.01 [-0.20 to 0.21] (*P*=0.94), in men the SMD was 0.14 [-0.24 to 0.53] (*P*=0.48).

For HbA_{1c}, a random-effects model was used, as the I^2 statistic indicated evidence for some heterogeneity (I^2=30%). The pooled SMD was -0.62 [-1.01 to -0.23], showing lower HbA_{1c} concentrations after alcohol consumption compared to no alcohol consumption (Figure 8.3; *P*<0.01).

Sensitivity analyses and meta-regression

Only the study of Contaldo et al. (20) used a high alcohol dosage (70 g/day) and measured insulin and glucose. Exclusion of this study from the meta-analysis resulted in generally similar results for insulin (SMD -0.18 [-0.36 to -0.01]) and glucose (SMD 0.06 [-0.12 to 0.23]). Combining the 2 intervention arms of the studies of Davies et al. (9) with 15 and 30 g alcohol per day and of Queipo-Ortuño et al. (35) with red wine and gin resulted in generally similar outcomes. The pooled SMD for insulin sensitivity (ISI & HOMA-IR) was SMD 0.06 [-0.11 to 0.24] overall and 0.15 [-0.08 to 0.38] in women. For insulin, SMD -0.18 [-0.38 to -0.01] overall and SMD -0.22 [-0.43 to -0.02] in women.

Including the non-randomized crossover study by Cordain et al. (13) resulted in generally similar results for insulin (SMD -0.17 [-0.33 to 0.00] and glucose (SMD 0.08 [-0.09 to 0.25].

Table 8.1. Characteristics of studies included in this systematic review and meta-analysis on the effect of alcohol consumption on insulin sensitivity.

Study reference	Design	Participants	Participant characteristics	Intervention	Alcohol dosage (g/day)	Intervention period (weeks)	Outcome measure	In meta-analysis
Bantle 2008 (31)	Randomized crossover	17 diabetic men and women	Age 64 years BMI 31.7 kg/m^2	Abstinence or white/ red wine during dinner	18	4	Insulin, glucose, HbA_{1c}	No*
Beulens 2006 (21)	Randomized crossover	17 healthy men, with waist circumference >94 cm	Age 53 (9) years BMI 29.1 (4.2) kg/m^2 Insulin 10.7 (5.6) units/L	Red wine or de-alcoholized red wine with dinner	40	4	ISI (HEGC)	Yes
Beulens 2007 (23)	Randomized crossover	19 healthy lean or overweight men	Lean: Age 21 (2) years BMI 21.4 (2.0) kg/m^2 Insulin 4.7 (1.2) units/L Overweight: Age 28 (6) y BMI 30.1 (3.4) kg/m^2 Insulin 11.0 (5.4) units/L	Whisky or mineral water	32	4	ISI (OGTT), HbA_{1c}	Yes
Beulens 2008 (22)	Randomized crossover	20 healthy lean or overweight men	Lean: Age 19 (2) years BMI 20.1 (1.0) kg/m^2 Overweight: Age 21 (2) years BMI 31.3 (3.9) kg/m^2	Beer or alcohol-free beer during dinner	40	3	ISI (OGTT)	Yes
Bhathena 1995 (27)	Randomized crossover	37 healthy premenopausal women	Age 30 (7) years BMI 24.4 (4.6) kg/m^2	Ethanol mixed with fruit juice or soft drink after dinner	30	12	Insulin	Yes
Cesena 2011 (28)	Parallel (one arm)	42 healthy men and women	Age 46 (9) years BMI 25.1 (2.8) kg/m^2	Abstinence or red wine during dinner	24	2	Glucose	No†
Chiva-Blanch 2013 (15)	Randomized crossover	52 healthy and 15 diabetic men	Age 60 (8) years BMI 29.6 (3.9) kg/m^2	Gin, red wine or dealcoholized red wine	30	4	HOMA-IR, insulin, glucose	Yes
Contaldo 1989 (20)	Randomized crossover	8 healthy men	BMI 25.4 (1.4) kg/m^2	Abstinence or red wine during dinner	75	2	Insulin, glucose	Yes
Cordain 1997 (13)	Randomized crossover	14 healthy men	Age 32 (9) years	Abstinence or wine	28	6	Insulin, glucose	No†
Cordain 2000 (8)	Randomized crossover	20 sedentary and overweight premenopausal women	BMI 29.8 (2.2) kg/m^2 Insulin 8.6 (3.3) units/L	Abstinence or red wine	20	10	ISI (FSIVGTT), insulin, glucose	Yes
Davies 2002 (9)	Randomized crossover	51 healthy post-menopausal women	Age 60 (8) years BMI 27.4 (5.7) kg/m^2 Insulin 6.5 (5.7) units/L	Alcohol or isocaloric beverage	15 or 30	8	ISI (MFFM), insulin, glucose	Yes

Study reference	Design	Participants	Participant characteristics	Intervention	Alcohol dosage (g/day)	Intervention period (weeks)	Outcome measure	In meta-analysis
Flechtner-Mors 2004 (33)	Randomized parallel	40 overweight men and women	Age 48 (11) years BMI 34.2 (6.4) kg/m^2	Grape juice or white wine during meals	17	12	Insulin, glucose	Yes
Joosten 2008 (24)	Randomized crossover	36 healthy postmenopausal women	Age 57 (4) years BMI 25.4 (3.3) kg/m^2 Insulin 37.4 (12.6) pmol/L	White wine or white grape juice daily during dinner	25	6	HOMA-IR, Insulin, glucose, HbA_{1c}	Yes
Joosten 2011 (25, 26)	Randomized crossover	24 healthy premenopausal women	Age 24 (4) years BMI 22.2 (1.6) kg/m^2 Insulin 41.7 (16.0) pmol/L	Beer or alcohol-free beer during dinner	26	3	HOMA-IR, Insulin, glucose, HbA_{1c}	Yes
Joosten 2012 (26, 34)	Randomized crossover	24 healthy men	Age 26 (3) years BMI 24 (3) kg/m^2	Vodka orange juice or orange juice during dinner	30	4	HOMA-IR, insulin, glucose	Yes
Kim 2009 (29)	Parallel	20 non-diabetic, insulin resistant men and women	Age 54 (7) years BMI 32 (5) kg/m^2	Abstinence or vodka or red wine during dinner	30	8	Steady state plasma glucose, glucose	No†
Lavy 1994 (14)	Randomized parallel	20 healthy men	–	Red or white wine	40	2	Glucose	No‡
Queipo-Ortuno 2012 (35)	Randomized crossover	10 healthy men	Age 48 (2) years BMI 27.6 (3.2) kg/m^2	Gin, red wine or dealcoholized red wine	30	3	Glucose	Yes
Romeo 2008 (30)	Parallel (one arm)	57 healthy men and women	Women: Age 38 (9) years BMI 24.4 (3.5) kg/m^2 Men: Age 35 (6) years BMI 25.5 (2.4) kg/m^2	Abstinence or beer during the meal	11 (♀) 22 (♂)	4	Glucose	No†
Shai 2007 (32)	Randomized parallel (multicentre)	91 diabetic men and women	Age 62 (6) years BMI 30.1 (4.6) kg/m^2	Wine or non-alcoholic beer during dinner	13	12	Glucose, HbA_{1c}	No*
Sierksma 2004 (10)	Randomized crossover	23 healthy men	Age 52 (5) years BMI 26.7 (3.0) kg/m^2 Insulin 8.9 (8.8) units/L	Whisky or tap water during dinner	40	2.5	ISI (HEGC)	Yes
Zheng 2012 (19)	Randomized parallel	45 healthy men and women	TFL: Age 24 (2) years BMI 21.3 (1.6) kg/m^2 TCL: Age 24 (1) years BMI 21.1 (2.2) kg/m^2	Tea-flavour liquor (TFL) or traditional Chinese liquor (TCL)	10	4	HOMA-IR, Insulin, glucose	No‡

Data are mean (SD) unless otherwise indicated. MFFM, whole body glucose disposal rate normalized to fat-free mass; TCL, traditional Chinese liquor; TFL tea-flavor liquor.
Reason for exclusion from meta-analysis: * Participants with type 2 diabetes. † No randomized design. ‡ No control group.

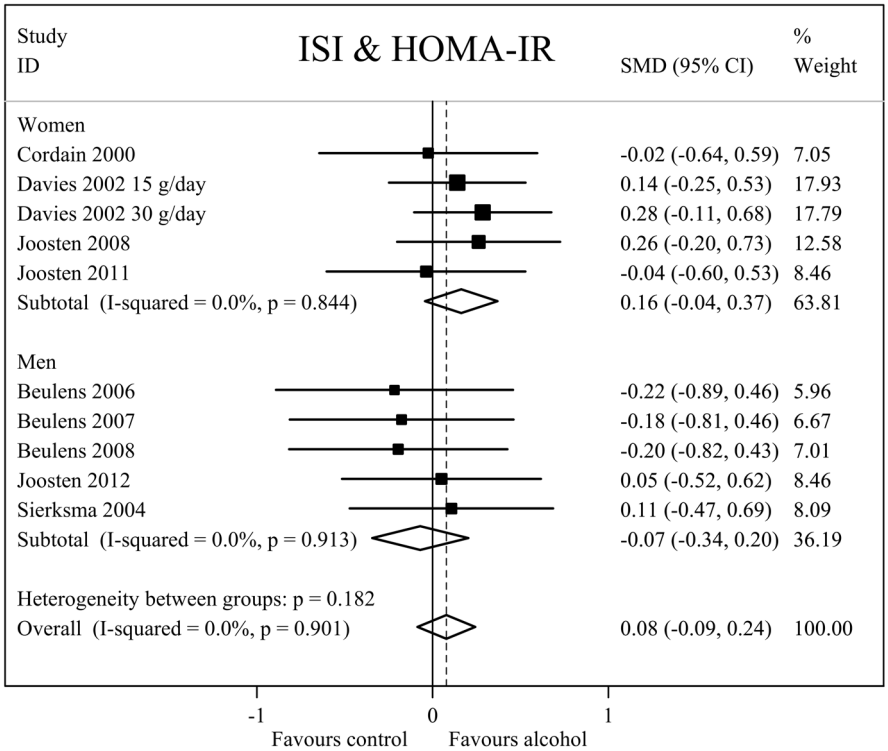


Figure 8.1. Forest plot of meta-analysis of the effect of alcohol consumption on insulin sensitivity. Data are pooled SMDs with 95% CIs and are calculated with exclusion of the results of the two study arms of Chiva-Blanch et al. (2013) (15), because they induced heterogeneity.

The meta-regression showed no influence of duration (all P_{trend}>0.60) and/or alcohol dosage (all P_{trend}>0.67) on the pooled SMD of ISI and HOMA-IR, insulin and glucose. Additionally, the meta-regression showed no differences between results from the studies using the HEGC to measured insulin sensitivity and the other studies (SMD -0.03 for HEGC studies vs. 0.09 for other studies, *P*=0.64).

Publication bias

Results of the Egger's and Begg's tests showed publication bias for the outcomes of ISI, ISI and HOMA-IR, and glucose (Supplementary Table 8.2). Visual inspection of the funnel plots showed some asymmetry, which was due to missing results in favor of alcohol treatment from smaller studies (Supplementary Figure 8.2). For ISI and HOMA-IR, we calculated an adjusted pooled SMD by using the trim and fill approach by Duval and Tweedie. This resulted in four extra study estimates (linear method used) and an adjusted pooled SMD of 0.17 [0.02 to

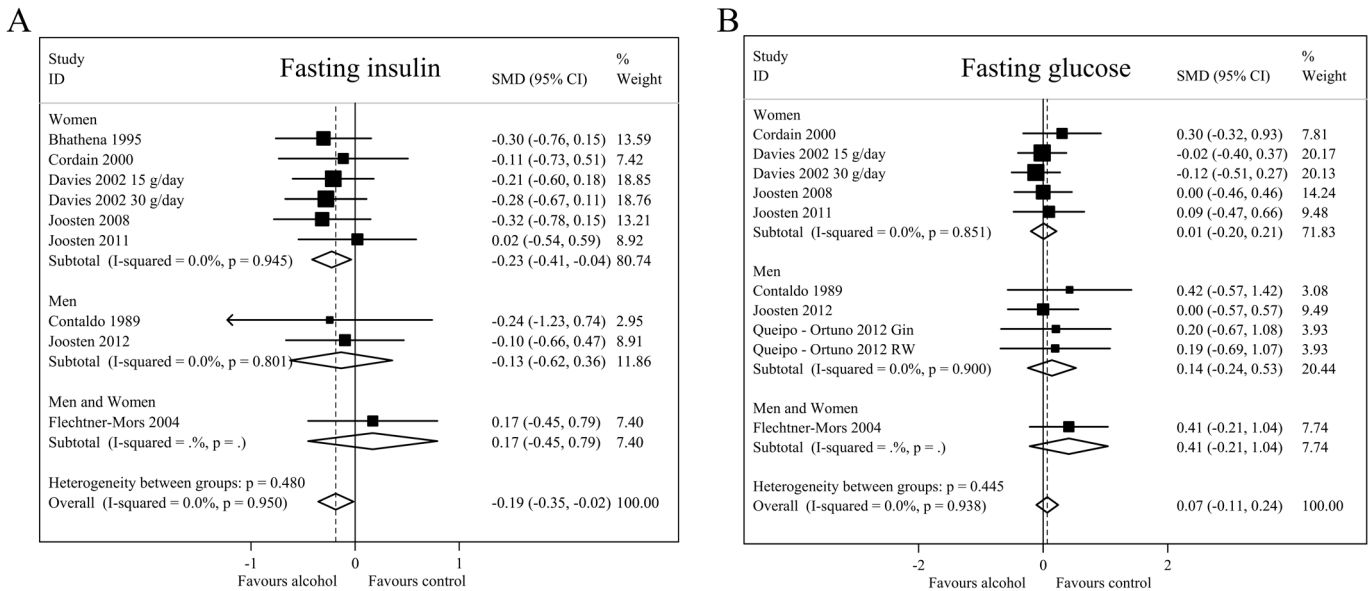


Figure 8.2. Forest plots of meta-analysis of the effect of alcohol consumption on fasting insulin (A) and fasting glucose (B).
Data are pooled SMDs with 95% CIs. RW, red wine.

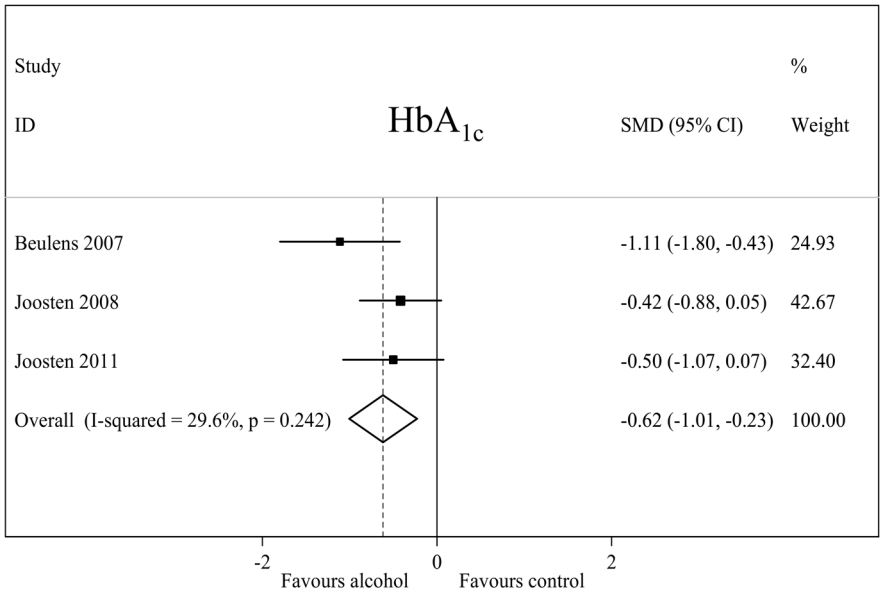


Figure 8.3. Forest plot of meta-analysis of the effect of alcohol consumption on HbA_{1c}.
Data are pooled SMDs with 95% CIs.

0.31], *P*=0.03. The trim and fill method shows that without publication bias, the pooled SMD would probably indicate a positive effect of alcohol consumption on insulin sensitivity, while the unadjusted SMD did not show an effect (SMD 0.08 [-0.09 to 0.24], *P*=0.35). Adjusted results and funnel plot are shown in Table 8.2 and Supplementary Figure 8.2.

Discussion

This meta-analysis shows that moderate alcohol consumption did not affect estimates of insulin sensitivity or fasting glucose levels, but it decreased fasting insulin concentrations and HbA_{1c}. Sex-stratified analysis suggested that moderate alcohol consumption may improve insulin sensitivity and decreased fasting insulin concentrations in women but not in men. The meta-regression suggested no influence of dosage and duration on the results. However, the number of studies may have been too low to detect influences by dosage and duration.

Comparison with other studies

The primary finding that alcohol consumption does not influence insulin sensitivity concords with the intervention studies included in the review of Hulthe and Fagerberg (5). This is in contrast with observational studies suggesting a significant association between moderate

alcohol consumption and improved insulin sensitivity (36, 37). However, the results of these studies might be biased through residual confounding because of their observational nature. Moreover, in contrast to intervention studies, observational studies are not designed to detect a causal relationship. On the other hand, we cannot exclude the possibility that the intervention studies in this review may have had an insufficient sample size or too short a duration to detect an effect of alcohol consumption on insulin sensitivity (10, 21, 23, 24).

We found lower fasting insulin levels after alcohol consumption. This finding agrees with the inverse relation between alcohol consumption and insulin levels observed in observational studies (38-41). However, in the DESIR (Data from an Epidemiological Study on the Insulin Resistance Syndrome) cohort, a longitudinal study, no relation between average or a change in alcohol consumption and fasting insulin levels was found, but this may be due to the inclusion of subjects with type 2 diabetes (42). Fasting insulin level is a surrogate marker of insulin sensitivity in healthy subjects, with lower insulin levels indicating higher insulin sensitivity (11, 43). Conversely, low insulin levels are a common phenomenon in subjects with type 2 diabetes due to impaired insulin secretion by β-cells. Because we excluded studies in subjects with type 2 diabetes, the results of lower fasting insulin levels may indicate higher insulin sensitivity. Additionally, we observed no change in glucose levels by alcohol consumption, and lower insulin levels coinciding with unchanged glucose levels suggest an improved insulin sensitivity.

The current meta-analysis suggests that men and women might respond differently to a period of alcohol consumption with regard to insulin sensitivity. Subgroup analysis showed that the effect of alcohol consumption on insulin sensitivity was only present among women, but the pooled effects in men and women were not significantly different. These results generally concord with observational studies showing a larger risk reduction of moderate alcohol consumption on risk of type 2 diabetes in women than in men (40% vs. 13%) (1) and with the study by Beulens et al. (44). The studies included in the review by Hulthe and Fagerberg (5), which were mainly cross-sectional, did not find sex differences in alcohol effects.

We observed lower levels of HbA_{1c} in subjects consuming moderate amounts of alcohol compared to abstainers. This has also been shown in several observational studies (41, 45, 46). Alcohol may decrease HbA_{1c} by suppressing the acute rise in blood glucose after a meal and increasing the early insulin response (47). This would result in lower glucose concentrations over time and, thus, lower HbA_{1c} concentrations. Unfortunately, the underlying mechanism of glycemic control by alcohol is not clearly understood.

Strengths and weaknesses of the study

A major strength of this meta-analysis is the inclusion of studies with a randomized controlled design and the inclusion of several complementary end points, providing a comprehensive overview of the evidence on this topic. There are also limitations that warrant consideration. As in any meta-analysis, the strength of our study is largely determined by the quality and

number of the included studies. The results of the quality assessment show that the larger part of the included studies did not report or did not take into account some important aspects, such as blinding. Nevertheless, randomization and the inclusion of an alcohol-free control group were the most important quality factors for this review and only six studies did not satisfy those criteria. Compliance was measured in most studies (17 of 22), but only reported in 13. However, of these 13 studies, 11 reported good or excellent compliance, suggesting that low compliance did not influence the results of the studies. Second, the analysis of several different outcomes resulted in inclusion of a small number of studies for certain end points, such as HbA_{1c}. Third, only two studies used the gold standard HEGC to estimate insulin sensitivity (11). Because this may lead to inconsistency in the results, we standardized the results of the different studies using Cohen's *d*. However, the results from the studies using HEGC were similar to the other intervention studies and no significant heterogeneity was present except for the combined meta-analysis of ISI and HOMA-IR. This was due to the study of Chiva-Blanch et al. (15), who reported a relatively small variation in HOMA-IR causing a relatively large SMD. Exclusion of this study removed heterogeneity without changing the effect. Fourth, because most studies used a crossover design, a carryover effect might have influenced the outcomes. Another limitation was the short duration and small sample sizes of the included studies. The average duration of 5.4 weeks may not have been long enough to show detectable differences in insulin sensitivity or glucose status. In addition, effects may change after longer-term intake of alcohol. Therefore, the short-term nature of the included studies does not allow us to draw conclusions on longer-term alcohol consumption.

It is important to note evidence for publication bias for certain outcomes in the current study. The publication bias unexpectedly suggested that smaller studies with positive results are missing. After adjustment for publication bias using the trim and fill method, even a significant increase in insulin sensitivity by alcohol consumption was shown. However, statistical tests for publication bias may yield biased results with small numbers of studies and are prone to heterogeneity (17).

Finally, the results of this research may not be generalizable to all healthy subjects because the selected studies included mainly light to moderate alcohol consumers. Therefore, the period of abstaining from alcohol might also be seen as an intervention, and subjects might have responded differently than alcohol abstainers.

Implications

To draw implications from the current research, the findings need to be placed in a clinical context. In this meta-analysis, we observed that alcohol consumption decreased fasting insulin levels by 0.19, which translates to an ~11% decrease in insulin (-20 pmol/L) in people with impaired glucose tolerance, as calculated from data of the Diabetes Prevention Program study (48), and a 13% decrease in insulin (-5.2 pmol/L) in normoglycemic people, as calculated from data of the Multiethnic Study of Atherosclerosis (MESA) (49). For comparison,

metformin treatment results in a 14% decrease in fasting insulin levels and a 40% lower risk of diabetes versus a control group (50). An 11% reduction of fasting insulin levels after alcohol consumption would result in an ~30% reduced risk of diabetes, which is in line with the 40% risk reduction observed among women.

