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Moderate alcohol consumption and lipoprotein-associated phospholipase A2 activity

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Abstract *