The reduced HbA_{1c} concentration found in the current study by alcohol consumption (SMD -0.62) is equal to a 5% reduction in HbA_{1c} concentration in both the MESA and the Diabetes Prevention Program study (from 5.4% [36 mmol/mol] to 5.1% [33 mmol/mol] and from 5.9% [41 mmol/mol] to 5.6% [38 mmol/mol], respectively (48, 51). The Diabetes Prevention Program study showed that 4 years of metformin medication and a lifestyle intervention both resulted in a reduction in HbA_{1c} of ~3% (52). Because type 2 diabetes is characterized by hyperglycemia, HbA_{1c} could be seen as a surrogate end point of the disease rather than an intermediate factor in the pathway toward type 2 diabetes. The World Health Organization indeed suggests that a level >6.5% (48 mmol/mol) be used as a cutoff point for diagnosing diabetes (53). In this respect, the current results for HbA_{1c} match with the reduced risk of type 2 diabetes with moderate alcohol consumption. Results of alcohol intake on HbA_{1c} should be carefully interpreted because we included only three intervention studies in the analysis. However, the results suggest that drinking a moderate amount of alcohol is not harmful with regard to insulin sensitivity and glycemic status in healthy adults without type 2 diabetes.

Conclusion

This systematic review and meta-analysis showed that moderate alcohol consumption decreased fasting insulin and HbA_{1c} concentrations among nondiabetics. Alcohol consumption might improve insulin sensitivity among women but did not do so overall. These results may partly explain the lower risk of type 2 diabetes with moderate alcohol consumption found in observational studies. However, more intervention studies with a longer intervention period are necessary to confirm the results.

Funding

I.C.S. and H.F.J.H. were supported both by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO. Their joint aim is to independently study the health effects of moderate alcohol consumption. The funding sources had no role in conducting, analyzing, or interpreting study results or in the decision to submit the manuscript for publication. A.L.J.H., K.J.M. and J.W.J.B. were independent from funders. No potential conflicts of interest relevant to this article were reported.

Parts of this study were presented in abstract form at the Epidemiology and Prevention/ Nutrition, Physical Activity and Metabolism 2014 Scientific Sessions of the American Heart Association, San Francisco, CA, 18-21 March 2014 and were presented orally at the 7th European Beer and Health Symposium, Brussels, Belgium, 30 September 2014.

Supplemental Data

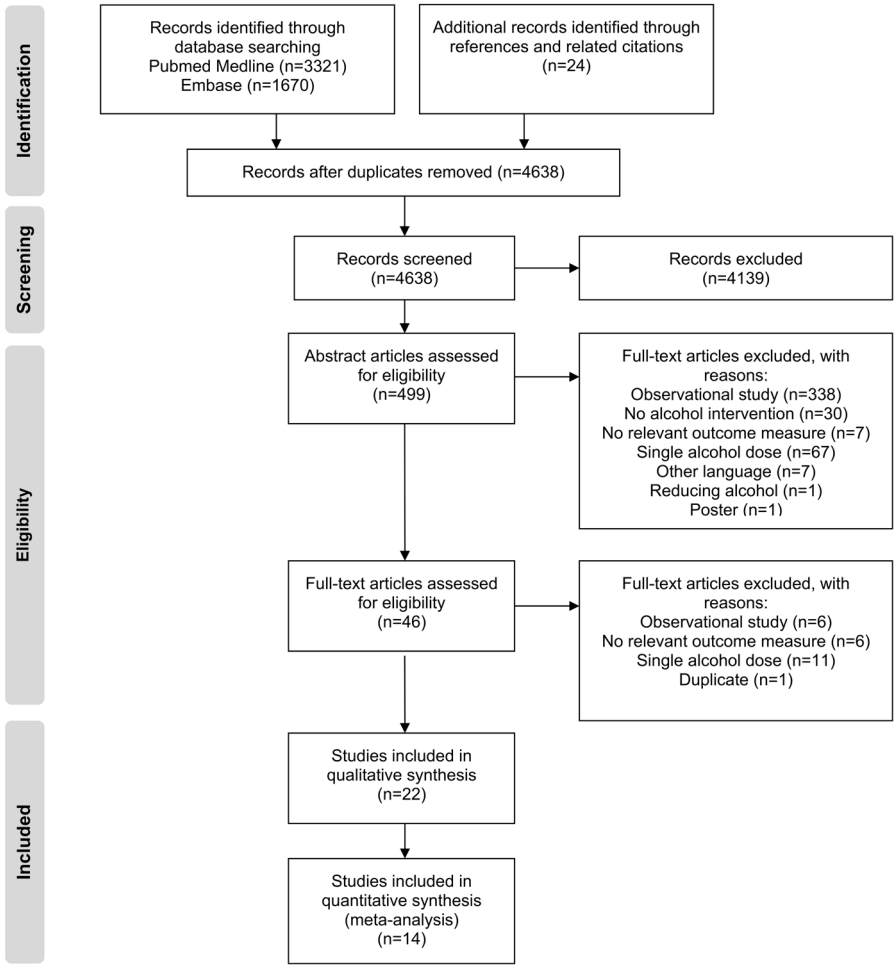
Supplementary Text 8.1. Pre-specified search string.

Pubmed Medline

"((alcohol [Title/Abstract] OR ethanol [Title/Abstract])
AND
(intake [Title/Abstract] OR consumption [Title/Abstract])) OR (alcoholic [Title/Abstract] AND (beverage [Title/Abstract] OR beverages [Title/Abstract] OR drink [Title/Abstract] OR drinks [Title/Abstract]))
AND
(Insulin [Title/Abstract] OR glycemic control [Title/Abstract] OR glycaemic control [Title/Abstract] OR glycemic response [Title/Abstract] OR glycaemic response [Title/Abstract] OR glucose [Title/Abstract] OR HbA1c [Title/Abstract] OR Hb A1c [Title/Abstract] OR HbA1 [Title/Abstract] OR HB A1 [Title/Abstract] OR Glycemic [Title/Abstract] OR Glycemia [Title/Abstract] OR Hemoglobin [Title/Abstract] OR Haemoglobin [Title/Abstract])
NOT (Animals [Mesh] NOT Humans [Mesh]))"

Embase

alcohol:ti:ab OR ethanol:ti:ab
AND
intake:ti:ab OR consumption:ti:ab OR alcoholic:ti:ab AND beverage:ti:ab OR beverages:ti:ab OR drink:ti:ab OR drinks:ti:ab
AND
Insulin:ti:ab OR 'glycemic control':ti:ab OR 'glycaemic control':ti:ab OR 'glycemic response':ti:ab OR 'glycaemic response':ti:ab OR glucose:ti:ab OR HbA1c:ti:ab OR 'Hb A1c':ti:ab OR HbA1:ti:ab OR 'HB A1':ti:ab OR Glycemic:ti:ab OR Glycemia:ti:ab OR Hemoglobin:ti:ab OR Haemoglobin:ti:ab
AND
[embase]/lim NOT [medline]/lim NOT ([animals]/lim NOT [humans]/lim)



Supplemental Figure 8.1. Flow chart of the multi-phase process for study selection.

Supplemental Table 8.1. Quality assessment of selected studies (n=22).

Study	Design	Randomized	Randomization procedure	Control group	Blinding	Drop out rates*	Measurement compliance	Intention to treat analysis	Funding	Jadad score (0-5)†	Included in meta-analysis‡
Bantle et al. (2008)	Crossover	Yes	NR	Yes	NR	Yes	Yes	Yes	Yes	2	No§
Beulens et al. (2006)	Crossover	Yes	NR	Yes	NR	Yes	Yes	No	Yes	2	Yes
Beulens et al. (2007)	Crossover	Yes	NR	Yes	NR	Yes	Yes	Yes	Yes	2	Yes
Beulens et al. (2008)	Crossover	Yes	Yes	Yes	NR	Yes	Yes	Yes	Yes	3	Yes
Bhathena et al. (1995)	Crossover	Yes	Yes	Yes	NR	NR	NR	NR	NR	2	Yes
Cesena et al. (2011)	Parallel	No	NA	Yes	NR	Yes	Yes	Yes	Yes	1	No‖
Chiva-Blanch et al. (2013)	Crossover	Yes	Yes	Yes	No	Yes	Yes	No	Yes	3	Yes
Contaldo et al. (1989)	Crossover	Yes	NR	Yes	NR	NR	NR	NR	Yes	1	Yes
Cordain et al. (1997)	Crossover	No	NA	Yes	NR	Yes	Yes	Yes	Yes	1	No‖
Cordain et al. (2000)	Crossover	Yes	NR	Yes	NR	Yes	Yes	Yes	NR	2	Yes
Davies et al. (2002)	Crossover	Yes	NR	Yes	NR	Yes	NR	No	NR	2	Yes
Flechtner-Mors et al. (2004)	Parallel	Yes	NR	Yes	NR	Yes	Yes	No	Yes	2	Yes
Joosten et al. (2008)	Crossover	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	3	Yes
Joosten et al. (2011)¶	Crossover	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	3	Yes
Joosten et al. (2012)¶	Crossover	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	3	Yes
Kim et al. (2009)	Parallel	No	NA	Yes	NR	Yes	Yes	No	Yes	1	No‖
Lavy et al. (1994)	Parallel	Yes	NR	No	NR	NR	NR	NR	NR	1	No#
Queipo-Ortuno et al. (2012)	Crossover	Yes	NR	Yes	NR	Yes	Yes	Yes	No	2	Yes
Romeo et al. (2008)	Parallel	No	NA	Yes	NR	Yes	Yes	No	Yes	1	No‖
Shai et al. (2007)	Parallel	Yes	NR	Yes	NR	Yes	Yes	No	Yes	2	No§
Sierksma et al. (2004)	Crossover	Yes	NR	Yes	No	Yes	Yes	No	Yes	2	Yes
Zheng et al. (2012)	Parallel	Yes	NR	No	NR	Yes	Yes	No	Yes	2	No#

* The studies that reported drop outs also reported the reasons for withdrawal. † Jadad score is based on description of randomization and randomization procedure, blinding and blinding procedure, and dropout rates and reasons for withdrawal. ¶ Results of the studies of Joosten et al. (2011 and 2012) are partly published in Joosten et al. (2014). ‡ Reason for exclusion from meta-analysis: § Participants with type 2 diabetes; ‖No randomized design; #No control group. NR: Not reported; NA: Not applicable

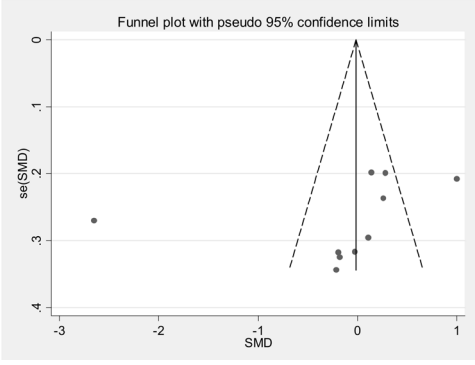
Supplemental Table 8.2. Publication bias calculated by Egger's and Begg's test.

	Egger's test	Begg's test	Begg test (continuity corrected)
ISI	0.01*	0.02*	0.04*
HOMA-IR	0.29	0.33	0.46
ISI & HOMA-IR	0.26	0.02*	0.02*
- Women	0.13	0.33	0.46
- Men	0.47	0.29	0.37
Insulin	0.18	0.30	0.35
- Women	0.17	0.85	1.00
- Men	NA	NA	NA
Glucose	0.01*	0.01*	0.01*
- Women	0.03*	0.05	0.09
- Men	0.05*	0.04	0.09
HbA_{1c}	0.28	0.12	0.30

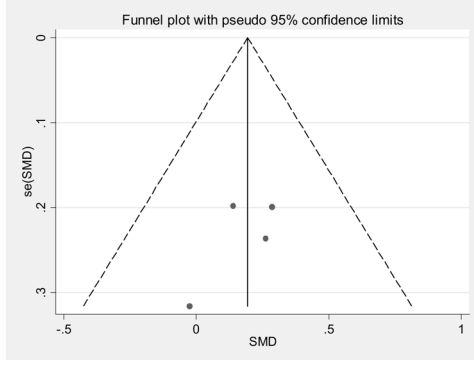
* *P*<0.05 indicates evidence for publication bias.

Supplemental Figure 8.2. Funnel plots of all endpoints for identification of publication bias.

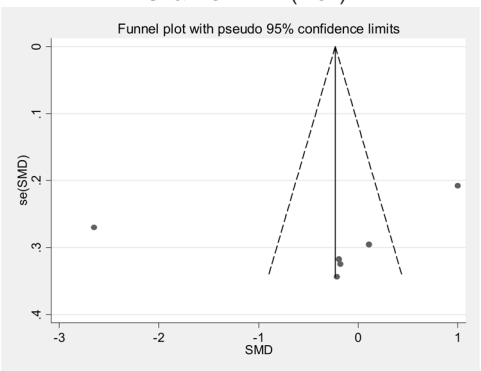
ISI & HOMA-IR (all)



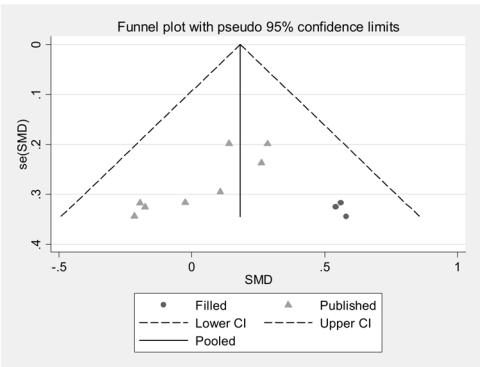
ISI & HOMA-IR (women)



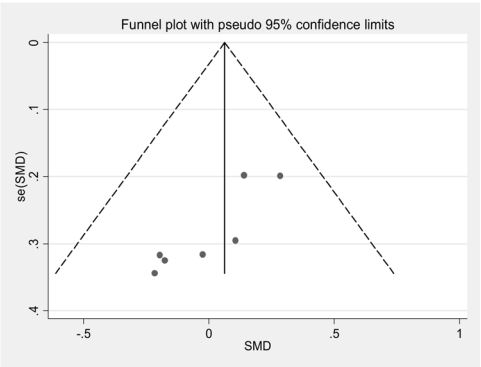
ISI & HOMA-IR (men)



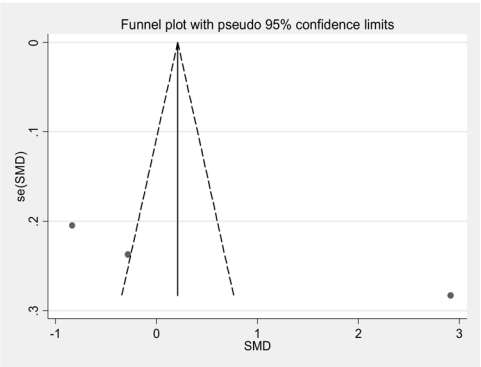
ISI & HOMA-IR (trim and fill method to adjust for publication bias)*



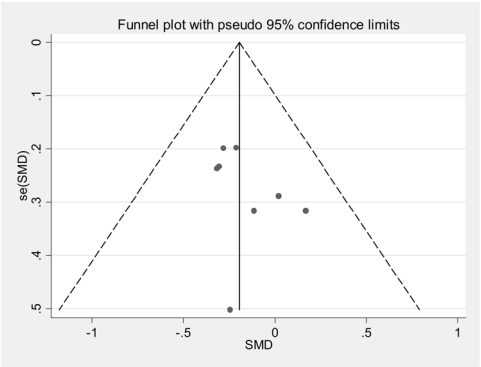
ISI (all)



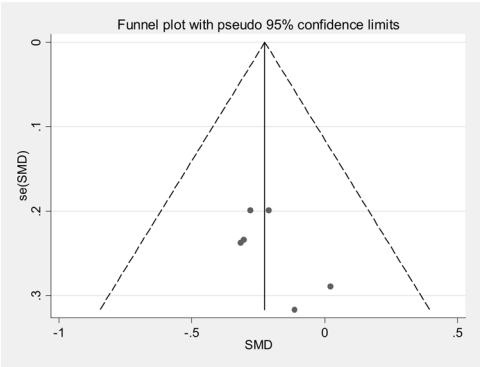
HOMA-IR (all)



Insulin (all)

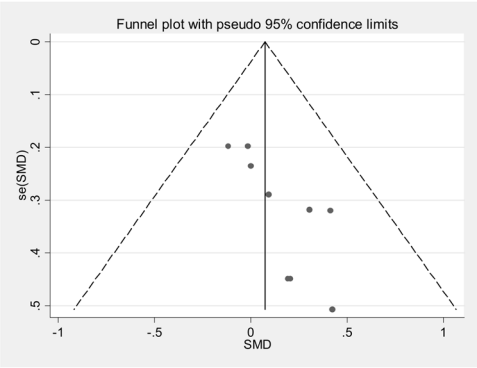


Insulin (women)

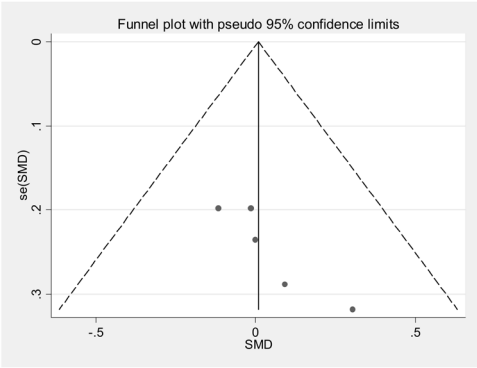


* The data from the study arms of Chiva-Blanch et al. (2013) were excluded, as they induced heterogeneity.

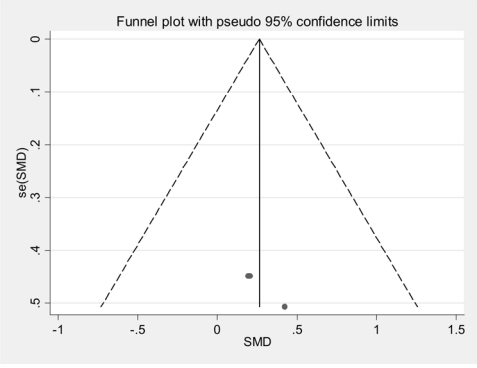
Glucose (all)



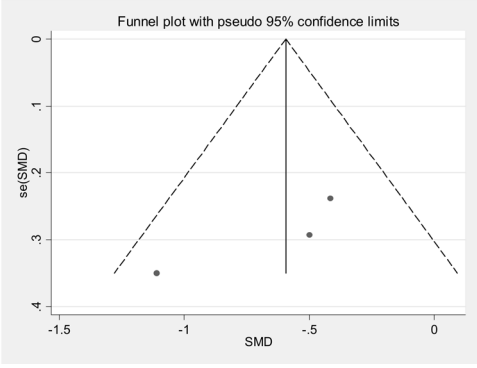
Glucose (women)



Glucose (men)



HbA_{1c} (all)



References

1. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, Rehm J. Alcohol as a risk factor for type 2 diabetes: A systematic review and meta-analysis. Diabetes Care. 2009;32(11):2123-32.
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(3):719-25.
3. Hendriks HFJ. Moderate alcohol consumption and insulin sensitivity: Observations and possible mechanisms. Ann Epidemiol. 2007 5;17(5):S40-2.
4. Brien SE, Ronksley PE, Turner BJ, Mukamal KJ, Ghali WA. Effect of alcohol consumption on biological markers associated with risk of coronary heart disease: Systematic review and meta-analysis of interventional studies. BMJ. 2011;342
5. Hulthe J, Fagerberg B. Alcohol consumption and insulin sensitivity: A review. Metab Syndr Relat D. 2005;3(1):45-50.
6. 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(3):608-12.
7. Flanagan D, Pratt E, Murphy J, Vaile J, Petley G, Godsland I, Kerr D. Alcohol consumption alters insulin secretion and cardiac autonomic activity. Eur J Clin Invest. 2002;32(3):187-92.
8. Cordain L, Melby CL, Hamamoto AE, O'Neill DS, Cornier M, Barakat HA, Israel RG, Hill JO. Influence of moderate chronic wine consumption on insulin sensitivity and other correlates of syndrome X in moderately obese women. Metab Clin Exp. 2000;49(11):1473-8.
9. 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. JAMA. 2002;287(19):2559-62.
10. Sierksma A, Patel H, Ouchi N, Kihara S, Funahashi T, Heine RJ, Grobbee DE, Kluft C, Hendriks HF. Effect of moderate alcohol consumption on adiponectin, tumor necrosis factor-α, and insulin sensitivity. Diabetes Care. 2004;27(1):184-9.
11. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: Advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab. 2008;294(1):E15-26.
12. Nathan DM, Turgeon H, Regan S. Relationship between glycated haemoglobin levels and mean glucose levels over time. Diabetologia. 2007 11/01;50(11):2239-44.
13. Cordain L, Bryan E, Melby C, Smith M. Influence of moderate daily wine consumption on body weight regulation and metabolism in healthy free-living males. J Am Coll Nutr. 1997;16(2):134-9.
14. Lavy A, Fuhrman B, Markel A, Dankner G, Ben-Amotz A, Presser D, Aviram M. Effect of dietary supplementation of red or white wine on human blood chemistry, hematology and coagulation: Favorable effect of red wine on plasma high-density lipoprotein. Ann Nutr Metab. 1994;38(5):287-94.
15. Chiva-Blanch G, Urpi-Sarda M, Ros E, Valderas-Martinez P, Casas R, Arranz S, Guillén M, Lamuela-Raventós RM, Llorach R, Andres-Lacueva C. Effects of red wine polyphenols and alcohol on glucose metabolism and the lipid profile: A randomized clinical trial. Clin Nutr. 2013;32:200-6.
16. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJM, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: Is blinding necessary? Control Clin Trials. 1996;17(1):1-12.
17. Higgins, JPT, Green, S. Cochrane Handbook for Systematic Reviews of Interventions. Version 5.1.0 ed. The

Cochrane Collaboration; 2011 [revised 2011 March; cited 2014 April 22]. Available from: www.cochrane-handbook.org

18. Duval S, Tweedie R. Trim and fill: A simple funnel-plot–based method of testing and adjusting for publication bias in meta-analysis. Biometrics. 2000;56(2):455-63.

19. Zheng J, Yang J, Huang T, Hu X, Luo M, Li D. Effects of chinese liquors on cardiovascular disease risk factors in healthy young humans. ScientificWorldJournal. 2012;2012:372143.

20. Contaldo F, D'Arrigo E, Carandente V, Cortese C, Coltorti A, Mancini M, Taskinen M, Nikkilä E. Short-term effects of moderate alcohol consumption on lipid metabolism and energy balance in normal men. Metab Clin Exp. 1989;38(2):166-71.

21. Beulens JW, Beers RM, Stolk RP, Schaafsma G, Hendriks HF. The effect of moderate alcohol consumption on fat distribution and adipocytokines. Obesity. 2006;14(1):60-6.

22. Beulens J, de Zoete E, Kok F, Schaafsma G, Hendriks H. Effect of moderate alcohol consumption on adipokines and insulin sensitivity in lean and overweight men: A diet intervention study. Eur J Clin Nutr. 2008;62(9):1098-105.

23. Beulens JW, van Loon LJ, Kok FJ, Pelsers M, Bobbert T, Spranger J, Helander A, Hendriks HF. The effect of moderate alcohol consumption on adiponectin oligomers and muscle oxidative capacity: A human intervention study. Diabetologia. 2007;50(7):1388-92.

24. Joosten M, Beulens J, Kersten S, Hendriks H. Moderate alcohol consumption increases insulin sensitivity and ADIPOQ expression in postmenopausal women: A randomised, crossover trial. Diabetologia. 2008;51(8):1375-81.

25. Joosten MM, Witkamp RF, Hendriks HF. Alterations in total and high–molecular-weight adiponectin after 3 weeks of moderate alcohol consumption in premenopausal women. Metab Clin Exp. 2011;60(8):1058-63.

26. Joosten MM, Schrieks IC, Hendriks HF. Effect of moderate alcohol consumption on fetuin-A levels in men and women: Post-hoc analyses of three open-label randomized crossover trials. Diabetol Metab Syndr. 2014 Feb 18;6(1):24,5996-6-24.

27. Bhathena SJ, Berlin E, Judd JT, Clevidence BA, Taylor PR, Campbell WS, Nair PP. Selective responses of hormones involved in carbohydrate and lipid metabolism and properties of erythrocyte membranes during the menstrual cycle in premenopausal women consuming moderate amounts of alcohol. Am J Clin Nutr. 1995;62(4):751-6.

28. Cesena FHY, Coimbra SR, Andrade ACM, da Luz PL. The relationship between body mass index and the variation in plasma levels of triglycerides after short-term red wine consumption. J Clin Lipidol. 2011;5(4):294-8.

29. Kim SH, Abbasi F, Lamendola C, Reaven GM. Effect of moderate alcoholic beverage consumption on insulin sensitivity in insulin-resistant, nondiabetic individuals. Metab Clin Exp. 2009;58(3):387-92.

30. Romeo J, González-Gross M, Wärnberg J, Díaz LE, Marcos A. Effects of moderate beer consumption on blood lipid profile in healthy spanish adults. Nutr Metab Cardiovasc Dis. 2008;18(5):365-72.

31. Bantle AE, Thomas W, Bantle JP. Metabolic effects of alcohol in the form of wine in persons with type 2 diabetes mellitus. Metab Clin Exp. 2008;57(2):241-5.

32. Shai I, Wainstein J, Harman-Boehm I, Raz I, Fraser D, Rudich A, Stampfer MJ. Glycemic effects of moderate alcohol intake among patients with type 2 diabetes: A multicenter, randomized, clinical intervention trial. Diabetes Care. 2007;30(12):3011-6.

33. Flechtner-Mors M, Biesalski H, Jenkinson C, Adler G, Ditschuneit H. Effects of moderate consumption of white wine on weight loss in overweight and obese subjects. Int J Obes. 2004;28(11):1420-6.

34. Joosten MM, van Erk MJ, Pellis L, Witkamp RF, Hendriks HFJ. Moderate alcohol consumption alters both leucocyte gene expression profiles and circulating proteins related to immune response and lipid metabolism in men. Br J Nutr. 2012;FirstView:1.

35. Queipo-Ortuño MI, Boto-Ordóñez M, Murri M, Gomez-Zumaquero JM, Clemente-Postigo M, Estruch R, Diaz FC, Andrés-Lacueva C, Tinahones FJ. Influence of red wine polyphenols and ethanol on the gut microbiota ecology and biochemical biomarkers. Am J Clin Nutr. 2012;95(6):1323-34.

36. Kawamoto R, Kohara K, Tabara Y, Miki T, Ohtsuka N, Kusunoki T, Abe M. Alcohol consumption is associated with decreased insulin resistance independent of body mass index in japanese community-dwelling men. Tohoku J Exp Med. 2009;218(4):331-7.

37. Ögge LE, Brohall G, Behre CJ, Schmidt C, Fagerberg B. Alcohol consumption in relation to metabolic regulation, inflammation, and adiponectin in 64-year-old caucasian women: A population-based study with a focus on impaired glucose regulation. Diabetes Care. 2006;29(4):908-13.

38. Mayer EJ, Newman B, Quesenberry CP, Friedman GD, Selby JV. Alcohol consumption and insulin concentrations. role of insulin in associations of alcohol intake with high-density lipoprotein cholesterol and triglycerides. Circulation. 1993 November 01;88(5):2190-7.

39. Kiechl S, Willeit J, Poewe W, Egger G, Oberhollenzer F, Muggeo M, Bonora E. Insulin sensitivity and regular alcohol consumption: Large, prospective, cross sectional population study (bruneck study). BMJ. 1996;313(7064):1040-4.

40. Lazarus R, Sparrow D, Weiss ST. Alcohol intake and insulin levels: The normative aging study. Am J Epidemiol. 1997 May 15;145(10):909-16.

41. Kroenke CH, Chu N, Rifai N, Spiegelman D, Hankinson SE, Manson JE, Rimm EB. A cross-sectional study of alcohol consumption patterns and biologic markers of glycemic control among 459 women. Diabetes Care. 2003;26(7):1971-8.

42. Vernay M, Balkau B, Moreau J, Sigalas J, Chesnier M, Ducimetiere P, the Desir Study Group. Alcohol consumption and insulin resistance syndrome parameters: Associations and evolutions in a longitudinal analysis of the french DESIR cohort. Ann Epidemiol. 2004 3;14(3):209-14.

43. Laakso M. How good a marker is insulin level for insulin resistance? Am J Epidemiol. 1993 May 01;137(9):959-65.

44. Beulens J, van der Schouw Y, Bergmann M, Rohrmann S, Schulze M, Buijsse B, Grobbee D, Arriola L, Cauchi S, Tormo M. Alcohol consumption and risk of type 2 diabetes in european men and women: Influence of beverage type and body size. the EPIC–InterAct study. J Intern Med. 2012;272(4):358-70.

45. Harding A, Sargeant L, Khaw K, Welch A, Oakes S, Luben R, Bingham S, Day N, Wareham N. Cross-sectional association between total level and type of alcohol consumption and glycosylated haemoglobin level: The EPIC-norfolk study. Eur J Clin Nutr. 2002;56(9):882-90.

46. Gulliford M, Ukoumunne O. Determinants of glycated haemoglobin in the general population: Associations with diet, alcohol and cigarette smoking. Eur J Clin Nutr. 2001;55(7):615-23.

47. McMonagle J, Felig P. Effects of ethanol ingestion on glucose tolerance and insulin secretion in normal and diabetic subjects. Metab Clin Exp. 1975;24(5):625-32.

48. Kitabchi AE, Temprosa M, Knowler WC, Kahn SE, Fowler SE, Haffner SM, Andres R, Saudek C, Edelstein SL, Arakaki R, Murphy MB, Shamoon H, Diabetes Prevention Program Research Group. Role of insulin secretion and sensitivity in the evolution of type 2 diabetes in the diabetes prevention program: Effects of lifestyle intervention and metformin. Diabetes. 2005 Aug;54(8):2404-14.

49. Bertoni AG, Wong ND, Shea S, Ma S, Liu K, Preethi S, Jacobs DR, Wu C, Saad MF, Szklo M. Insulin resistance, metabolic syndrome, and subclinical atherosclerosis: The multi-ethnic study of atherosclerosis (MESA). Diabetes Care. 2007 November 01;30(11):2951-6.

50. Salpeter SR, Buckley NS, Kahn JA, Salpeter EE. Meta-analysis: Metformin treatment in persons at risk for diabetes mellitus. Am J Med. 2008;121(2):149-57.

51. McNeely MJ, McClelland RL, Bild DE, Jacobs DR, Tracy RP, Cushman M, Goff DC, Astor BC, Shea S, Siscovick DS. The association between A1C and subclinical cardiovascular disease: The multi-ethnic study of atherosclerosis. Diabetes Care. 2009 September 01;32(9):1727-33.

52. Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, Nathan DM, Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. N Engl J Med. 2002 Feb 7;346(6):393-403.

53. World Health Organization. Use of glycated haemoglobin (HbA1c) in the diagnosis of diabetes mellitus. 2011. Report No.: 11.1

Chapter 9

Bidirectional associations between alcohol consumption and health-related quality of life among young and middle-aged women

Ilse C. Schrieks
Melissa Y. Wei
Eric B. Rimm
Olivia I. Okereke
Ichiro Kawachi
Henk F. J. Hendriks
Kenneth J. Mukamal

Submitted

Abstract

Background: Cross-sectional studies have suggested a positive association between moderate alcohol consumption and health-related quality of life, but prospective data remain scarce. We examined the bidirectional relationships between alcohol consumption and health-related quality of life in women using a longitudinal design.

Methods: A total of 92,448 participants of the Nurses' Health Study II (mean age 42.8 years) reported their alcohol consumption 4 times (1991, 1995, 1999, 2003) and health-related quality of life 3 times (1993, 1997, 2001) on biennial questionnaires. Using generalized estimating equations, we modeled the physical and mental component summary (PCS and MCS) scores (means ~50, SDs ~9) as a function of alcohol consumption 2 years earlier, and vice versa. Models were also adjusted for demographic and clinical covariates.

Results: Greater alcohol consumption was associated with better PCS scores 2 years later in a dose-response relationship up to ~1 serving daily (mean difference (β)=0.67 ± 0.06 units of PCS; *P*<0.001, for moderate vs. infrequent drinkers). After adjustment for previous PCS, the association demonstrated a similar but attenuated pattern (β=0.33 ± 0.07; *P*<0.001). Daily moderate alcohol consumption was not related to MCS, although moderate to heavy drinkers had a lower MCS score than infrequent drinkers (β=−0.34 ± 0.15; *P*=0.027). When modelling the prospective association between HRQOL and alcohol consumption, higher PCS scores were associated with greater alcohol consumption 2 years later (β=0.58 ± 0.06 g/d; *P*<0.001), which was again attenuated after adjustment for previous alcohol consumption (β=0.53 ± 0.05 g/d; *P*<0.001). MCS was not related to alcohol consumption 2 years later.

Conclusions: Among young and middle-aged women, greater alcohol intake (up to ~1 serving daily) was associated with a small improvement in physical health-related quality of life two years later, and vice versa. No significant relation was observed between moderate alcohol consumption and mental health-related quality of life in either direction.

Introduction

Cross-sectional studies suggest an association between moderate alcohol consumption and higher health-related quality of life (HRQOL) (1-6). However, excessive alcohol consumption and binge-drinking have been associated with poorer subjective health (7, 8). Indeed, moderate alcohol consumption has been associated with physical and mental health benefits in longitudinal studies, such as a reduced risk of type 2 diabetes, rheumatoid arthritis and incident depression (9-11). Moreover, moderate alcohol consumption has been related to lower psychological distress, increased sociability and mood enhancement (12). However, these associations may be biased by reverse causation. For example, moderate drinkers may also engage in more social activities (13). A few small longitudinal studies have examined the associations between alcohol consumption and HRQOL prospectively. Kaplan et al. (2012) and Byles et al. (2006) found that persistent moderate alcohol consumption was related to a higher mental and physical HRQOL compared to abstaining or decreasing alcohol consumption (14, 15). This suggests that alcohol consumption may influence subsequent quality of life. However, the reverse association, in which HRQOL influences subsequent alcohol consumption, may also be true. Bell & Britton (2014) suggested that the relationship between mental health and alcohol consumption is driven by mental health, meaning that mental health influences change in alcohol but not vice versa. Specifically, they showed that people with better mental HRQOL and high alcohol consumption showed a larger decrease in alcohol consumption in the next 5 years (16). Furthermore, people with poorer self-perceived health status tend to be more likely to reduce or stop drinking alcohol than people with excellent health status (17).
These studies provide evidence for a more complex, bidirectional relationship between alcohol consumption and the physical and mental components of HRQOL. However, to our knowledge, these relationships have not been investigated in any large-scale prospective studies with repeated measures of both alcohol consumption and HRQOL. Therefore, the aim of this study was to examine these bidirectional relations in young and middle-aged women who were followed for 12 years in the Nurses' Health Study II.

Methods

Study population

The Nurses' Health Study II (NHS II) was established in 1989, when 116,430 U.S. female nurses aged 25-42 years responded to a mailed questionnaire regarding their diet and medical history. The participants have been followed every 2 years with mailed questionnaires that collect diet, lifestyle and medical information. We excluded women with missing data on alcohol consumption or HRQOL on every questionnaire throughout the study. Furthermore, we excluded women who were diagnosed with multiple sclerosis or cancer (except for those with nonmelanoma skin cancer) before 1991, when follow-up for these analyses started,

because these diseases have a large negative impact on health-related quality of life (18, 19). Additionally, in each cycle, we excluded women who were pregnant in the period from 2 years before exposure until the outcome measurement time, as pregnancy causes most women to stop drinking and reduces quality of life (20). After these exclusions, 186,845 observations (from 88,363 participants) remained for the analysis of the association between alcohol consumption and subsequent HRQOL and 178,849 observations (from 84,621 participants) remained for the analysis of the reverse association. The study flow is shown in Supplemental Figure 9.1. The study protocol was approved by the institutional review board of Partners Health Care System. The completion and return of the self-administered questionnaires was considered to represent informed consent.

Assessment of alcohol consumption

Alcohol consumption was assessed by a semi-quantitative food frequency questionnaire (FFQ) in 1991 and every 4 years subsequently. The FFQ included separate items for regular beer, light beer, white wine, red wine, and liquor with 9 frequency responses ranging from never or less than 1/month up to 6+ times/day over the previous year. We calculated total alcohol intake by multiplying the average consumption of each beverage by the published alcohol content of the specified portion size based on periodically updated U.S. Department of Agriculture food consumption tables and then summing across beverages (21). We previously assessed the reproducibility and validity of the self-reported alcohol intake with the FFQ against one-week dietary records completed every three months for a year among 173 Boston-area participants of Nurses' Health Study, a similar cohort of female nurses. The spearman correlation coefficient between these two measures of alcohol intake was 0.90 (22).

Assessment of HRQOL

Health-related quality of life was measured using the Medical Outcomes Study 36-Item Short-Form Health Survey (SF-36) in 1993, 1997 and 2001. The SF-36 is a self-administered questionnaire that comprises 8 scales of HRQOL: 1) physical functioning, 2) role limitations due to physical health problems (role physical), 3) bodily pain, 4) general health perceptions, 5) vitality, 6) social functioning, 7) role limitations due to emotional problems (role emotional), and 8) mental health. Each scale was scored separately from 0 to 100, with higher scores reflecting better HRQOL (Ware et al. 1992). There were two primary outcomes of this study: the physical component summary (PCS) and the mental component summary (MCS) scores. These component summary scores reflect overall physical and mental HRQOL. By design, the PCS and MCS scales represent orthogonal (i.e., uncorrelated) constructs (23). Summary scores were standardized by using the mean, standard deviation and factor score coefficients for the SF-36 scales in the US general population, so that a mean PCS and MCS score of 50 (standard deviation = 10) reflects the mean in the general US population (24). The instrument has been extensively validated, has good construct validity, and high test-retest ability and

internal consistency (25-27).

Covariates

In follow-up biennial questionnaires, we obtained self-reported information on demographics, lifestyle factors (28), social integration (29), diseases, medication and other characteristics that were included as covariates in multivariable analyses: age (years), race (white or non-white), region of residence (Northeast, Midwest, South, West, Non-US), body mass index (<18.5 kg/m^2 ,18.5-22.9 kg/m^2, 23-24.9 kg/m^2, 25-29.9 kg/m^2, 30-34.9 kg/m^2, ≥35 kg/m^2), smoking status (never, past and current smokers), physical activity (MET-h/week), energy intake (kcal/d), marital status (married or not married), living arrangement (alone or with others), parity, employment status (employed or not employed), night shift work, (30) arthritis (i.e. osteoarthritis and rheumatoid arthritis), diabetes mellitus, hypertension, hypercholesterolemia, asthma, premenstrual syndrome, regular use (during the past 2 years) of antidepressants (selective serotonin reuptake inhibitors, tricyclic antidepressants or other), anxiolytics, analgesics (acetaminophen, aspirin, or non-steroidal anti-inflammatory drugs), or oral contraceptives, menopausal status. Dietary information (in addition to alcohol) was obtained from repeated FFQs. To reflect overall diet quality, a diet score (without alcohol) was calculated based on the 2010 Alternative Healthy Eating Index (AHEI), where a higher score denotes better overall dietary quality (31). In addition, the frequency of sugar-sweetened beverages consumption and candy consumption were added as covariates, since moderate alcohol consumption has been related to lower intake of sugar-sweetened beverages and candy (32, 33).

Statistical analysis

Primary analyses

We conducted two sets of analyses to examine the bidirectional associations between alcohol consumption and HRQOL (PCS and MCS). In the first analysis, we examined the association between alcohol consumption and HRQOL two years later. For example, we used alcohol consumption in 1991 to predict HRQOL scores in 1993, and then alcohol consumption in 1995 to predict HRQOL scores in 1997. The following alcohol consumption categories were used: former drinkers, long-term abstainers, and infrequent (0.1-1.24 g/d), light (1.25-4.9 g/d), moderate (5.0-19.9 g/d) and moderate to heavy drinkers (≥ 20 g/d). We used infrequent drinkers as the reference group. In the second analysis, we examined the association between HRQOL (quintiles of PCS and MCS) and alcohol consumption (continuous, g/d) two years later. In total, there were three cycles for both directions of the association (Supplemental Figure 9.1).

In both sets of analyses, the following covariates were included in the model: age, race, region of residence, body mass index, smoking status, physical activity, energy intake, marital status, living arrangement, parity, employment status, working rotating night shifts. This model

was called the lifestyle-adjusted model. A second model, the morbidity-adjusted model, was further adjusted by arthritis, diabetes mellitus, hypertension, hypercholesterolemia, asthma, premenstrual syndrome, antidepressant use, anxiolytic use, regular analgesic use, oral contraceptive use and menopausal status. Finally, a third model, referred to as the diet-adjusted model, was further adjusted for the Alternative Healthy Eating Index score without alcohol, sugar-sweetened beverages consumption, and candy consumption.

To account for the effect of previous outcomes on current ones, we further adjusted for baseline outcome variables (HRQOL in the first analysis, alcohol consumption in the second analysis) in each model. For example, when we used alcohol consumption in 1995 to predict HRQOL scores in 1997, we additionally adjusted for HRQOL scores in 1993.

Because each individual contributed repeated measures of HRQOL and alcohol consumption, we used generalized estimating equations (GEE; "PROC GENMOD" in SAS) with an identity link and exchangeable correlation matrix to account for the correlation of within-person repeated measures. Models that used linear mixed models yielded very similar results.

Secondary analyses

We examined the potential non-linear relation between alcohol consumption and PCS and MCS non-parametrically with restricted cubic splines (34). Tests for non-linearity used the likelihood ratio test, comparing the model with only the linear term to the model with the linear and the cubic spline terms. Results using loess smoothers and fractional polynomials yielded similar findings.

In addition, we examined the association between alcohol consumption and the 8 HRQOL subscales two years later with GEE models. The same covariates were used as in the primary analysis.

Because no single summary score for HRQOL exists in the SF-36, we examined the associations between alcohol consumption and "overall HRQOL" using both PCS and MCS. In these, we defined "good HRQOL" as having both a high PCS and MCS score and "poor HRQOL" as having both a low PCS and MCS score (defined as being above the 60th percentile for good HRQOL and below the 40th percentile for poor HRQOL). These cut off levels were chosen a priori to yield ~15% of women in the good and poor HRQOL groups. GEE models were used (PROC GENMOD in SAS with a Poisson distribution and a log link) to calculate odds ratios (OR) for good and poor overall HRQOL vs. being not in one of these categories (the 'intermediate' group).

In addition to examining total alcohol consumption as a primary outcome variable, we separately examined the likelihood of being a drinker and the amount of alcohol consumed among drinkers, we examined the association between PCS and MCS and the prevalence ratio of any alcohol consumption with GEE models (PROC GENMOD in SAS with a Poisson distribution and a log link). Former drinkers were excluded in this analysis.

All analyses were performed using SAS software, version 9.2 (SAS Institute, North Carolina).

A two-tailed *P* value of 0.05 was considered statistically significant.

Results

Alcohol consumption and subsequent HRQOL

Baseline characteristics

Supplemental Table 1 summarizes the characteristics of participants in the Nurses' Health Study II by baseline alcohol consumption in 1991. Women drinking higher amounts of alcohol had a lower BMI and higher physical activity. They were less often unemployed, married or with children. Prevalence of hypertension, hypercholesterolemia, osteoarthritis, diabetes, and asthma was lower among women drinking alcohol up to a moderate dosage (5-19.9 g/d). However, a regular use of analgesics was more common in higher alcohol consumers.

Alcohol consumption and subsequent PCS

In Table 9.1, the associations between alcohol consumption and subsequent PCS and MCS are reported. The diet-adjusted model showed that alcohol consumption was associated with higher PCS scores in a dose-response relationship, with women consuming ≥20 g/d having the highest PCS scores compared to the infrequent drinkers reference group (β=0.83 ± 0.11, *P*<0.001). After further adjustment for previous PCS scores, the association between alcohol consumption and PCS was attenuated. The dose-response relationship was only evident up to a moderate dose of 5-19.9 g/d (β=0.33 ± 0.07, *P*<0.001, for moderate vs. infrequent drinkers). For comparison, in the lifestyle-adjusted model (also adjusted for previous PCS), a 1-unit increment in BMI was associated with a decrement of 0.20 in PCS score, and a 1-year increment in age was associated with a 0.10 decrement. Furthermore, smoking was associated with a decrement of 0.93 in PCS score (current vs. never smokers). The non-linear dose-response relationship between alcohol intake and PCS was confirmed in a restricted cubic spline (Figure 9.1, test for non-linear relationship *P*<0.001).

Alcohol consumption and subsequent MCS

The diet-adjusted model showed that light to moderate alcohol consumption was not associated with higher MCS scores compared to infrequent drinking. However, compared to infrequent drinkers, women drinking ≥20 g/d had a lower MCS score (β=−0.36 ± 0.15, *P*=0.017), and long-term abstainers had a higher MCS score (β=0.34 ± 0.08, *P*<0.001). After additional adjustment for previous MCS, only moderate to high alcohol consumption remained associated with lower MCS (β=−0.34 ± 0.15, *P*=0.027). This was further confirmed by results from a restricted cubic spline, which showed an inverse linear relation between alcohol consumption and MCS (*P*=0.032).

Alcohol consumption and subsequent HRQOL scales and overall HRQOL

The associations between alcohol consumption and eight scales of the SF-36 are shown in Supplemental Figure 9.2. Compared to infrequent drinkers, abstainers and former drinkers scored lower on all scales while moderate drinkers (5-19.9 g/d) scored higher on all scales, except for role-emotional and mental health. Moderate to heavy drinkers (≥20 g/d) scored higher on role-physical and vitality, but lower on role-emotional and mental health.

Moderate drinkers had a lower likelihood of poor overall HRQOL (OR=0.90 [0.84-0.97]) and a higher likelihood of good overall HRQOL (OR=1.09 [1.02-1.16]) as compared to infrequent drinkers (diet-adjusted model, additionally adjusted for previous PCS and MCS).

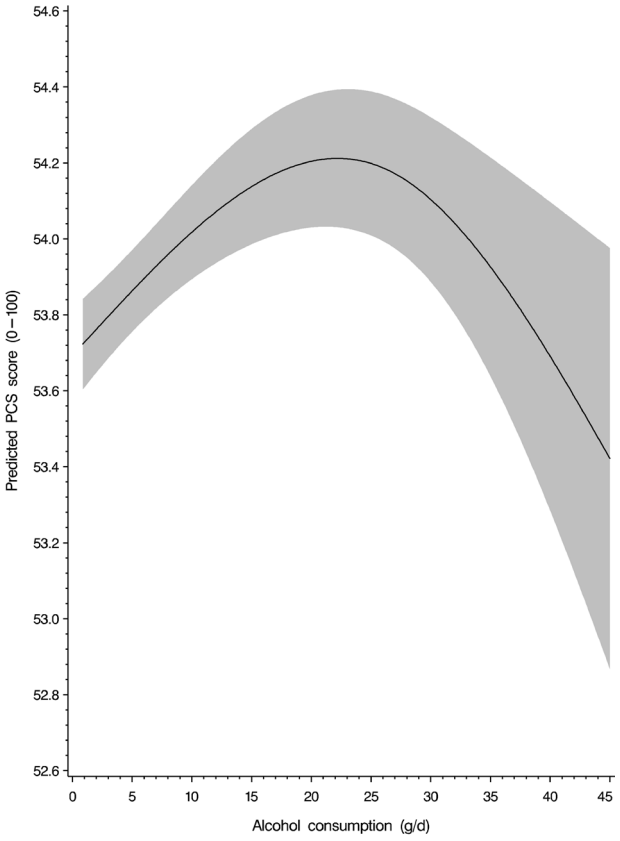


Figure 9.1. Non-linear dose-response relationship between alcohol consumption and subsequent physical component summary (PCS) scores.

Data derive from a restricted cubic spline GEE model (lifestyle-adjusted model) in alcohol consumers. The model is adjusted for previous PCS, age, physical activity, energy intake, body mass index, smoking, marital status, employment status, night shift work, race, region, living arrangement, parity. Alcohol consumption is winsorized on the 99.5 percentile. The 95% confidence interval (CI) is indicated by the gray cloud. *P*<0.001 for test for non-linear relationship.

HRQOL and subsequent alcohol consumption

Baseline characteristics

Supplemental Tables 2 and 3 summarize the characteristics of the participants in the Nurses' Health Study II by baseline PCS and MCS scores. Women with lower PCS and MCS scores had higher BMI and energy intake and lower physical activity. In addition, they more often worked rotating night shifts and used antidepressants. Women with lower PCS scores were older, while women with lower MCS scores were younger.

PCS and subsequent alcohol consumption

Table 9.2 provides the associations between PCS and MCS and subsequent alcohol consumption. Subsequent alcohol consumption was higher in women with higher initial PCS. Compared with the lowest quintile of PCS, women in the highest quintile consumed 0.58 ± 0.06 g alcohol per day more (*P*<0.001, diet-adjusted model). After further adjustment for previous alcohol consumption, the association was attenuated; the difference in alcohol consumption between the highest and the lowest quintile was 0.53 ± 0.05 g/d.

To further investigate this positive association between PCS and subsequent alcohol consumption, we analyzed if the association was also evident amongst alcohol drinkers only. In addition, we analyzed the association between PCS and the likelihood of drinking alcohol. Amongst alcohol drinkers, women in the highest quintile consumed 0.40 ± 0.08 g alcohol per day more than women in the lowest quintile (*P*<0.001). The association between PCS scores and subsequent alcohol consumption was linear, with higher PCS scores associated with higher alcohol consumption in a dose-response manner (*P*<0.001, Figure 9.2).

We next tested the association of PCS with likelihood of any alcohol intake. In the diet-adjusted model we observed a linear association for PCS: women with a higher PCS score had a higher chance of drinking (OR=1.11 [1.10-1.13], *P*<0.001 for highest vs. lowest quintile). Further adjustment for previous alcohol intake did not change the results.

MCS and subsequent alcohol consumption

No association was found between MCS scores and subsequent alcohol consumption in both diet-adjusted models with and without adjustment for previous alcohol consumption (highest vs. lowest quintile: -0.03 ± 0.06 and 0.03 ± 0.05 g/d, respectively).

Table 9.1. Association between alcohol consumption and 2 years later physical (PCS) and mental (MCS) component summary scores in the Nurses' Health Study II.

	Alcohol consumption category					
Outcome	Abstainer 0 g/d	Former drinker 0 g/d	Infrequent drinker 0.1-1.24 g/d	Light drinker 1.25-4.9 g/d	Moderate drinker 5-19.9 g/d	Moderate/heavy drinker ≥20 g/d
Physical component summary (PCS)						
Unadjusted for previous PCS (n=186,864)						
Lifestyle-adjusted model[a]	-0.84 (0.07)*	-0.53 (0.06)*	0.00 (referent)	0.36 (0.06)*	0.78 (0.06)*	0.91 (0.12)*
Morbidity-adjusted model[b]	-0.81 (0.06)*	-0.48 (0.06)*	0.00 (referent)	0.31 (0.06)*	0.69 (0.06)*	0.86 (0.11)*
Diet-adjusted model[c]	-0.79 (0.06)*	-0.47 (0.06)*	0.00 (referent)	0.30 (0.06)*	0.67 (0.06)*	0.83 (0.11)*
Adjusted for previous PCS (n=114,404)						
Lifestyle-adjusted model[a]	-0.53 (0.07)*	-0.45 (0.08)*	0.00 (referent)	0.20 (0.07)~	0.34 (0.07)*	0.39 (0.12)~
Morbidity-adjusted model[b]	-0.54 (0.07)*	-0.36 (0.08)*	0.00 (referent)	0.19 (0.07)~	0.35 (0.07)*	0.45 (0.12)*
Diet-adjusted model[c]	-0.52 (0.07)*	-0.36 (0.08)*	0.00 (referent)	0.17 (0.07)^	0.33 (0.07)*	0.43 (0.12)*
Mental component summary (MCS)						
Unadjusted for previous MCS (n=186,864)						
Lifestyle-adjusted model[a]	0.28 (0.08)*	-0.17 (0.07)^	0.00 (referent)	0.12 (0.07)	0.16 (0.08) ^	-0.24 (0.15)
Morbidity-adjusted model[b]	0.31 (0.08)*	-0.12 (0.07)	0.00 (referent)	0.09 (0.07)	0.12 (0.08)	-0.26 (0.15)
Diet-adjusted model[c]	0.34 (0.08)*	-0.10 (0.07)	0.00 (referent)	0.06 (0.07)	0.06 (0.08)	-0.36 (0.15)^
Adjusted for previous MCS (n=114,404)						
Lifestyle-adjusted model[a]	0.11 (0.08)	-0.21 (0.09)^	0.00 (referent)	0.06 (0.08)	0.08 (0.08)	-0.25 (0.15)
Morbidity-adjusted model[b]	0.15 (0.08)	-0.14 (0.09)	0.00 (referent)	0.04 (0.08)	0.06 (0.08)	-0.25 (0.15)
Diet-adjusted model[c]	0.17 (0.08)^	-0.12(0.09)	0.00 (referent)	0.01 (0.08)	0.00 (0.08)	-0.34 (0.15)^

Mean difference (β-coefficient (SE)) in PCS and MCS between the alcohol consumption category and the reference category. ^P<0.05; ~P<0.01; *P<0.001.

[a] Lifestyle-adjusted model: adjusted for time, age, physical activity, energy intake, body mass index, smoking, marital status, employment status, night shift work, race, region, living arrangement, parity. [b] Morbidity-adjusted model: lifestyle-adjusted model + adjusted for arthritis (osteoarthritis and rheumatoid arthritis), diabetes mellitus, hypertension, hypercholesterolemia, asthma, premenstrual syndrome, antidepressant use, anxiolytic use, analgesic use, oral contraceptive use, menopausal status. [c] Diet-adjusted model: morbidity-adjusted model + adjusted for Alternative Healthy Eating Index-2010 without alcohol, sweetened beverages, candy.

Table 9.2. Association between physical (PCS) and mental (MCS) component summary scores and 2 years later alcohol consumption in the Nurses' Health Study II.

	Quintiles of physical component summary score				
Outcome: alcohol intake (g/d)	Q1 (8-46)	Q2 (47-52)	Q3 (53-55)	Q4 (56-58)	Q5 (59-75)
Unadjusted for previous alcohol intake (n=178,849)					
Lifestyle-adjusted model[a]	0.00 (referent)	0.32 (0.05)*	0.53 (0.05)*	0.63 (0.05)*	0.61 (0.06)*
Morbidity-adjusted model[b]	0.00 (referent)	0.31 (0.05)*	0.51 (0.05)*	0.62 (0.05)*	0.61 (0.06)*
Diet-adjusted model[c]	0.00 (referent)	0.30 (0.05)*	0.50 (0.05)*	0.60 (0.05)*	0.58 (0.06)*
Adjusted for previous alcohol intake (n=165,693)					
Lifestyle-adjusted model[a]	0.00 (referent)	0.23 (0.04)*	0.38 (0.04)*	0.48 (0.04)*	0.55 (0.05)*
Morbidity-adjusted model[b]	0.00 (referent)	0.23 (0.04)*	0.38 (0.04)*	0.49 (0.05)*	0.57 (0.05)*
Diet-adjusted model[c]	0.00 (referent)	0.22 (0.04)*	0.37 (0.04)*	0.47 (0.05)*	0.53 (0.05)*
	Quintiles of mental component summary score				
Outcome: alcohol intake (g/d)	Q1 (0-42)	Q2 (43-49)	Q3 (50-53)	Q4 (54-56)	Q5 (57-71)
Unadjusted for previous alcohol intake (n=178,849)					
Lifestyle-adjusted model[a]	0.00 (referent)	0.00 (0.05)	-0.02 (0.05)	-0.05 (0.05)	-0.03 (0.06)
Morbidity-adjusted model[b]	0.00 (referent)	0.01 (0.05)	-0.01 (0.05)	-0.03 (0.05)	0.00 (0.06)
Diet-adjusted model[c]	0.00 (referent)	0.00 (0.05)	-0.02 (0.05)	-0.05 (0.05)	-0.03 (0.06)
Adjusted for previous alcohol intake (n=165,693)					
Lifestyle-adjusted model[a]	0.00 (referent)	0.02 (0.05)	0.01 (0.04)	0.04 (0.04)	0.04 (0.05)
Morbidity-adjusted model[b]	0.00 (referent)	0.03 (0.05)	0.03 (0.05)	0.07 (0.05)	0.07 (0.05)
Diet-adjusted model[c]	0.00 (referent)	0.02 (0.05)	0.02 (0.05)	0.04 (0.05)	0.03 (0.05)

Mean difference (β-coefficient (SE)) in PCS and MCS between the alcohol consumption category and the reference category. *P<0.001.

[a] Lifestyle-adjusted model: adjusted for time, age, physical activity, energy intake, body mass index, smoking, marital status, employment status, night shift work, race, region, living arrangement, parity. [b] Morbidity-adjusted model: lifestyle-adjusted model + adjusted for arthritis (osteoarthritis and rheumatoid arthritis), diabetes mellitus, hypertension, hypercholesterolemia, asthma, premenstrual syndrome, antidepressant use, anxiolytic use, analgesic use, oral contraceptive use, menopausal status. [c] Diet-adjusted model: morbidity-adjusted model + adjusted for Alternative Healthy Eating Index-2010 without alcohol, sweetened beverages, candy.

Discussion

This large longitudinal cohort provides evidence that the association between alcohol consumption and HRQOL is bidirectional. Moderate alcohol consumption was associated with better subsequent PCS, but not with subsequent MCS. In the reverse direction, we observed an association between higher PCS and higher subsequent alcohol consumption. MCS was not related to subsequent alcohol consumption.

Comparison with other work

Our study confirms previous studies that have reported a positive association between moderate alcohol consumption and physical health-related quality of life. We observed a non-linear dose-response relationship between alcohol consumption and 2 years later PCS, with highest PCS scores in women drinking moderately. A similar association was observed between alcohol consumption and scales of PCS, i.e. physical functioning, role-physical, bodily pain and general health. Previous studies also reported higher scores for PCS and its scales in moderate drinkers as compared to long-term abstainers or light drinkers (1, 2, 5, 15). Although the association found in this study is consistent with previous studies, persists after multivariate adjustment, and remains consistent through PCS scales, the strength of the association was modest. The 0.33-units higher PCS score observed in moderate alcohol drinkers was comparable to women with a 1.7 unit lower BMI or 3.5 years younger age in our sample.

We observed no association between alcohol consumption and MCS. This has been investigated by two other (cross-sectional) studies. Valencia-Martin et al. (2013) did not find an association in male and female adults, whereas Chan et al. (2009) reported higher MCS scores in moderate drinkers in men only. However, several studies reported a positive association between moderate alcohol consumption and scales of MCS, such as mental health (1, 15), vitality (1, 2), and social functioning (1, 15). This parallels the higher vitality and social functioning scores in moderate drinkers observed in the present study.

Overall, the likelihood of having a good HRQOL in both physical and mental domains was highest among moderate drinkers. A similar positive association between moderate alcohol consumption and quality of life (3, 14), subjective wellbeing (35), subjective health (7) and life satisfaction (3) has been reported. Since for SF-36 there is no measure available in which PCS and MCS sores are integrated, we created this overall score to explore the association between alcohol and overall HRQOL. However, this overall score has limitations, because PCS and MCS are designed to be uncorrelated.

To our knowledge, this is the first study showing a positive association between PCS per se and subsequent alcohol consumption. However, previous studies have reported an association between subjective health status and alcohol use. Specifically, suboptimal health has been related to quitting or reducing alcohol consumption (17, 36). Additionally, abstainers have

more characteristics related to a poor health status compared to light or moderate drinkers (37). However, after excluding abstainers and former drinkers from the analysis, the positive relationship between PCS and subsequent alcohol consumption persisted.

MCS was neither associated with subsequent alcohol consumption nor the likelihood of drinking alcohol. This finding contrasts with the study of Bell & Britton (2014), which showed an inverse association between MCS and change in alcohol consumption in 6,330 mainly male British civil servants. Participants with good mental health appeared to reduce their alcohol consumption, whereas people with poor mental health increased their alcohol consumption or maintained high alcohol consumption. We cannot directly compare our results to theirs, as most women (~70%) did not change their alcohol consumption between measures conducted 4 years apart.

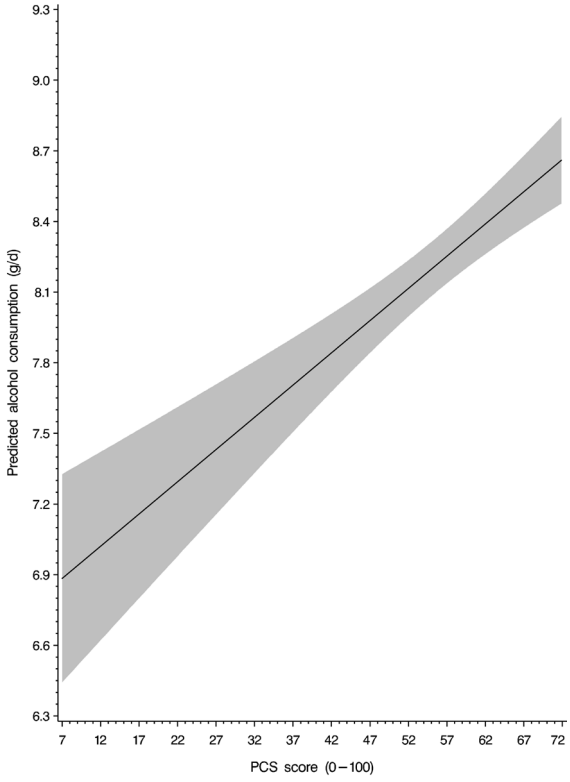


Figure 9.2. Linear dose-response relationship between physical component summary (PCS) scores and subsequent alcohol consumption.

Data derive from a restricted cubic spline GEE model (lifestyle-adjusted model) in alcohol consumers. The model is adjusted for previous alcohol consumption, age, physical activity, energy intake, body mass index, smoking, marital status, employment status, night shift work, race, region, living arrangement, parity. PCS scores are winsorized on the 99.5 percentile. The 95% confidence interval (CI) is indicated by the gray cloud. $P<0.001$ for test for linear relationship.

Limitations

Some limitations of our study warrant consideration. First, alcohol intake was self-reported by a semi-quantitative FFQ, which generally causes underreporting of alcohol intake, especially at higher levels of alcohol (38). However, a validation study in nurses showed a high correlation between alcohol consumption from the FFQ and four one-week dietary recalls and HDL-C, a strong biomarker of alcohol intake (22).

Second, the time lag of 2 years between alcohol consumption and HRQOL measurements may have introduced misclassification bias, since changes in alcohol intake or HRQOL could have occurred during that period. However, alcohol intake and HRQOL both tended to be stable over 4 years, as most women did not change their alcohol intake and average changes in PCS and MCS were small. Therefore, we do not expect this to have a large influence on our results.

Third, the distribution of the PCS and MCS scales was not as widespread as the distribution of the US general population. In particular, the standard deviations were somewhat smaller (SD~9 vs. SD of 10). However, average PCS and MCS scores were comparable to US population data for women of 35-44 years (51.9 and 49.1 in our sample vs. 51.7 and 47.8 in the US population, respectively), indicating that the women in the cohort had HRQOL that was comparable to other American women of their age (39). We excluded women who were pregnant in a given cycle from that time period, but the fact that pregnant women could re-enter the cohort in the next cycle was an important advantage of our longitudinal design.

Fourth, although we controlled for a large number of health behavior factors and socio-demographic factors, residual confounding remains possible. Particularly, limited information was available on socioeconomic status, sleep quality, and disorders such as depression and anxiety. However, related covariates such as antidepressant and anxiolytic medication use, and socio-demographic factors (race, region, marital status, employment status and night shift work) were examined in the model. In addition, our study populations primarily consisted of white educated US women with higher and more homogeneous socioeconomic status. Therefore, we expect that the influence of residual confounding on our results will be limited. However, this may potentially limit generalizability to other ethnic groups and socioeconomic groups (as well as males).

Finally, a limited number of participants were heavy alcohol consumers (>40 g/d). Therefore, we could not examine the association between heavy drinking and HRQOL and results are not generalizable to heavy alcohol consumers.

Conclusion

In summary, this study supports the presence of a bidirectional relation between alcohol consumption and health-related quality of life. Among young and middle-aged women, greater alcohol intake (up to ~1 serving daily) was associated with a small improvement of physical health-related quality of life two years later, and vice versa. No significant relation

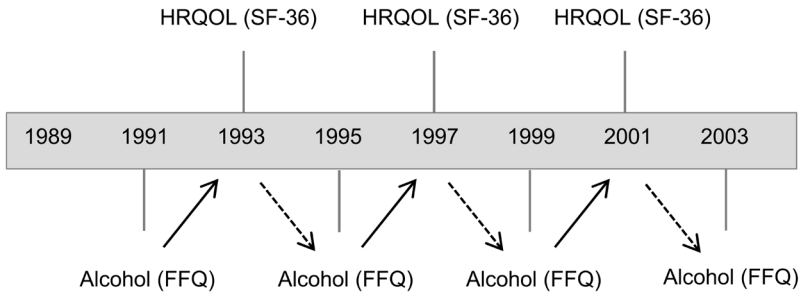
was observed between moderate alcohol consumption and mental health-related quality of life in either direction, although drinking ≥20 g/d was associated with lower mental health-related quality of life.

These results indicate the importance of exploring bidirectional associations in studies concerning alcohol consumption. Additionally, the current American guidelines on alcohol consumption are to consume alcohol in moderation (up to 1 drink per day for women) if alcohol is consumed (40). Our results are in agreement with these guidelines: moderate alcohol consumption may be beneficial for physical health-related quality of life, whereas drinking more than 1 drink per day may be harmful for mental health-related quality of life.

Funding

This work was supported by the Nurses' Health Study II grant CA50385 from the National Institutes of Health. ICS and HFJH were supported both by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO (Grant EZ1503). Their joint aim is to independently study the health effects of moderate alcohol consumption. The funding sources had no role in conducting, analyzing, or interpreting study results or in the decision to submit the manuscript for publication.

Supplemental data



Supplemental Figure 9.1. Schematic overview of the study design.
Solid arrows indicate the relationship between alcohol consumption and health-related quality of life 2 years later; Dashed arrows indicate the relationship between health-related quality of life and alcohol consumption 2 years later. HRQOL, health-related quality of life; SF-36, the 36-Item Short Form Health Survey; FFQ, food frequency questionnaire.

Supplemental Table 9.1. Characteristics of the study population at 1991 by alcohol consumption categories.

	Alcohol consumption category					
	Abstainer	Former drinker	Infrequent drinker (reference)	Light drinker	Moderate drinker	Moderate/ heavy drinker
Alcohol consumption, g/d, range	0.0	0.0	0.1 - 1.24	1.25 - 4.9	5.0 - 19.9	≥20
Alcohol consumption, g/d, median	0.0	0.0	0.9	2.7	8.8	29.8
No. of participants	14258	10353	7621	14589	9824	1490
Age, years	38.4 (3.4)	36.5 (4.9)	37.3 (4.3)	37.1 (4.4)	37.2 (4.5)	38.2 (4.1)
Race, white, %	94.7	95.8	97.6	97.8	98.0	98.5
Region						
- Northeast, %	29.9	33.2	34.4	37.1	36.1	34.2
- Midwest, %	37.0	35.8	35.2	32.7	28.4	23.4
- South, %	19.4	16.2	15.5	14.8	15.8	19.0
- West, %	13.0	14.2	14.3	14.9	18.8	22.4
- Non-U.S., %	0.7	0.7	0.6	0.5	0.8	1.0
Unemployed, %	10.2	7.7	6.9	6.6	6.8	6.1
Working rotating night shifts, %	22.0	23.6	22.3	21.9	20.6	18.7
Currently married, %	81.1	75.0	75.8	73.2	70.0	67.6
Parity (0 vs. ≥1 children), %	79.6	73.8	73.5	69.8	63.4	58.8
Physical activity, MET-h/week[a]	17.9 (24.4)	19.4 (25.7)	20.4 (26.2)	22.1 (27.8)	24.3 (30.1)	23.2 (28.2)
Smoking status						
- Never, %	77.8	66.1	66.0	61.0	50.7	35.4
- Past, %	14.1	20.8	21.0	26.1	32.1	33.8
- Current, %	8.1	13.1	13.0	12.9	17.2	30.8
Body mass index, kg/m²	25.8 (6.2)	25.6 (6.0)	24.9 (5.5)	24.3 (5.1)	23.4 (4.1)	23.7 (4.2)
Energy intake, kcal/d	1736 (545)	1726 (543)	1711 (525)	1772 (535)	1815 (533)	1925 (546)
AHEI-2010 score (0-100)[b]	43.3 (10.7)	43.6 (10.8)	44.3 (10.4)	45.2 (10.3)	45.8 (10.0)	44.3 (10.1)
Oral contraceptive use, %	79.2	84.0	85.3	86.4	88.8	88.3
Postmenopausal status, %	5.2	4.9	4.7	4.0	3.3	3.9
Self-reported conditions						
- Hypertension, %	5.0	5.0	4.2	3.3	2.9	5.2
- High cholesterol, %	11.3	12.1	11.1	10.1	8.8	10.2
- Osteoarthritis, %	5.4	5.3	5.0	4.2	3.9	4.7
- Rheumatoid arthritis, %	1.1	1.2	1.0	1.0	0.8	0.2
- Diabetes, %	1.4	1.0	0.7	0.6	0.2	0.3
- Asthma, %	8.2	7.9	7.9	7.4	6.9	8.2
Regular analgesic use[c], %	41.9	45.8	45.2	45.1	46.0	50.4

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

[a] Met-h indicates metabolic equivalent hours; [b] AHEI, Alternative Healthy Eating Index without alcohol consumption;

[c] using analgesics at least twice a week.

Supplemental Table 9.2. Characteristics of the study population at 1993 by physical component summary (PCS) scores.

	Quintiles of physical component summary scores				
	Q1	Q2	Q3	Q4	Q5
MCS, range (median)	8-46 (41)	47-52 (50)	53-55 (54)	56-58 (57)	59-75 (60)
No. of participants	9301	9864	10145	13153	10711
Age, years	40.1 (4.1)	39.8 (4.1)	39.6 (4.2)	39.4 (4.2)	39.2 (4.3)
Race, white, %	96.7	96.7	96.8	96.9	97.0
Region					
- Northeast, %	32.5	33.0	33.5	34.9	35.5
- Midwest, %	34.6	35.1	35.1	33.1	31.8
- South, %	17.0	16.8	16.4	16.1	15.5
- West, %	15.2	14.4	14.3	15.2	16.7
- Non-U.S., %	0.7	0.7	0.7	0.7	0.6
Unemployed, %	10.1	5.5	5.2	6.0	6.1
Working rotating night shifts, %	14.2	14.3	13.0	11.9	12.5
Currently married, %	73.1	76.3	77.7	77.4	73.0
Parity (0 vs. ≥1 children), %	71.3	73.7	73.7	74.4	73.4
Living alone, %	12.1	10.2	10.5	10.7	13.1
Physical activity, MET-h/week[a]	17.5 (24.8)	18.1 (23.4)	19.2 (24.4)	23.0 (29.0)	24.3 (30.8)
Smoking status, %					
- Never, %	62.3	62.8	65.2	66.1	64.3
- Past, %	24.0	23.8	23.4	23.6	25.5
- Current, %	13.7	13.4	11.4	10.3	10.2
Body mass index, kg/m²	28.3 (7.6)	26.8 (6.3)	25.5 (5.5)	24.2 (4.5)	23.6 (4.0)
Energy intake, kcal/d	1794 (533)	1777 (519)	1760 (515)	1728 (507)	1710 (503)
AHEI-2010 score (0-100)[b]	43.4 (10.3)	43.6 (10.1)	44.2 (10.2)	45.1 (10.3)	45.5 (10.3)
Oral contraceptive use, %	85.6	85.2	85.3	84.4	85.2
Postmenopausal status, %	10.0	7.3	6.2	5.3	4.9
Self-reported conditions					
- Hypertension, %	12.6	8.3	6.0	4.0	2.8
- High cholesterol, %	23.5	18.8	15.5	11.9	11.4
- Osteoarthritis, %	20.7	10.7	6.0	3.3	2.3
- Rheumatoid arthritis, %	4.4	1.9	0.9	0.5	0.4
- Diabetes, %	3.0	1.7	1.0	0.5	0.3
- Asthma, %	16.7	10.9	8.3	6.9	6.0
Antidepressants use, %	27.1	14.8	10.5	8.3	11.6
Regular analgesic use[c], %	61.6	47.1	33.6	24.1	20.1

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

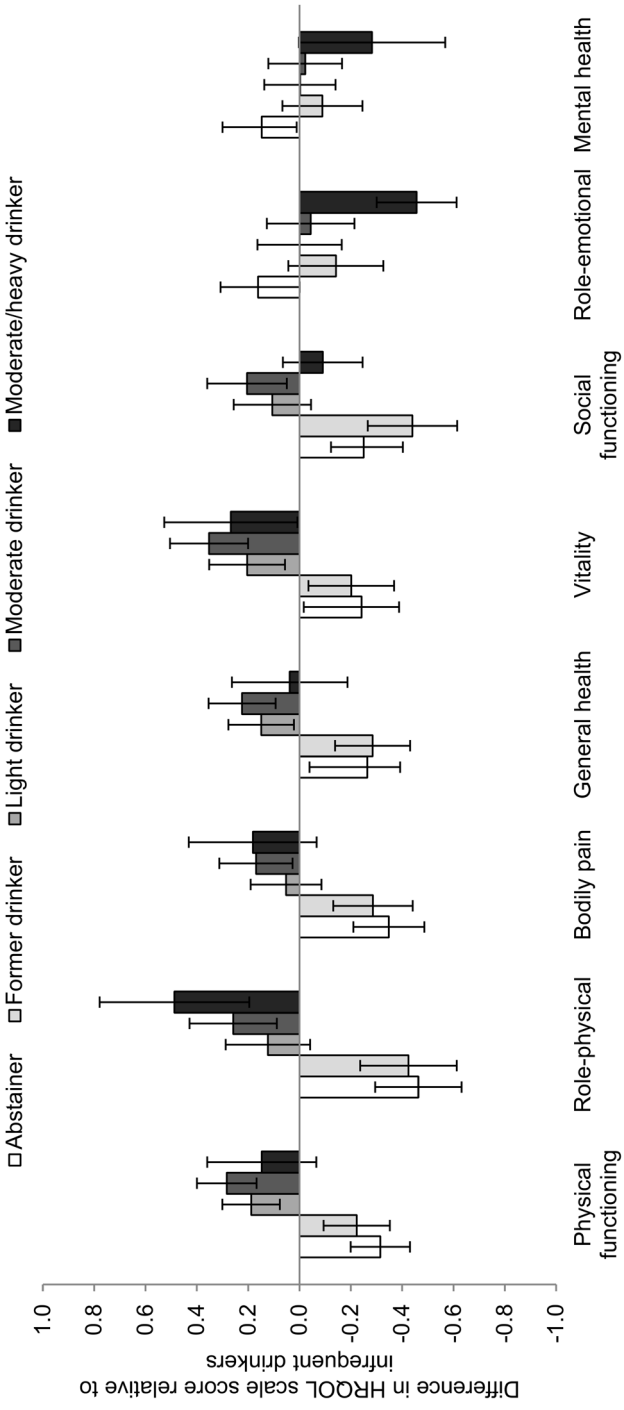
[a] Met-h indicates metabolic equivalent hours; [b] AHEI, Alternative Healthy Eating Index without alcohol consumption; [c] using analgesics at least twice a week.

Supplemental Table 9.3. Characteristics of the study population at 1993 by mental component summary (MCS) scores.

	Quintiles of mental component summary scores				
	Q1	Q2	Q3	Q4	Q5
MCS, range (median)	0-42 (35)	43-49 (47)	50-53 (52)	54-56 (55)	57-71 (58)
No. of participants	12913	11337	11368	9782	7774
Age, years	39.4 (4.2)	39.5 (4.2)	39.5 (4.2)	39.7 (4.2)	40.1 (4.1)
Race, white, %	96.7	97.0	96.9	96.9	96.8
Region					
- Northeast, %	33.0	34.3	34.9	34.1	33.8
- Midwest, %	33.5	33.6	34.3	34.3	33.3
- South, %	17.0	16.0	15.7	16.3	16.7
- West, %	15.8	15.4	14.4	14.5	15.5
- Non-U.S., %	0.6	0.7	0.7	0.8	0.6
Unemployed, %	6.5	5.6	6.0	6.6	8.2
Working rotating night shifts, %	15.0	13.6	12.2	12.0	11.5
Currently married, %	69.8	75.5	78.8	78.3	77.3
Parity (0 vs. ≥1 children), %	72.7	74.1	74.7	73.9	70.7
Living alone, %	14.0	11.4	9.7	10.2	10.7
Physical activity, MET-h/week[a]	19.0 (25.5)	19.4 (25.4)	20.4 (26.9)	21.6 (27.4)	24.4 (31.1)
Smoking status, %					
- Never, %	61.0	63.4	65.4	66.4	66.8
- Past, %	23.7	24.4	24.2	23.9	24.1
- Current, %	15.3	12.2	10.4	9.8	9.1
Body mass index, kg/m²	26.0 (6.2)	25.6 (5.8)	25.4 (5.7)	25.1 (5.6)	25.5 (6.0)
Energy intake, kcal/d	1772 (527)	1757 (515)	1747 (512)	1731 (508)	1742 (510)
AHEI-2010 score (0-100)[b]	43.8 (10.2)	44.1 (10.2)	44.2 (10.2)	45.0 (10.3)	45.7 (10.3)
Oral contraceptive use, %	86.2	85.3	85.0	84.2	84.1
Postmenopausal status, %	7.5	6.3	6.5	5.8	6.7
Self-reported conditions					
- Hypertension, %	8.3	6.5	6.1	5.0	6.1
- High cholesterol, %	18.7	16.8	14.7	13.8	13.7
- Osteoarthritis, %	9.9	8.2	7.2	6.3	8.8
- Rheumatoid arthritis, %	1.6	1.5	1.3	1.4	1.6
- Diabetes, %	1.4	1.2	1.1	1.2	1.2
- Asthma, %	11.0	9.6	8.7	8.4	8.9
Antidepressants use, %	25.0	14.1	9.9	8.3	8.0
Regular analgesic use[c], %	41.6	38.0	33.7	30.9	33.4

Values are means (SD) or percentages and are standardized to the age distribution of the study population.

[a] Met-h indicates metabolic equivalent hours; [b] AHEI, Alternative Healthy Eating Index without alcohol consumption; [c] using analgesics at least twice a week.



Supplemental Figure 9.2. Association between alcohol consumption and scales of health-related quality of life.

Data derive from generalized linear models and represent the difference in HRQOL scale score (95% CI) relative to the reference category infrequent drinkers. Data are standardized to data from the US general population (with mean=50 and SD=10). The model is adjusted for previous outcome, time, age, physical activity, energy intake, body mass index, smoking, marital status, employment status, shift working, race, region, living alone, parity, arthritis (osteoarthritis and rheumatoid arthritis), diabetes mellitus, hypertension, hypercholesterolemia, asthma, premenstrual syndrome, antidepressant use, anxiolytic use, analgesic use, oral contraceptive use, and menopausal status.

References

1. Van Dijk AP, Toet J, Verdurmen J. The relationship between health-related quality of life and two measures of alcohol consumption. Journal of Studies on Alcohol and Drugs. 2004;65(2):241.
2. Saito I, Okamura T, Fukuhara S, Tanaka T, Suzukamo Y, Okayama A, Ueshima H. A cross-sectional study of alcohol drinking and health-related quality of life among male workers in japan. Journal of occupational health. 2005;47(6):496-503.
3. Chan AM, von Mühlen D, Kritz-Silverstein D, Barrett-Connor E. Regular alcohol consumption is associated with increasing quality of life and mood in older men and women: The rancho bernardo study. Maturitas. 2009;62(3):294-300.
4. Saarni SI, Joutsenniemi K, Koskinen S, Suvisaari J, Pirkola S, Sintonen H, Poikolainen K, Lonnqvist J. Alcohol consumption, abstaining, health utility, and quality of life--a general population survey in finland. Alcohol Alcohol. 2008;43(3):376-86.
5. Valencia-Martín JL, Galan I, Guallar-Castillón P, Rodriguez-Artalejo F. Alcohol drinking patterns and health-related quality of life reported in the spanish adult population. Prev Med. 2013;57(5):703-7.
6. Stranges S, Notaro J, Freudenheim JL, Calogero RM, Muti P, Farinaro E, Russell M, Nochajski TH, Trevisan M. Alcohol drinking pattern and subjective health in a population-based study. Addiction. 2006;101(9):1265-76.
7. Poikolainen K, Vartiainen E, Korhonen HJ. Alcohol intake and subjective health. Am J Epidemiol. 1996;144(4):346-50.
8. Okoro CA, Brewer RD, Naimi TS, Moriarty DG, Giles WH, Mokdad AH. Binge drinking and health-related quality of life: Do popular perceptions match reality? Am J Prev Med. 2004;26(3):230-3.
9. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, Rehm J. Alcohol as a risk factor for type 2 diabetes: A systematic review and meta-analysis. Diabetes Care. 2009;32(11):2123-32.
10. Gea A, Martinez-Gonzalez MA, Toledo E, Sanchez-Villegas A, Bes-Rastrollo M, Nunez-Cordoba JM, Sayon-Orea C, Beunza JJ. A longitudinal assessment of alcohol intake and incident depression: The SUN project. BMC Public Health. 2012;12:954,2458-12-954.
11. Jin Z, Xiang C, Cai Q, Wei X, He J. Alcohol consumption as a preventive factor for developing rheumatoid arthritis: A dose-response meta-analysis of prospective studies. Ann Rheum Dis. 2014;73(11):1962-7.
12. Peele S, Brodsky A. Exploring psychological benefits associated with moderate alcohol use: A necessary corrective to assessments of drinking outcomes? Drug Alcohol Depend. 2000;60(3):221-47.
13. Graham K. Alcohol abstention among older adults: Reasons for abstaining and characteristics of abstainers. Addiction Research & Theory. 1998;6(6):473-87.
14. Kaplan MS, Huguet N, Feeny D, McFarland BH, Caetano R, Bernier J, Giesbrecht N, Oliver L, Ross N. Alcohol use patterns and trajectories of health-related quality of life in middle-aged and older adults: A 14-year population-based study. J Stud Alcohol Drugs. 2012;73(4):581-90.
15. Byles J, Young A, Furuya H, Parkinson L. A drink to healthy aging: The association between older women's use of alcohol and their health-related quality of life. J Am Geriatr Soc. 2006;54(9):1341-7.
16. Bell S, Britton A. An exploration of the dynamic longitudinal relationship between mental health and alcohol consumption: A prospective cohort study. BMC medicine. 2014;12(1):91.
17. Liang W, Chikritzhs T. Reduction in alcohol consumption and health status. Addiction. 2011;106(1):75-81.

18. Nortvedt MW, Riise T, Myhr KM, Nyland HI. Quality of life in multiple sclerosis: Measuring the disease effects more broadly. Neurology. 1999;53(5):1098-103.

19. Trentham-Dietz A, Sprague BL, Klein R, Klein BE, Cruickshanks KJ, Fryback DG, Hampton JM. Health-related quality of life before and after a breast cancer diagnosis. Breast Cancer Res Treat. 2008;109(2):379-87.

20. Otchet F, Carey MS, Adam L. General health and psychological symptom status in pregnancy and the puerperium: What is normal? Obstetrics & Gynecology. 1999;94(6):935-41.

21. U.S. Department of Agriculture ARS. USDA Nutrient Database for Standard Reference. Washington, DC: U.S. Government Printing Office; 1999.

22. Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L, Willett WC. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol. 1991;133(8):810-7.

23. Ware Jr JE, Kosinski M, Bayliss MS, McHorney CA, Rogers WH, Raczek A. Comparison of methods for the scoring and statistical analysis of SF-36 health profile and summary measures: Summary of results from the medical outcomes study. Med Care. 1995:AS264-79.

24. Ware, J, Kosinski, M, Keller, S. SF-36 Physical and Mental Health Summary Scales: A User's Manual. Boston, MA: The Health Institute; 1994.

25. Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L. Validating the SF-36 health survey questionnaire: New outcome measure for primary care. BMJ. 1992;305(6846):160-4.

26. McHorney CA, Ware Jr JE, Rogers W, Raczek AE, Lu JR. The validity and relative precision of MOS short-and long-form health status scales and dartmouth COOP charts: Results from the medical outcomes study. Med Care. 1992:MS253-65.

27. Ware Jr JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36): I. conceptual framework and item selection. Med Care. 1992:473-83.

28. Duncan MJ, Kline CE, Vandelanotte C, Sargent C, Rogers NL, Di Milia L. Cross-sectional associations between multiple lifestyle behaviors and health-related quality of life in the 10,000 steps cohort. PloS one. 2014;9(4):e94184.

29. Achat H, Kawachi I, Levine S, Berkey C, Coakley E, Colditz G. Social networks, stress and health-related quality of life. Quality of life research. 1998;7(8):735-50.

30. Kaliterna LL, Prizmic LZ, Zganec N. Quality of life, life satisfaction and happiness in shift-and non-shiftworkers. Revista de Saúde Pública. 2004;38:3-10.

31. Chiuve SE, Fung TT, Rimm EB, Hu FB, McCullough ML, Wang M, Stampfer MJ, Willett WC. Alternative dietary indices both strongly predict risk of chronic disease. J Nutr. 2012;142(6):1009-18.

32. Colditz GA, Giovannucci E, Rimm EB, Stampfer MJ, Rosner B, Speizer FE, Gordis E, Willett WC. Alcohol intake in relation to diet and obesity in women and men. Am J Clin Nutr. 1991;54(1):49-55.

33. Joosten MM, Grobbee DE, van der ADL, Verschuren WM, Hendriks HF, Beulens JW. Combined effect of alcohol consumption and lifestyle behaviors on risk of type 2 diabetes. Am J Clin Nutr. 2010;91(6):1777-83.

34. Durrleman S, Simon R. Flexible regression models with cubic splines. Stat Med. 1989;8(5):551-61.

35. Lang I, Wallace RB, Huppert FA, Melzer D. Moderate alcohol consumption in older adults is associated with better cognition and well-being than abstinence. Age Ageing. 2007;36(3):256-61.

36. Powers JR, Young AF. Longitudinal analysis of alcohol consumption and health of middle-aged women in australia. Addiction. 2008;103(3):424-32.

37. Hansel B, Thomas F, Pannier B, Bean K, Kontush A, Chapman M, Guize L, Bruckert E. Relationship between alcohol intake, health and social status and cardiovascular risk factors in the urban paris-ile-de-france cohort: Is the cardioprotective action of alcohol a myth? Eur J Clin Nutr. 2010;64(6):561-8.

38. Feunekes GI, van 't Veer P, van Staveren WA, Kok FJ. Alcohol intake assessment: The sober facts. Am J Epidemiol. 1999;150(1):105-12.

39. Ware, J, Kosinski, M, Bjorner, J, Turner-Bowker, D, Gandek, B, Maruish, M. User's Manual for the SF-36v2® Health Survey. QualityMetric Incorporated, Lincoln; 2007.

40. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary guidelines for Americans. 7th ed. Washington D.C.: available from: http://www.health.gov/dietaryguidelines/2010.asp; 2010

Chapter 10

General discussion

The research presented in this thesis focused on the acute and long-term effects of moderate alcohol consumption on emotional well-being, and on its possible connections with physiological determinants and health outcomes. This final chapter starts with an overview of the main findings, followed by a section on methodological considerations, a discussion of the findings and a comparison with other studies. Finally, implications and suggestions for future research are given.

Main findings

The main findings of the studies conducted and described in this thesis are summarized and presented in Table 10.1.

Acute effects on emotional well-being

First, it was shown that moderate alcohol consumption may acutely lead to an improvement of emotional well-being shortly after consumption (**chapter 2-5**). Happiness scores increased shortly after alcohol consumption, but only in those situations in which an unpleasant mood state had been created in an artificial way. The mood effects of alcohol consumed with a meal were found to follow a biphasic pattern. Sympathetic nervous system activity increased in parallel to the stimulating effects of alcohol (**chapter 2**). Circulating endocannabinoids were not influenced by alcohol consumption, and showed an inconsistent correlation with mood states. However, they were highly correlated with plasma free fatty acids and cortisol levels (**chapter 3**).

Furthermore, we showed that moderate alcohol consumption increased subsequent indicators for reward of savoury foods. This suggests that the effects of alcohol on the reward system may lead to an increased rewarding value of certain foods.

Both in a resting and a stressed state, moderate alcohol consumption caused a further decline in plasma cortisol as compared to situations in which no alcohol was consumed. Moderate drinking also resulted in a more pronounced decline of plasma ACTH, the inflammatory marker IL-8 and percentage monocytes in blood during stress recovery, suggesting that moderate alcohol consumption shorty after a stressor improves stress recovery and may also modulate a stress-induced inflammatory response.

Short-term influence on physical well-being

We showed that moderate alcohol consumption may positively influence acute and short-term physical determinants related to emotional well-being, apparently in a gender-specific manner (**chapter 6-8**). Moderate alcohol consumption acutely attenuated meal-induced oxidative stress (**chapter 6**). Furthermore, short-term moderate alcohol intake reduced fetuin-A levels in men but not in women (**chapter 7**). Our meta-analysis showed that only in women, short-term moderate alcohol consumption tended to improve insulin sensitivity and reduced fasting

insulin levels (**chapter 8**). This suggests that alcohol consumption may influence different pathways and intermediates in men and in women leading to a reduced type 2 diabetes risk.

Long-term association with emotional and physical well-being
In **chapter 9** we showed that moderate alcohol consumption was associated with a better physical health-related quality of life, but not with a better mental health-related quality of life.

Methodological considerations

In this section we consider some methodological issues that are important to take into account before the results are being discussed and interpreted. Acute intervention studies, short-term intervention studies and longitudinal observational studies will be discussed separately.

Acute intervention studies

Study design
All acute intervention studies described in this thesis used a randomized crossover design, allowing within-subject comparisons. A possible issue in crossover studies, in which subjects receive a stress intervention or perform a task twice, is that a learning effect may occur, which may interfere with the treatment. This was also observed in our study; the second time subjects conducted a mental stress test, the test did not induce as much stress as it did the first time, because subjects had learned to adapt to the type of stressor applied (1-3). Therefore, we decided to use data from the first treatment day only, and analysed the data as if it was a parallel study. We have checked the influence of a learning effect in our other studies by adding 'period' as a factor in the statistical models and found that learning effects did not occur.

Blinding and expectation effects
We used different alcoholic beverages, varying from wine to beer to spirits. All intervention studies were placebo-controlled, using the most comparable control study substance available in order to study pure alcohol effects and to blind the subjects to the alcohol condition. This is important, since expectancy effects may induce part of alcohol's behavioural effects (4-10). The magnitude of the expectancy effects, however, is varying largely between individuals, outcomes and experimental settings (7). For example, drinking in a group induces more pronounced expectancy effects as compared to drinking alone (9). Expectancy effects seem to be large for outcomes like aggression, sexual arousal and stress reduction (5, 7). In our studies on mood and food reward, the subjects were blinded for the alcohol treatment. However, blinding might not have been complete due to the physiological and behavioural effects of alcohol. Indeed, most participants guessed correctly whether they consumed alcohol

Table 10.1. Summary of the main findings of the studies described in this thesis.

Acute intervention studies on alcohol and emotional well-being				
Chapter	**Population**	**Intervention**	**Outcome**	**Results and Conclusions**
2	28 young women	Sparkling white wine (30 g alcohol) or alcohol-free white wine with dinner in a pleasant or unpleasant ambiance	Mood	During ascending BACs, alcohol increased stimulation, reduced calmness and increased happiness (latter only in unpleasant ambiance). During descending BACs, alcohol increased sedation and sleepiness.
			Autonomic nervous system activity	Alcohol increased SNS activity during ascending BACs, and decreased PNS activity during both ascending and descending BACs.
3	28 young women	Sparkling white wine (30 g alcohol) or alcohol-free white wine with dinner in a pleasant or unpleasant ambiance	Plasma endocannabinoids (ECs) and related NAEs	Alcohol had no influence on plasma ECs or related NAEs. The NAEs PEA and SEA were acutely increased in the unpleasant ambiance, but decreased in the pleasant ambiance.
			Correlations with mood, free fatty acids and cortisol	ECs and NAEs were correlated with free fatty acids and cortisol in general, and correlated with mood states only in the pleasant ambiance without alcohol. Alcohol caused a stronger decline in cortisol after the meal.
4	24 young and middle-aged men	Vodka/orange juice (20 g alcohol) or orange juice	Food intake and food reward	Alcohol increased intake of an ad libitum lunch, specifically of high-fat savoury foods. This effect was related to the higher explicit liking of high-fat savoury foods and higher implicit wanting of savoury foods.
5	24 young men	Beer (26 g alcohol) or alcohol-free beer after a mental stressor	Stress hormones and immune response	Alcohol caused a stronger decline in cortisol and ACTH after stress, but had no influence on heart rate. Alcohol reduced IL-8 and monocytes after stress.

Short-term intervention studies on alcohol and physical well-being

Chapter	Population	Intervention	Outcome	Results and Conclusions
6	19 men with increased waist circumference	Red wine (41 g alcohol) or dealcoholized red wine daily with dinner for 4 weeks	Acute: oxidative capacity, NF-κB	Alcohol acutely increased oxidative capacity and suppressed NF-κB induced by a meal.
			4 weeks: F2-isoprostanes	Alcohol increased oxidative lipid damage marker F2-isoprostanes after 4 weeks
7	36 post-menopausal women	White wine (25 g alcohol) or white grape juice daily with dinner for 6 weeks	Fetuin-A	Alcohol decreased fetuin-A in the trial performed in men, but not in the trials performed in women. Changes in fetuin-A and were not correlated with HOMA-IR and adiponectin.
	24 young women	Beer (26 g alcohol) or alcohol-free beer daily with dinner for 3 weeks		
	24 young men	Vodka/orange juice (30 g alcohol) or orange juice daily with dinner for 4 weeks		
8	381 men and women from 14 intervention studies	≥ 2 weeks (moderate) alcohol consumption	insulin sensitivity and glycemic status	Alcohol tended to improve insulin sensitivity in women, but not in men. Alcohol decreased fasting insulin and HbA1c concentrations in both men and women.

Long-term study on alcohol and emotional and physical well-being

Chapter	Population	Exposure	Outcome	Results and Conclusions
9	88,363 young and middle-aged women	Habitual alcohol consumption	2 years later mental and physical HRQOL	Habitual moderate alcohol consumption (up to 1 drink daily) was associated with a small improvement in subsequent physical HRQOL, but not in mental HRQOL.
	84,621 young and middle-aged women	Mental and physical HRQOL	2 years later habitual alcohol consumption	Better physical HRQOL was associated with higher habitual alcohol consumption 2 years later. Mental HRQOL was not associated with subsequent habitual alcohol consumption.

or not. Although subjects were drinking alone, which may have reduced expectancy effects, a certain influence of expectancy in our results should be taken into account during interpretation of the results.

Dose and blood alcohol concentration

The amount of alcohol used in the acute intervention trials varied between 30 gram in women and 20 to 26 gram in men. These dosages were chosen to induce a specific blood alcohol concentration (BAC), taking into account gender differences and influence of food intake. In the study in women, alcohol was served with a meal, which reduces the BAC. Here, average peak BAC was 0.53‰ w/v (11.5 mmol/L). In the study in men, average peak BAC was almost similar, 0.52‰ w/v, although less alcohol was consumed (20 g alcohol). A BAC of 0.5‰ w/w corresponds with the Dutch legal limit for drinking and driving for experienced drivers (0.2‰ for inexperienced drivers). In addition, we chose these BACs because of the behavioural effects we expected, i.e. stimulant-like effects (euphoria, arousal) and tension-reduction effects (11, 12).

Study population

The subjects that participated in the studies described in this thesis were all healthy adults. They were all non-smokers, habitual moderate alcohol consumers, without a (family) history of alcoholism. In one study female participants were included, whereas the other two studies used men. Since men and women have different emotional and physiological responses on food intake and on stress (13-17), the findings on mood in women cannot be directly generalized to men, and the findings on food reward and stress recovery cannot be directly generalized to women. Nevertheless, gender did not influence the mood effects of alcohol in another study (11). Therefore, it seems unlikely that the findings on mood are limited to women.

Outcome measures

We used both subjective and objective measures to study the influence of alcohol on emotional well-being in our interventions. As subjective measures we used mood questionnaires and appetite, food liking and food wanting ratings (visual analogue scales). Subjective measures are generally seen as the gold standard for evaluating behavioural outcomes like mood and food reward. Nevertheless, it should be noted that with subjective measures it is not the behaviour itself that is measured, but the person's perception of his or her behaviour (18). Furthermore, large inter-individual differences in behavioural effects of alcohol are known (11, 19). Therefore, it is important to compare within-subject measurements.

In the study on mental stress where we did not compare outcomes within subjects, we did not include subjective measures but focused on the physiological outcomes. This prevented us from comparing physiological outcomes directly with subjective feelings of tension and

anxiety.
However, in the other trials we combined subjective and objective to explore physiological mechanisms. As objective measures for mood we used indicators of autonomic nervous system activity (skin conductance, heart rate variability). The autonomic nervous system is thought to play a role in mood regulation. Its activity has been related to the arousal dimension of mood, such that sympathetic nervous system activity correlates with high arousal mood states. This indicates that physiological markers, such as indicators of autonomic nervous system activity, may provide insight in the underlying mechanism of effects of alcohol on well-being.

Short-term intervention studies

Study design
All short-term intervention studies described in this thesis (including the intervention studies in the meta-analysis) used a randomized controlled design. Except for one study which had a parallel design in the meta-analysis, all studies had a crossover design. In short-term trials, a disadvantage of the crossover design is the risk of carry-over effects. We adjusted for these effects by adding treatment order to the model. We did not observe an effect by treatment order, and therefore we concluded that the results were not influenced by carry-over effects. However, most of the studies included in the meta-analysis did not indicate if the results were influenced by carry-over effects. Therefore, the findings from the meta-analysis on insulin sensitivity may have been influenced by carry-over effects if effects of alcohol consumption in one treatment period were still present in the alcohol-free treatment period and interfered with the effects from the alcohol-free control in this period. This would have resulted in attenuation of the alcohol effect.

Study population
Participants in the studies consisted of both men and women. The influence of alcohol on oxidative stress was only tested in men. Our findings on oxidative stress are therefore not directly generalizable to women, although one other study by Hartman et al. (2005) observed also an increase in lipid oxidation in women (20). The influence of alcohol on fetuin-A and insulin sensitivity were measured in both men and women, and gender-specific effects were observed. The potential influence of age on alcohol's effect on oxidative stress, fetuin-A and insulin sensitivity needs further clarification.

Alcohol dosage and intervention duration
Alcohol doses used varied between the different studies in this thesis. Doses were in the range of 15 - 40 g alcohol daily. This would be equal to 1.5 - 4 Dutch units or around 1 - 3 U.S. units, which exceeds current recommendation for moderate consumption. Because the

intervention period was relatively short, compared to sustainable moderate drinking in life, we chose to use an alcohol amount in the upper range of moderate consumption (25 - 40 g alcohol per day). We also included studies using doses up to 40 g alcohol per day in the meta-analysis and categorized these as moderate alcohol consumption. Our intervention studies were conducted in experimental settings with the aim to explore the physiological determinants of emotional well-being, and they are therefore not meant to be translated directly into public health advices.

The duration of intervention was on average ~5 weeks for studies measuring insulin sensitivity in the meta-analysis. The intervention duration of other studies varied between 3 and 6 weeks. These durations may not have been long enough to result in detectable changes in insulin sensitivity in men, and of fetuin-A in women. However, we observed that the outcomes of the studies were generally not influenced by duration, suggesting that study duration has been sufficiently long.

Outcomes

The short-term trials described in this thesis included several different outcome measurements for oxidative stress, inflammation and insulin sensitivity.

Markers of oxidative stress were determined using previously validated assays. Although there are many markers available to measure oxidative stress, they all have their limitations (21). Therefore, we chose multiple measures of oxidative stress that reflect different aspects. We measured total Trolox equivalent antioxidant capacity (TEAC) because it could be related to the antioxidant capacity of the wine and dealcoholized wine used in the study and because changes in TEAC reflect acute effects. A limitation of TEAC is that outcomes cannot be interpreted quantitatively but only for comparison of antioxidant status between groups. Therefore, the TEAC observed in our study, cannot be quantitatively compared to findings in other studies. An advantage, however, is that it is a good measure of the total activity of all antioxidants in plasma, including non-S-H containing antioxidants and unknown antioxidants (22). Antioxidants act together, and therefore the measurement of total antioxidant capacity gives a better reflection of antioxidant balance and yields more valuable information as compared to measuring single antioxidants. F-2 isoprostanes are the most widely used biomarker of oxidative stress and are generally accepted as the best currently available biomarker of lipid oxidation. Changes in urinary F2-isoprostanes may reflect changes in lipid oxidation by chronic oxidative stress (23). Therefore, we used this biomarker to evaluate the effect of long-term alcohol consumption on lipid oxidation. However, F2-isoprostanes are oxidative products of arachidonic acid, and outcomes may thus be influenced by nutritional intervention studies affecting the arachidonic acid concentration in tissues.

The intervention studies included in the meta-analysis measured insulin sensitivity in different ways, with only two of them using the gold standard; the euglycemic hyperinsulinemic clamp technique. All other studies used indirect measures for insulin sensitivity, but these measures

gave similar outcomes as those using the gold standard. Therefore, overall findings are not expected to be influenced by the predominant use of indirect measures.

Long-term observational studies

Compared with acute and short-term intervention studies, findings from longitudinal observational studies are more appropriate to generalize to a larger population, as they are based on a more heterogeneous sample population and measuring the natural development of a trait in a real-life setting.

The observational study described in this thesis had several strengths, such as repeated measurements by follow-up and a large sample size. However, the study had, as with many longitudinal observational studies, also certain limitations. Since these are already discussed in detail in chapter 9, only some limitations that are specific for longitudinal studies in alcohol research on well-being will be addressed here.

Reference group

In our study we used infrequent drinkers as the reference group instead of abstainers. The abstaining group may be mixed with people who stopped drinking due to illness, former alcohol abuse or because of interaction with their medication. This concerns the 'sick quitters' hypothesis, which was first proposed by Shaper et al. (1988) (24). The inclusion of 'sick quitters' in the abstaining group may cause a higher incidence of disease or a lower quality of life among abstainers as compared to moderate alcohol consumers. In our study on quality of life we separated long-term abstainers from former drinkers to exclude potential sick-quitters from the abstainers. However, in many high-income countries, alcohol consumption is normative and long-term abstainers differ from current drinkers in socio-demographic status and other health determinants. Therefore, potential confounding is higher in studies using long-term or lifetime abstention and thus it appears to be a better approach to use light or infrequent drinkers as the reference group, as we did in our study.

Assessment of alcohol intake

Alcohol intake was assessed with a semi-quantitative food frequency questionnaire (FFQ). Self-reported alcohol intake has been shown to be generally underreported, especially at higher levels of alcohol consumption (25). Nevertheless, a validation study showed a high correlation between alcohol consumption from the FFQ and four one-week dietary recalls ($r=0.90$). Furthermore, self-reported alcohol intake by the FFQ was linearly associated with HDL-cholesterol concentrations ($r=0.40$), which is a strong biomarker of alcohol intake (26). This means that the FFQ for assessment of alcohol intake is a valid method for ranking subjects according to their intake rather than for estimation of absolute amounts.

External validity and interpretation of the findings

The aims of this thesis were 1) to further explore the acute effects of moderate alcohol consumption on emotional well-being and the association between habitual alcohol consumption and emotional well-being and 2) to provide more insight in the influence of moderate alcohol consumption on physiological determinants and health outcomes related to emotional well-being.

Emotional well-being

Beneficial effects of moderate alcohol consumption on acute emotional well-being have been shown previously (4, 12, 27-31). An overview of these effects is presented in chapter 1 (Figure 1.2). In accordance with previous studies (11, 29, 30, 32, 33) we observed a biphasic response in mood when alcohol was consumed with a meal, showing first stimulation effects, followed by sedative effects (chapter 2). Though, the onset of sedative effects occurred immediately after consumption instead of at the peak BAC. These delayed sedative effects may have been due to an increased tiredness induced by food intake. The tension reducing effects of alcohol are suggested to be dose-dependent (12). Our study may not have shown a reduction in tension because of the slightly higher dose (30 g alcohol) used, although we did observe a stronger decline in cortisol after alcohol consumption (chapter 3). Surprisingly, mood was only improved when alcohol was consumed in a created unpleasant ambiance in which subjects had a lower mood state. This suggests that moderate alcohol consumption may improve emotional well-being only when a person is not feeling happy.

Furthermore, we showed that moderate alcohol consumption increased subsequent food reward of savoury foods (chapter 4). With this study we showed that, in addition to the research available on the rewarding effects of alcohol itself, alcohol increases the rewarding value of intake of certain foods, thereby increasing the enjoyment of eating. An increased rewarding value of eating may contribute to the improvement of emotional well-being induced by alcohol.

In line with research showing the stress dampening effects of alcohol when consumed before a mental stressor, we observed an improved stress recovery when alcohol was consumed after the stressor. This implies that alcohol may attenuate stress, either when it is consumed before or after a stressor (chapter 5). Together, these results suggest that moderate alcohol consumption may have a buffering effect on emotional well-being, such that moderate alcohol consumption only acutely improves emotional well-being in case it is disturbed. Furthermore, emotional well-being may be further improved via alcohol's enhanced enjoyment of other rewarding behaviours.

A positive association between habitual moderate alcohol consumption and well-being has been suggested by cross-sectional studies. In our longitudinal observational study, moderate alcohol consumption was associated with better health-related quality of life in women

(chapter 9). However, this effect was only shown for physical health-related scales, vitality and social functioning, and not for the mental health and role emotional scales. The last two were actually scored lower in women drinking more than 20 gram alcohol (2 Dutch units) per day. Some studies did show a positive association between moderate alcohol consumption and mental health (both scale or summary score) (34-36). This difference may be explained by the use of other study populations, designs and reference categories in these studies. A good health-related quality of life contributes to well-being. Nevertheless, the positive effects of moderate alcohol consumption on short-term emotional well-being do not seem to influence long-term emotional well-being via an increased mental health-related quality of life in this young/middle-aged population. Whether other aspects of emotional well-being, such as life satisfaction and finding purpose and meaning in one's life are increased by habitual moderate alcohol intake requires further investigation. The acute effects of moderate alcohol consumption on emotional well-being may, however, translate into a better long-term physical well-being.

Physiological determinants and health outcomes

Whether alcohol consumption improves markers of oxidative stress and insulin sensitivity has been investigated in several intervention trials. Most trials showed that red wine consumption increases antioxidant capacity, but whether this was due to the polyphenols or alcohol in the wine was not clear. We showed that red wine consumption acutely attenuated meal-induced oxidative stress as compared to dealcoholized red wine. This indicates that the reduction of meal-induced oxidative stress is an effect of alcohol per se or a combined effect of alcohol and polyphenols in red wine (chapter 6). This is in agreement with the effects observed for moderate alcohol consumption and inflammation, suggesting that probably both alcohol and polyphenols possess anti-inflammatory effects (37). However, our study showed that the improved oxidative capacity was not maintained after short-term daily alcohol intake in healthy subjects. In line with other studies, short-term daily alcohol intake was even found to increase lipid oxidation (20, 38). Nevertheless, both a high intake of polyphenols and moderate alcohol consumption have been related to a lower risk for cardiovascular mortality (39, 40). It appears that alcohol only reduces oxidative stress when it is consumed with food that induces oxidative stress, such that alcohol may restore oxidative balance. The contribution of alcohol to an oxidative balance may be one of the mediating factors in alcohol's effect on diseases as cardiovascular disease and type 2 diabetes.

The apparently protective effect of moderate alcohol consumption on type 2 diabetes has been well described in two meta-analyses (41, 42). The mechanism behind this reduced risk for type 2 diabetes is not completely clear yet. In recent observational studies, fetuin-A has been proposed to play a role in this association (43-45), but the effect of alcohol intake on fetuin-A was not yet investigated. We showed that moderate alcohol intake reduced fetuin-A levels in men (chapter 7). Thereby we provided evidence for a mediating role of fetuin-A in the

reduction of type 2 diabetes risk by moderate alcohol consumption in men. Insulin sensitivity is another factor that may explain the association, but intervention studies reported inconsistent results. Our meta-analysis showed that this inconsistency may be due to gender differences, because only in women moderate alcohol consumption tended to improve insulin sensitivity and reduced fasting insulin levels (chapter 8). This is in line with observational studies showing a larger prospective effect of alcohol on type 2 diabetes in women than in men. Together, these results provide evidence for potential mediators explaining the reduced risk of chronic diseases, such as type 2 diabetes, by moderate alcohol consumption. An improved oxidative balance and reduced innate inflammatory response may also explain part of the association suggested between alcohol consumption and reduced risk of diseases such as rheumatoid arthritis (46-48).

Public health implications

The results presented in this thesis underline that moderate alcohol consumption has acute beneficial effects on emotional well-being, by enhancing mood, inducing the rewarding value of food, and facilitating mental stress recovery. In addition, the results show that moderate alcohol consumption may improve short-term physiological determinants and physical outcomes related to emotional well-being, as shown by a reduction of oxidative stress and fetuin-A, and an improvement of insulin sensitivity. Finally, the results suggest that the beneficial effects of moderate alcohol consumption on short-term emotional well-being are probably not translated into an improved long-term emotional well-being, although they may translate into better long-term physical well-being.

The significance of well-being and quality of life as a public health concern has been endorsed for over half a century. Already in 1948 the WHO stated that health is ‘a complete state of physical, mental and social well-being and not merely the absence of disease or infirmity’ (49). Furthermore, in 1995, the WHO stressed the importance of assessing and improving people’s well-being (50). Therefore, the results from this thesis would best be interpreted within this framework of health. Because people are living longer than before, it is important to look beyond physical diseases and mortality. However, public health guidelines on alcohol intake have so far been focused primarily on risk of mortality, morbidity and injuries. An average daily intake of one to two alcohol beverages is associated with the lowest risk of all-cause mortality (51) mainly attributed to a reduced risk of cardiovascular disease (40). The lowest risk of type 2 diabetes has been observed around two alcoholic beverages per day (41, 42), while every glass of alcohol increases the risk of breast cancer (52). The risk of injuries and violation rises with heavier alcohol intake (53).

Ours and previous research on the relation between alcohol and well-being showed that

there is a J-shaped association, with moderate alcohol consumption being associated with better well-being. We showed that for physical well-being the optimal alcohol consumption would be around 20 gram per day, whereas emotional well-being decreases with daily intakes higher than 20 gram per day. In addition, we showed that moderate alcohol consumption may immediately improve emotional well-being. Higher doses of alcohol are known to result in unpleasant behavioural effects. Taking into account influences of alcohol on both physical and emotional well-being, from acute to long-term, the current guidelines for alcohol consumption are in line with effects on mortality and disease outcomes.

The current American and Dutch guidelines on alcohol consumption, if any alcohol is consumed at all, are to consume alcohol in moderation (up to 1 drink per day for women and 2 drinks per day for men) (54, 55). Our results are in agreement with these guidelines: moderate alcohol consumption may be beneficial for physical well-being, whereas drinking above moderate may be harmful for emotional well-being.

There are several risk groups who are advised not to drink at all by the U.S. National Institute of Alcohol Abuse and Alcoholism and the Netherlands Health Council: children and adolescents, women who are pregnant or planning to get pregnant, individuals with specific medical condition or taking medications that may interact with alcohol, individuals planning to engage in activities that alertness and skills, or individuals who cannot restrict their drinking to moderate levels or are recovering from alcoholism (55, 56).
Research has also shown that people with a family history of alcoholism have an altered response to alcohol consumption, including attenuated stress and mood responses (57-60). This may make them more at risk to heavy drinking and alcohol abuse. Therefore, it would be better to restrict alcohol consumption in this high-risk group.

Recommendations for future research

Moderate alcohol consumption and stress adaptation
The research presented in this thesis suggests that alcohol may improve emotional well-being. We showed that moderate alcohol consumption reduces stress and tension shortly after intake. However, the question whether alcohol consumption improves short-term (e.g. after several weeks) stress resilience remains unanswered and requires further investigation. Several studies reported that repeated exposure to the same stressor leads to a reduced HPA-axis mediated stress response (1-3). The adaptive capacity of a person to respond to stressors experienced during life may influence the vulnerability to stress-related disorders on the long term. Stress adaptation involves activation of neural, cardiovascular, neuroendocrine and immune systems. In case these systems are not turned down efficiently after the stressor

or fail to respond adequately to the stressor initially, this may have consequences for the systems involved. For example, chronic overactivation of the cardiovascular system can lead to hypertension, whereas chronic overactivation of the neuroendocrine system can lead to depression (61, 62). The current high incidence of stress-related disorders, such as depression, coronary heart disease, type 2 diabetes and ulcerative colitis, warrants more in-depth investigation of stress resilience. This could include further studies on the influence of alcohol consumption on stress resilience. The latter should preferably be carried out following an integrated approach, in which all involved systems are evaluated. This includes, but is not limited to subjective measures of stress and mood, and physiological measures of autonomic nervous system, blood pressure, HPA-axis and immune system.

Bidirectional association between alcohol and mental stress

Many observational studies investigated the association between stress and alcohol consumption. These studies suggest that high work stress or stressful experiences may induce drinking and alcohol abuse (63-65). Our research showed that alcohol consumption may help to reduce stress shortly after drinking, suggesting that moderate alcohol consumption might also reduce stress in the long-term. Therefore, it is important to investigate whether the association between stress and alcohol consumption is bidirectional. Societal disease burden due to high perceived mental stress load is increasing. This underlines the relevance of further investigation into cause-effect relationships between stress and lifestyle factors, including moderate alcohol consumption and other dietary habits.

Interestingly, Vasse et al. (1998) showed that in situations of high perceived stress, abstainers were more likely to be absent due to sickness than moderate drinkers (66). Therefore, also the possible associations between alcohol, stress and illness are of interest, as moderate alcohol consumption may not only reduce the perceived stress burden, but could also modulate stress-related illness.

Epigenetic effects of moderate alcohol consumption

The last several years, the importance of flexibility and adaptability as components of health has been increasingly recognised. This led to a renewed definition (in 2011) of health, namely ‘the ability to adapt and to self-manage’ (67). This development coincides with new insights into mechanisms by which DNA can adapt to environmental influences, such as stress and inflammation, via epigenetic regulation (68). Epigenetic events have been defined as ‘the structural adaptation of chromosomal regions so as to register, signal or perpetuate altered activity states’ (69). Alcohol consumption has been shown to cause epigenetic changes via an increased NADH/NAD^+ ratio, the formation of reactive oxygen species (ROS) and of acetate resulting from alcohol metabolism (70). For example, heavy alcohol consumption has been shown to influence epigenetic factors related to dysregulation of the immune system (71). However, moderate alcohol consumption, in contrast to heavy alcohol consumption, has

been suggested to influence the immune system in a positive way (37). Therefore, moderate alcohol consumption may result in beneficial epigenetic changes as well. Exploring the epigenetic changes induced by moderate alcohol consumption may provide further insight in the underlying mechanisms of the observed positive effects of moderate alcohol consumption on the immune system and metabolism. This could also generate a better understanding of the underlying mechanisms by which moderate alcohol consumption reduces the risk for diseases, such as type 2 diabetes and cardiovascular disease.

Physiological mechanisms explaining the relation between alcohol and type 2 diabetes
Despite the extensive evidence on the association of moderate alcohol consumption with a reduced risk of type 2 diabetes (41, 42), the underlying mechanisms are still not completely understood. The research in this thesis underlines that moderate alcohol consumption may influence different pathways and intermediates possibly involved in the reduced risk of type 2 diabetes in men and women. Several factors and processes have been identified that may mediate this relation, such as insulin sensitivity, inflammatory processes, effects of lipids and adiponectin (72). The relation was suggested to be explained for ~25% by adiponectin in middle-aged women (73). Future research should preferably include intervention trials in which the effect of moderate alcohol consumption on different pathways and mediators is studied in parallel, in relation to type 2 diabetes risk. These intervention trials should preferably use the gold standard of insulin sensitivity (the euglycemic hyperinsulinemic clamp technique) and include both men and women to investigate gender-related differences in mechanisms.

Concluding remarks

In conclusion, research presented in this thesis has added to our existing knowledge on the acute effects of moderate alcohol consumption on emotional well-being in common situations, such as consumption with a meal or in a mentally stressed state. In addition, we have further elucidated the influence of moderate alcohol consumption on physiological determinants related to emotional well-being, such as oxidative stress, inflammation and insulin sensitivity, and its gender-related differences. Finally, we provided new insights in the association between moderate alcohol consumption and physical and mental-health related quality of life.

References

1. Petrowski K, Wintermann G, Siepmann M. Cortisol response to repeated psychosocial stress. Appl Psychophysiol Biofeedback. 2012;37(2):103-7.
2. Schommer NC, Hellhammer DH, Kirschbaum C. Dissociation between reactivity of the hypothalamus-pituitary-adrenal axis and the sympathetic-adrenal-medullary system to repeated psychosocial stress. Psychosom Med. 2003;65(3):450-60.
3. Wüst S, Federenko IS, van Rossum EF, Koper JW, Hellhammer DH. Habituation of cortisol responses to repeated psychosocial stress—further characterization and impact of genetic factors. Psychoneuroendocrinology. 2005;30(2):199-211.
4. Levenson RW, Sher KJ, Grossman LM, Newman J, Newlin DB. Alcohol and stress response dampening: Pharmacological effects, expectancy, and tension reduction. J Abnorm Psychol. 1980 08;89(4):528-38.
5. Balodis IM, Wynne-Edwards KE, Olmstead MC. The stress–response-dampening effects of placebo. Horm Behav. 2011;59(4):465-72.
6. Yeomans MR. Short term effects of alcohol on appetite in humans. Effects of context and restrained eating. Appetite. 2010 12;55(3):565-73.
7. Testa M, Fillmore MT, Norris J, Abbey A, Curtin JJ, Leonard KE, et al. Understanding Alcohol Expectancy Effects: Revisiting the Placebo Condition. Alcoholism: Clinical and Experimental Research. 2006;30(2):339-48.
8. Maisto SA, Connors GJ, Sachs PR. Expectation as a mediator in alcohol intoxication: A reference level model. Cognitive Therapy and Research. 1981;5(1):1-18.
9. Sher KJ. Subjective effects of alcohol: the influence of setting and individual differences in alcohol expectancies. J Stud Alcohol. 1985 03;46(0096-882; 0096-882; 2):137-46.
10. Leigh BC. In search of the Seven Dwarves: issues of measurement and meaning in alcohol expectancy research. Psychol Bull. 1989;105(3):361.
11. Holdstock L, de Wit H. Individual Differences in the Biphasic Effects of Ethanol. Alcohol Clin Exp Res. 1998;22(9):1903-11.
12. Mick I, Spring K, Uhr M, Zimmermann US. Alcohol administration attenuates hypothalamic–pituitary–adrenal (HPA) activity in healthy men at low genetic risk for alcoholism, but not in high-risk subjects. Addict Biol. 2013;18(5):863-71.
13. Mumenthaler MS, Taylor JL, O'Hara R, Yesavage JA. Gender differences in moderate drinking effects. Alcohol Res Health. 1999;23(1):55-64.
14. Wells AS, Read NW, Uvnas-Moberg K, Alster P. Influences of fat and carbohydrate on postprandial sleepiness, mood, and hormones. Physiol Behav. 1997 5;61(5):679-86.
15. Bianchin M, Angrilli A. Gender differences in emotional responses: A psychophysiological study. Physiol Behav. 2012;105(4):925-32.
16. Kelly MM, Tyrka AR, Anderson GM, Price LH, Carpenter LL. Sex differences in emotional and physiological responses to the Trier Social Stress Test. Journal of Behavior Therapy and Experimental Psychiatry. 2008 03;39(1):87-98.
17. Kudielka BM, Kirschbaum C. Sex differences in HPA axis responses to stress: a review. Biol Psychol. 2005;69(1):113-32.

18. Kahneman D, Krueger AB. Developments in the measurement of subjective well-being. The journal of economic perspectives. 2006;20(1):3-24.

19. Eckardt MJ, File SE, Gessa GL, Grant KA, Guerri C, Hoffman PL, et al. Effects of moderate alcohol consumption on the central nervous system. Alcohol Clin Exp Res. 1998;22(5):998-1040.

20. Hartman T, Baer D, Graham L, Stone W, Gunter E, Parker C, et al. Moderate alcohol consumption and levels of antioxidant vitamins and isoprostanes in postmenopausal women. Eur J Clin Nutr. 2005;59(2):161-8.

21. Ho E, Galougahi KK, Liu C, Bhindi R, Figtree GA. Biological markers of oxidative stress: applications to cardiovascular research and practice. Redox biology. 2013;1(1):483-91.

22. van den Berg R, Haenen GR, 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 Chem. 1999;66(4):511-7.

23. Halliwell B, Lee CYJ. Using isoprostanes as biomarkers of oxidative stress: some rarely considered issues. Antioxidants & redox signaling. 2010;13(2):145-56.

24. Shaper AG, Wannamethee G, Walker M. Alcohol and mortality in British men: explaining the U-shaped curve. The Lancet. 1988;332(8623):1267-73.

25. Feunekes GI, van 't Veer P, van Staveren WA, Kok FJ. Alcohol intake assessment: the sober facts. Am J Epidemiol. 1999 Jul 1;150(1):105-12.

26. Giovannucci E, Colditz G, Stampfer MJ, Rimm EB, Litin L, Sampson L, et al. The assessment of alcohol consumption by a simple self-administered questionnaire. Am J Epidemiol. 1991 Apr 15;133(8):810-7.

27. Peele S, Brodsky A. Exploring psychological benefits associated with moderate alcohol use: a necessary corrective to assessments of drinking outcomes? Drug Alcohol Depend. 2000 11/10;60(3):221-47.

28. Baum-Baicker C. The psychological benefits of moderate alcohol consumption: A review of the literature. Drug Alcohol Depend. 1985 08;15(4):305-22.

29. Hendler RA, Ramchandani VA, Gilman J, Hommer DW. Stimulant and Sedative Effects of Alcohol. Curr Top Behav Neurosci. 2013 01/01;13:489-509.

30. King A, de Wit H. Rewarding, stimulant, and sedative alcohol responses and relationship to future binge drinking. Arch Gen Psychiat. 2011 04/04;68(4):389-99.

31. Dai X, Thavundayil J, Gianoulakis C. Response of the Hypothalamic-Pituitary-Adrenal Axis to Stress in the Absence and Presence of Ethanol in Subjects at High and Low Risk of Alcoholism. Neuropsychopharmacology. 2002 print;27(3):442-52.

32. King AC, Houle T, de Wit H, Holdstock L, Schuster A. Biphasic Alcohol Response Differs in Heavy Versus Light Drinkers. Alcohol Clin Exp Res. 2002;26(6):827-35.

33. Holdstock L, King AC, de Wit H. Subjective and Objective Responses to Ethanol in Moderate/Heavy and Light Social Drinkers. Alcoholism: Clinical and Experimental Research. 2000;24(6):789-94.

34. Byles J, Young A, Furuya H, Parkinson L. A Drink to Healthy Aging: The Association Between Older Women's Use of Alcohol and Their Health-Related Quality of Life. J Am Geriatr Soc. 2006;54(9):1341-7.

35. Chan AM, von Mühlen D, Kritz-Silverstein D, Barrett-Connor E. Regular alcohol consumption is associated with increasing quality of life and mood in older men and women: the Rancho Bernardo Study. Maturitas. 2009;62(3):294-300.

36. Van Dijk AP, Toet J, Verdurmen J. The relationship between health-related quality of life and two measures of alcohol consumption. Journal of Studies on Alcohol and Drugs. 2004;65(2):241.

37. Romeo J, Warnberg J, Nova E, Diaz LE, Gomez-Martinez S, Marcos A. Moderate alcohol consumption and the immune system: a review. Br J Nutr. 2007 10;98 Suppl 1:S111-5.

38. Caccetta RA, Burke V, Mori TA, Beilin LJ, Puddey IB, Croft KD. Red wine polyphenols, in the absence of alcohol, reduce lipid peroxidative stress in smoking subjects. Free Radical Biology and Medicine. 2001;30(6):636-42.

39. Arts IC, Hollman PC. Polyphenols and disease risk in epidemiologic studies. Am J Clin Nutr. 2005 Jan;81(1 Suppl):317S-25S.

40. Ronksley PE, Brien SE, Turner BJ, Mukamal KJ, Ghali WA. Association of alcohol consumption with selected cardiovascular disease outcomes: a systematic review and meta-analysis. BMJ. 2011 Feb 22;342:d671.

41. Baliunas DO, Taylor BJ, Irving H, Roerecke M, Patra J, Mohapatra S, et al. Alcohol as a Risk Factor for Type 2 Diabetes: A systematic review and meta-analysis. Diabetes Care. 2009;32(11):2123-32.

42. 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(3):719-25.

43. Ley SH, Sun Q, Jimenez MC, Rexrode KM, Manson JE, Jensen MK, et al. Association between alcohol consumption and plasma fetuin-A and its contribution to incident type 2 diabetes in women. Diabetologia. 2014;57(1):93-101.

44. Stefan N, Fritsche A, Weikert C, Boeing H, Joost HG, Haring HU, et al. Plasma fetuin-A levels and the risk of type 2 diabetes. Diabetes. 2008 Oct;57(10):2762-7.

45. Ix JH, Biggs ML, Mukamal KJ, Kizer JR, Zieman SJ, Siscovick DS, et al. Association of fetuin-a with incident diabetes mellitus in community-living older adults: the cardiovascular health study. Circulation. 2012 May 15;125(19):2316-22.

46. Lu B, Solomon DH, Costenbader KH, Keenan BT, Chibnik LB, Karlson EW. Alcohol consumption and markers of inflammation in women with preclinical rheumatoid arthritis. Arthritis & Rheumatism. 2010;62(12):3554-9.

47. Scott IC, Tan R, Stahl D, Steer S, Lewis CM, Cope AP. The protective effect of alcohol on developing rheumatoid arthritis: a systematic review and meta-analysis. Rheumatology (Oxford). 2013 May;52(5):856-67.

48. Jin Z, Xiang C, Cai Q, Wei X, He J. Alcohol consumption as a preventive factor for developing rheumatoid arthritis: a dose-response meta-analysis of prospective studies. Ann Rheum Dis. 2014 Nov;73(11):1962-7.

49. World Health Organization. Preamble to the constitution of the WHO as adopted by the International Health Conference, New York, 19–22 June 1946. Geneva: WHO. 1948.

50. WHOQOL group. The World Health Organization quality of life assessment (WHOQOL): position paper from the World Health Organization. Soc Sci Med. 1995;41(10):1403-9.

51. Di Castelnuovo A, Costanzo S, Bagnardi V, Donati MB, Iacoviello L, de Gaetano G. Alcohol dosing and total mortality in men and women: an updated meta-analysis of 34 prospective studies. Arch Intern Med. 2006;166(22):2437-45.

52. Hamajima N, Hirose K, Tajima K, Rohan T, Calle EE, Heath CW,Jr, et al. Alcohol, tobacco and breast cancer--collaborative reanalysis of individual data from 53 epidemiological studies, including 58,515 women with breast cancer and 95,067 women without the disease. Br J Cancer. 2002 Nov 18;87(11):1234-45.

53. Taylor B, Irving H, Kanteres F, Room R, Borges G, Cherpitel C, et al. The more you drink, the harder you fall: a systematic review and meta-analysis of how acute alcohol consumption and injury or collision risk increase together. Drug Alcohol Depend. 2010;110(1):108-16.

54. U.S. Department of Agriculture, U.S. Department of Health and Human Services. Dietary guidelines for Americans. 7th ed. Washington D.C.: available from: http://www.health.gov/dietaryguidelines/2010.asp; 2010.

55. Kok F, Van Binsbergen J, Breedveld B, Büller H, Feskens E, Van der Greft A. Richtlijnen goede voeding 2006. Den Haag: Gezondheidsraad. 2006.

56. NIAAA. Helping patients who drink too much: A clinician's guide. 2005. Report No.: 2015.

57. Schuckit MA. Subjective responses to alcohol in sons of alcoholics and control subjects. Arch Gen Psychiatry. 1984;41(9):879-84.

58. Schuckit MA, Gold E, Risch C. Plasma cortisol levels following ethanol in sons of alcoholics and controls. Arch Gen Psychiatry. 1987;44(11):942-5.

59. Schuckit MA. Low level of response to alcohol as a predictor of future alcoholism. Am J Psychiatry. 1994 Feb;151(2):184-9.

60. Schuckit MA, Smith TL. An 8-year follow-up of 450 sons of alcoholic and control subjects. Arch Gen Psychiatry. 1996;53(3):202-10.

61. McEwen BS. Stress, adaptation, and disease: Allostasis and allostatic load. Ann N Y Acad Sci. 1998;840(1):33-44.

62. De Kloet ER, Joëls M, Holsboer F. Stress and the brain: from adaptation to disease. Nature Reviews Neuroscience. 2005;6(6):463-75.

63. Dawson DA, Grant BF, Ruan WJ. The association between stress and drinking: modifying effects of gender and vulnerability. Alcohol Alcohol. 2005 Sep-Oct;40(5):453-60.

64. Crum RM, Muntaner C, Eaton WW, Anthony JC. Occupational stress and the risk of alcohol abuse and dependence. Alcoholism: Clinical and Experimental Research. 1995;19(3):647-55.

65. Ragland DR, Greiner BA, Yen IH, Fisher JM. Occupational Stress Factors and Alcohol-Related Behavior in Urban Transit Operators. Alcoholism: Clinical and Experimental Research. 2000;24(7):1011-8.

66. Vasse R, Nijhuis F, Kok G. Associations between work stress, alcohol consumption and sickness absence. Addiction. 1998;93(2):231-41.

67. Huber M, Knottnerus JA, Green L, van der Horst H, Jadad AR, Kromhout D, et al. How should we define health? BMJ. 2011 Jul 26;343:d4163.

68. Kanherkar RR, Bhatia-Dey N, Csoka AB. Epigenetics across the human lifespan. Frontiers in cell and developmental biology. 2014;2.

69. Bird A. Perceptions of epigenetics. Nature. 2007;447(7143):396-8.

70. Zakhari S. Alcohol metabolism and epigenetics changes. Alcohol Res. 2013;35(1):6-16.

71. Curtis BJ, Zahs A, Kovacs EJ. Epigenetic targets for reversing immune defects caused by alcohol exposure. Alcohol Res. 2013;35(1):97-113.

72. Hendriks HFJ. Moderate Alcohol Consumption and Insulin Sensitivity: Observations and Possible Mechanisms. Ann Epidemiol. 2007 5;17(5):S40-2.

73. Beulens JW, Rimm EB, Hu FB, Hendriks HF, Mukamal KJ. Alcohol consumption, mediating biomarkers, and risk of type 2 diabetes among middle-aged women. Diabetes Care. 2008 Oct;31(10):2050-5.

Summary

Moderate alcohol consumption has been suggested to contribute to emotional well-being. However, several knowledge gaps remain, in particular on the effects of moderate alcohol consumption on emotional well-being in common drinking situations. Furthermore, it is not well understood to what extent alcohol-induced emotional well-being influences physical well-being.
This thesis aimed to further explore the acute effects of moderate alcohol consumption on emotional well-being and the association between habitual alcohol consumption and emotional well-being. Next, this thesis aimed to provide more insight into physiological determinants that may be related to alcohol-induced emotional well-being.

Acute effects on emotional well-being
Alcohol is often consumed around mealtimes. Despite the evidence that moderate amounts of alcohol can improve well-being shortly after consumption, the effects of moderate alcohol consumption with a meal are not well understood. Therefore, we have investigated the influence of moderate alcohol consumption with dinner on mood in women (**chapter 2**). Happiness scores increased shortly after alcohol consumption, but only in those situations where an unpleasant current mood state was induced. The results suggest that alcohol consumption with a meal acutely improves well-being, but in an unpleasant ambiance only.

Endocannabinoids have been suggested to play a role in the behavioural effects of alcohol. Therefore, we examined the effects of mood changes induced by a meal ambiance and moderate alcohol consumption on plasma endocannabinoids and some N-acylethanolamine congeners in women (**chapter 3**). Circulating endocannabinoids were not influenced by alcohol consumption, and showed an inconsistent correlation with mood states. These findings do not suggest, at least under these conditions, that plasma endocannabinoids are important for mood regulation.

When alcohol is consumed before or with a meal, it increases food intake. Despite the well-known rewarding properties of alcohol itself, the effects of alcohol on the reward response of subsequent food intake have not been measured before. In **chapter 4** we described whether alcohol increases food intake by increasing food reward in men. Indeed, data obtained showed that moderate alcohol consumption is able to increase subsequent intake and rewarding value of savoury foods.

Although several studies have shown a stress-dampening effect of alcohol when consumed before a mental stressor, data on such an effect of alcohol when consumed after a stressor are limited. Therefore, we investigated whether moderate alcohol consumption shortly after a mental stressor attenuated the stress response and stress-related immune response in men (**chapter 5**). Moderate alcohol consumption shortly after a stressor improved the recovery

of cortisol and ACTH. Furthermore, the inflammatory marker IL-8 and the percentage of monocytes were decreased after alcohol consumption. This implies that alcohol may improve stress recovery and that it may modulate a stress-induced inflammatory response.

Short-term influence on physical well-being

To further explore the effect of moderate alcohol consumption on physiological determinants of emotional well-being, we investigated the influence of moderate alcohol consumption with a meal on postprandial plasma total oxidative capacity and NF-κB (nuclear transcription factor playing an important role in inflammation) levels in men (**chapter 6**). Here we found that a moderate amount of alcohol acutely attenuated meal-induced oxidative stress.

There is a clear association between habitual moderate alcohol consumption and a lower risk of type 2 diabetes. However, the acute effects of moderate drinking on inflammatory status and insulin sensitivity, two factors that might explain this relation, are poorly understood. To determine the effects of several weeks of moderate alcohol consumption on inflammation makers and insulin sensitivity, we conducted two studies. In **chapter 7** we described post hoc analyses on alcohol's influence on fetuin-A in three randomized intervention studies in both men and women. Additionally, we conducted a meta-analysis on intervention studies measuring the influence of short-term alcohol consumption (≥2 weeks) on insulin sensitivity and glycemic status (**chapter 8**).

Short-term moderate alcohol intake reduced fetuin-A levels in men but not in women. Our meta-analysis showed that in women only, short-term moderate alcohol consumption tended to improve insulin sensitivity and reduced fasting insulin levels. This suggests that alcohol consumption may influence different pathways and intermediates in men and in women, leading to a reduced type 2 diabetes risk.

Long-term association with emotional and physical well-being

The association between habitual alcohol consumption and well-being has thus far mainly been measured by cross-sectional studies, which demands for further substantiation by prospective observational studies. We therefore investigated the bidirectional association between alcohol consumption and health-related quality of life in a longitudinal study in women (**chapter 9**). Moderate alcohol consumption was associated with a better physical health-related quality of life, but not with a better mental health-related quality of life.

The main findings, methodological considerations and interpretation of findings of the studies described in this thesis are discussed in **chapter 10**. In this chapter, also directions for further research and implications for public health are mentioned.

In conclusion, data presented in this thesis add to the existing knowledge on acute improvement of emotional well-being by moderate alcohol consumption in common situations, such as consumption with a meal or during a mentally stressed state. In addition, we further elucidated the influence of moderate alcohol consumption on emotional well-being related physiological determinants, such as oxidative stress, inflammation and insulin sensitivity. Finally, we extended evidence on the association between moderate alcohol consumption and physical and mental-health related quality of life.

Lay summary in Dutch
(Samenvatting voor niet-ingewijden)

De invloed van matige alcoholconsumptie op het geestelijk en lichamelijk welbevinden

Alcohol wordt al sinds mensenheugenis gedronken, vooral om te ontspannen en plezier te hebben. Het drinken van alcohol heeft daarnaast ook duidelijke effecten op de gezondheid: overmatige alcoholconsumptie heeft sterke gezondheidsnadelen, terwijl matige alcoholconsumptie daarentegen overwegend gezondheidsvoordelen heeft. De afgelopen jaren is er vooral veel onderzoek gedaan naar de invloed van alcoholconsumptie op chronische ziekten, zoals hart- en vaatziekten en type 2 diabetes. De invloed van alcohol op het geestelijk welbevinden is nog niet duidelijk voor veel voorkomende drinkmomenten, zoals het drinken van een glaasje alcohol bij de maaltijd, of na een stressvolle dag op het werk. Ook is nog onvoldoende bekend wat de invloed is van matige alcoholconsumptie op gezondheidsparameters die gerelateerd zijn aan het geestelijk welbevinden. Een langdurige periode van stress kan bijvoorbeeld leiden tot meer ontstekingsfactoren in het bloed en een lagere insulinegevoeligheid. Dit zijn twee belangrijke factoren voor het ontstaan van type 2 diabetes.

Definitie van matige alcoholconsumptie:
Onder matige alcoholconsumptie verstaat de Nederlandse Gezondheidsraad:
Maximaal één standaardglas alcohol per dag voor vrouwen en maximaal twee standaardglazen per dag voor mannen. Eén standaardglas wijn, bier of gedistilleerd bevat ongeveer 10 gram alcohol.

Alcoholconsumptie in Nederland:
Volwassen mannen en vrouwen drinken in Nederland gemiddeld respectievelijk 1,6 en 0,9 glazen per dag.

Het doel van dit proefschrift is daarom tweeledig:

1. Het verder onderzoeken van de invloed van matige alcoholconsumptie op het geestelijk welbevinden. Dit is gedaan door middel van interventiestudies naar de directe effecten op het geestelijk welbevinden en door middel van een bevolkingsonderzoek naar de relatie tussen matige alcoholconsumptie en de kwaliteit van leven op de lange termijn.
2. Meer inzicht verkrijgen in de effecten van matige alcoholconsumptie op gezondheidsparameters die gerelateerd zijn aan geestelijk welbevinden. Dit is gedaan met interventiestudies en een meta-analyse waarin de effecten van alcoholconsumptie op oxidatieve stress, fetuin-A en insulinegevoeligheid zijn onderzocht.

Invloed op geestelijk welbevinden

We hebben door middel van drie interventiestudies de directe effecten van matige alcoholconsumptie op het geestelijk welbevinden onderzocht. Bij interventiestudies wordt aan

een relatief kleine groep mensen een bepaalde stof of behandeling gegeven. In de beschreven onderzoeken werden alcoholhoudende en alcoholvrije dranken aan de proefpersonen gegeven. Vervolgens werden de effecten op stemming, stress en de beloningsgevoelens van het consumeren van voeding gemeten. In de onderzoeken kwamen de proefpersonen meerdere keren naar de testlocatie om de testen een keer te doen met alcoholhoudende drank en een keer met alcoholvrije drank. De volgorde hiervan was willekeurig. De resultaten na het drinken van alcoholhoudende of alcoholvrije dranken werden vergeleken om te kunnen bepalen wat de effecten van alcohol zijn.
Om de invloed van matige alcoholconsumptie op het geestelijk welbevinden op de lange termijn te onderzoeken, hebben we de relatie tussen dagelijkse alcoholconsumptie en de kwaliteit van leven in een groot bevolkingsonderzoek onder Amerikaanse vrouwen onderzocht.

Effect van alcohol op stemming

In het eerste onderzoek kregen 28 vrouwelijke proefpersonen bij een avondmaaltijd 3 glazen bruisende witte wijn of alcoholvrije bruisende witte wijn te drinken. Dit deden zij in een gezellige of ongezellige kamer die de proefpersonen in een goede of slechte stemming bracht. Hierdoor konden wij onderzoeken of alcoholconsumptie de stemming anders beïnvloedt wanneer men alcohol drinkt in een positieve of negatieve stemming. In **hoofdstuk 2 en 3** beschrijven we de resultaten van dit onderzoek. Direct na het drinken van 3 glazen bruisende witte wijn gaven de proefpersonen in vragenlijsten aan vrolijker te zijn. Dit was echter alleen wanneer de wijn in een ongezellige kamer werd gedronken en niet wanneer deze in een gezellige kamer werd gedronken.

Effect van alcohol op het genieten van eten

In het tweede onderzoek kregen 24 mannelijke proefpersonen een mix van wodka en sinaasappelsap of alleen sinaasappelsap te drinken (**hoofdstuk 4**). Hierna werd met een vragenlijst op de computer getest of ze op dat moment meer zin hadden in zoete, hartige, vetrijke of vetarme snacks (‘wanting’). Ook werd getest hoe lekker ze de snacks op dat moment vonden (‘liking’). Deze twee componenten bepalen samen het beloningsgevoel; hoe belonend het eten van voeding wordt ervaren (‘rewarding value’). Een half uur na het drinken van de alcoholhoudende of alcoholvrije drank kregen ze een lunch waarbij ruim voldoende eten aanwezig was, zodat ze konden eten tot ze verzadigd waren. De belegproducten waren gelijkelijk verdeeld volgens de zoete, hartige, vetrijke en vetarme categorieën. Na het drinken van alcohol hadden de proefpersonen meer voorkeur voor hartige producten en aten ook meer van het hartige beleg, voornamelijk van het vetrijke hartige beleg.

Effect van alcohol op stress

In het derde onderzoek kregen 24 mannelijke proefpersonen 2 blikjes bier of alcoholvrij bier te

drinken na het uitvoeren van een mentale stresstest. Deze stresstest bestond onder andere uit het geven van een presentatie over de eigen sterke en zwakke punten. Deze presentatie werd gefilmd, en acteurs waren ingehuurd om kritische non-verbale signalen te geven aan de proefpersonen om de stress nog verder te verhogen. De resultaten van dit onderzoek zijn beschreven in **hoofdstuk 5**. De mentale stresstest zorgde voor een verhoging van de stresshormonen cortisol en ACTH, die weer daalden nadat de stresstest voorbij was. Er was een sterkere daling te zien van de stresshormonen cortisol en ACTH na het drinken van bier dan na het drinken van alcoholvrij bier na de stresstest. Ook werden er ontstekingsfactoren gemeten die tijdelijk vrijkomen bij stress. Twee van deze factoren, interleuking-8 (IL-8) en het percentage monocyten (van de totale hoeveelheid witte bloedcellen), waren lager na het drinken van bier dan na het drinken van alcoholvrij bier.

Langdurige alcoholconsumptie en kwaliteit van leven
Met data van een groot bevolkingsonderzoek dat is uitgevoerd onder Amerikaanse vrouwen, hebben we de relatie tussen matige alcoholconsumptie en de kwaliteit van leven onderzocht (**hoofdstuk 9**). Kwaliteit van leven wordt gedefinieerd als het functioneren van personen op fysiek, psychisch en sociaal gebied zoals zij dat zelf ervaren. In het bevolkingsonderzoek zijn met een vragenlijst de lichamelijke en psychische aspecten van de kwaliteit van leven gemeten. De alcoholconsumptie (in glazen alcohol per week) is gemeten met een voedselfrequentie-vragenlijst. Matige alcoholconsumptie was gerelateerd aan een betere kwaliteit van leven voor het lichamelijke gezondheidsaspect hiervan, maar was niet aan het psychische gezondheidsaspect.

Invloed op lichamelijk welbevinden

De effecten van matige alcoholconsumptie op het lichamelijk welbevinden zijn onderzocht met interventiestudies en een meta-analyse. Deze interventiestudies zijn vergelijkbaar qua opzet met de eerder beschreven interventiestudies. In de interventiestudies werd voor een aantal weken alcoholhoudende of alcoholvrije drank gedronken om de korte termijn effecten op gezondheidsparameters van het geestelijk welbevinden te meten. Een meta-analyse is een onderzoek waarbij de resultaten van alle studies (in dit geval interventiestudies) over een onderwerp worden samengevoegd zodat de uiteindelijke uitkomst betrouwbaarder is. De kwaliteit van de interventiestudies wordt meegenomen en de resultaten van de studies worden gewogen, zodat studies met secuurdere resultaten meer invloed hebben op de uiteindelijke uitkomst van de meta-analyse.

Effecten van alcohol op oxidatieve stress
Tijdens de stofwisseling ontstaat een kleine hoeveelheid oxidanten of reactieve

zuurstofverbindingen. Dit is een normaal verschijnsel. Oxidatieve stress is een stofwisselingstoestand waarbij er meer oxidanten vrij komen dan gebruikelijk. In een interventiestudie kregen 19 mannelijke proefpersonen bij hun avondmaaltijd thuis 4 weken lang rode wijn of alcoholvrije rode wijn te drinken. Na deze 4 weken kwamen ze naar TNO voor een testdag waar ze bij een maaltijd weer dezelfde wijn te drinken kregen. Voor en na deze maaltijd werd gemeten hoe goed het bloed in staat is om oxidanten onschadelijk te maken ('totale antioxidant capaciteit'). Ook werd de hoeveelheid NF-κB in het bloed gemeten, een stofje dat meer aanwezig is bij oxidatieve stress en die de productie van ontstekingsfactoren stimuleert. De resultaten van het onderzoek zijn beschreven in **hoofdstuk 6**. De totale antioxidant capaciteit was hoger na de maaltijd met rode wijn dan na de maaltijd met alcoholvrije rode wijn. Daarnaast was de hoeveelheid NF-κB verhoogd na de maaltijd met alcoholvrije rode wijn, terwijl de hoeveelheid NF-κB in het bloed onveranderd was na de maaltijd met rode wijn. Dit wijst op een lagere oxidatieve stress na het drinken van rode wijn bij de maaltijd.

Effecten van alcohol op insulinegevoeligheid

Insulinegevoeligheid is de gevoeligheid van de weefsels, zoals lever en spieren, voor insuline. Insuline zorgt ervoor dat glucose in de weefsels komt. Een verlaagde insulinegevoeligheid kan een voorspeller zijn van type 2 diabetes. In deze meta-analyse zijn de resultaten van 14 interventiestudies meegenomen waarin het effect van tenminste 2 weken alcoholconsumptie op de insulinegevoeligheid en de controle van de bloedsuikerspiegel is gemeten. De resultaten hiervan zijn beschreven in **hoofdstuk 8**. Matige alcoholconsumptie verhoogt mogelijk de insulinegevoeligheid bij vrouwen.

Effecten van alcohol op fetuin-A

Fetuin-A is een stofje dat door de lever gemaakt wordt en de insulinegevoeligheid verlaagt. Daarnaast is het gerelateerd aan een slechter functioneren van het immuunsysteem. In drie interventiestudies met wijn, bier en wodka en hun alcoholvrije variant, is in mannen en vrouwen het effect van een aantal weken matige alcoholconsumptie op fetuin-A onderzocht (**hoofdstuk 7**). In de studie met mannen was er een verlaging van fetuin-A door matige alcoholconsumptie, maar niet in de studies met vrouwen.

Algemene discussie

Tot slot wordt in **hoofdstuk 10** een kort overzicht gegeven van de belangrijkste bevindingen van dit proefschrift. Ook worden de voor- en nadelen van de gebruikte onderzoeksmethoden besproken en worden de gevonden resultaten vergeleken met eerdere bevindingen in de wetenschappelijke literatuur. Vervolgens wordt de relevantie van de uitkomsten voor de volksgezondheid beschreven. Tenslotte worden suggesties gedaan voor verder onderzoek

op het gebied van matige alcoholconsumptie en geestelijk en lichamelijk welbevinden.

Kortom, dit proefschrift heeft de kennis over de invloed van matige alcoholconsumptie op het geestelijk en lichamelijk welbevinden uitgebreid door aan te tonen dat:

1. Matige alcoholconsumptie op korte termijn het geestelijk welbevinden kan verbeteren. Dit volgt uit een directe verbeterde stemming in een ongezellige omgeving, een sterkere afname in stresshormonen na stress en het meer genieten van met name hartige voedingsmiddelen.
2. Matige alcoholconsumptie op lange termijn gerelateerd is aan een betere kwaliteit van leven voor het lichamelijke gezondheidsaspect, maar niet gerelateerd is aan het mentale gezondheidsaspect van kwaliteit van leven bij vrouwen.
3. Matige alcoholconsumptie op korte termijn het lichamelijk welbevinden kan verbeteren. Dit volgt uit een verlaging van de maaltijd-geïnduceerde oxidatieve stress en de hoeveelheid fetuin-A bij mannen en een mogelijke verbetering van insulinegevoeligheid bij vrouwen.

About the author

Curriculum Vitae

Ilse Corine Schrieks was born on August 25, 1987 in Gouda, the Netherlands. After completing secondary school at the 'Comenius College' in Capelle a/d IJssel, she started the Bachelor's program 'Nutrition and Health' at the Wageningen University. After having received her Bachelor's degree in 2008, she enrolled in the Master program in 'Nutrition in Health'. She specialized herself in nutrition physiology and eating behavior. During her Masters she did two theses: a minor thesis on the influence of snack consumption on compensation behavior and energy balance, and a major thesis on responses to a high fat intake in men with different metabolic risk phenotypes. She went to New Zealand where she did an internship on the methodology and applicability of maximal exercise tests at the Exercise and Sport Science department of Massey University.

In May 2011, Ilse was appointed as a PhD candidate to the division of Human Nutrition of Wageningen University and TNO (The Netherlands Organization for Applied Scientific Research) to perform research on moderate alcohol consumption, immune defense and well-being. She conducted human intervention studies at TNO and the Center for Human Drug Research. She performed a meta-analysis on alcohol consumption and insulin sensitivity in collaboration with Dr. Joline W. J. Beulens from the Julius Center, University Medical Center Utrecht. During the last year of her PhD project she went to Boston, USA, to conduct epidemiological research at the Harvard School of Public Health (HSPH) in close collaboration with Dr. Kenneth J. Mukamal and Prof. Dr. Eric B. Rimm. Ilse presented her research at several international conferences and was involved in teaching. In 2014, she was awarded with the Young Scientist Award at the 7th European Beer and Health Symposium in Brussels, Belgium.

List of publications

Peer reviewed publications

Schrieks IC, Heil ALJ, Hendriks HFJ, Mukamal KJ, and Beulens JWJ (2015) The effect of alcohol consumption on insulin sensitivity and glycemic status: A systematic review and meta-analysis of intervention studies. Diabetes Care, 38(4):723-732.

Schrieks IC, Ripken D, Stafleu A, Witkamp RF, and Hendriks HFJ. Effects of mood Inductions by meal ambiance and moderate alcohol consumption on endocannabinoids and N-acylethanolamines in humans: A Randomized Crossover Trial. Accepted for publication, PLoS ONE.

Schrieks IC, Stafleu A, Griffioen-Roose S, de Graaf C, Witkamp RF, Boerrigter-Rijneveld R, and Hendriks HFJ (2015) Moderate alcohol consumption stimulates food intake and food reward of savoury foods. Appetite, 89: 77-83.

Schrieks IC, Stafleu A, Kallen VL, Grootjen M, Witkamp RF, and Hendriks HFJ (2014) The biphasic effects of moderate alcohol consumption with a meal on ambiance-induced mood and autonomic nervous system balance: A randomized crossover trial. PLoS ONE, 9(1): e86199.

Joosten MM, **Schrieks IC**, and HFJ Hendriks (2014) Effect of moderate alcohol consumption on fetuin-A levels in men and women: Post-hoc analyses of three open-label randomized crossover trials. Diabetology & Metabolic Syndrome, 6:24.

Schrieks IC, van den Berg R, Sierksma A, Beulens JWJ, Vaes WHJ, and Hendriks HFJ (2013) Effect of red wine consumption on biomarkers of oxidative stress. Alcohol and Alcoholism, 48: 153-159.

Schrieks IC, Barnes MJ, and Hodges LD (2011) Comparison study of treadmill vs arm ergometry. Clinical Physiology and Functional Imaging, 31: 326-331.

Submitted papers

Schrieks IC, Wei M, Rimm EB, Okereke OI, Kawachi I, Hendriks HFJ, and Mukamal KJ. Bidirectional associations between alcohol consumption and health-related quality of life among young and middle-aged women.

Schrieks IC, Joosten MM, Klöpping WAA, Witkamp RF, and Hendriks HFJ. Moderate alcohol consumption after a mental stressor attenuates the stress response.

Other publications

Schrieks IC and Hendriks HFJ. De gezondheidseffecten van matige alcoholconsumptie (2012) Voeding Nu, 10:24-26.

Abstracts and presentations

Schrieks IC, Heil ALJ, Hendriks HFJ, Mukamal KJ, and Beulens JWJ The effect of alcohol consumption on insulin sensitivity and glycemic status: A systematic review and meta-analysis of intervention studies. 7th European Beer and Health Symposium, 30 September 2014, Belgium. *Oral presentation Young Scientist Award.*

Schrieks IC, Heil ALJ, Hendriks HFJ, Mukamal KJ, and Beulens JWJ. The effect of alcohol consumption on insulin sensitivity and glycemic status: A systematic review and meta-analysis of intervention studies. AHA Annual Conference on Cardiovascular Disease Epidemiology and Prevention - Nutrition, Physical Activity and Metabolism, 18-21 March 2014, USA; Circulation 2014, 129:AP149. *Poster presentation.*

Schrieks IC, Stafleu A, and Hendriks HFJ. Moderate alcohol consumption stimulates food intake and food reward of savoury food. Swiss Winter Conference on Ingestive Behavior, 1-6 March 2014, Switzerland. *Oral presentation.*

Schrieks IC, Ripken D, Stafleu A, Witkamp RF, and Hendriks HFJ. Effect of moderate alcohol consumption and ambiance during a meal on mood and plasma endocannabinoids in humans. 23rd Annual Symposium of the International Cannabinoid Research Society, 21-26 June 2013, Canada. *Poster presentation.*

Schrieks IC, Stafleu A, Kallen VL, Grootjen M, Witkamp RF, and Hendriks HFJ. Moderate alcohol consumption, autonomic nervous system and mood. Appetite 2013, 71:485. 37th British Feeding and Drinking Group Annual Meeting, 4-5 April 2013, UK. *Poster presentation.*

Overview of completed training activities

Description	Organizer, location	Year
Discipline specific activities		
Courses and training		
Epigenesis and epigenetics	WIAS, Wageningen (NL)	2011
Regulation of food intake and it's implication for nutrition and obesity	VLAG, Wageningen (NL)	2012
Summer school 'Food for thought'	Full4Health, Frauenwörth (DE)	2013
Online course medical neuroscience	Duke University, Coursera	2013
Master class Longitudinal data analysis (Mixed models)	VLAG, Wageningen (NL)	2013
Master class Confounding	VLAG, Wageningen (NL)	2014
Nutritional epidemiology	UMC Utrecht, Utrecht (NL)	2014
Training period at the Harvard School of Public Health	Harvard, Boston (USA)	2014
Conferences and meetings		
European Beer and Health Symposium	Beer and Health, Brussels (BE)	2011, 2014
Annual meetings NWO	NWO, Deurne (NL)	2011-2015
MWD Chronic inflammation	FMVW, Leiden (NL)	2011
NWO-STW Symposium on Sensory satiety	NWO-STW, Wageningen (NL)	2012
British Feeding and Drinking Group Annual Meeting	BFDG, Brighton/Loughborough (GB)	2012-2013
International Cannabinoid Research Society Symposium	ICRS, Vancouver (CA)	2013
Swiss Winter Conference on Ingestive Behaviour	SSIB, St Moritz (CH)	2014
AHA\NPAM Annual congress Epidemiology and Prevention	AHA, San Francisco (USA)	2014
General courses		
Good Clinical Practice	UMC Utrecht, Utrecht (NL)	2011
Communication with the media and the general public	WGS, Wageningen (NL)	2012
Project and time management	WGS, Wageningen (NL)	2012
Scientific writing	WGS, Wageningen (NL)	2013
Philosophy and ethics of food science and technology	WGS, Wageningen (NL)	2013
Communication in interdisciplinary research	WGS, Wageningen (NL)	2013
Effective behaviour in your professional surroundings	WGS, Wageningen (NL)	2014
Career perspectives	WGS, Wageningen (NL)	2015
Optional courses and activities		
Preparing PhD research proposal	WUR/TNO, Wageningen/Zeist (NL)	2011
PhD excursion Australia	WUR (NL)	2013
Research presentations TNO	TNO Zeist (NL)	2011-2015

The research described in this thesis was financially supported by by the Dutch Ministry of Economic Affairs, Agriculture and Innovation and by the Dutch Foundation for Alcohol Research (SAR) representing Dutch producers of and traders in beer, wine and spirits and TNO (Grant EZ1503). Their joint aim is to independently study the health effects of moderate alcohol consumption.

The printing of this thesis was kindly supported by:
Wageningen University, Wageningen
Nederlandse Brouwers, Den Haag
SpiritsNL, Den Haag
Koninklijke Vereniging van Nederlandse Wijnhandelaren (KVNW), Leidschendam
Stichting Verantwoord Alcoholgebruik (STIVA), Den Haag

Cover design & layout: Ilse C. Schrieks
Printed by: GVO drukkers & vormgevers B.V.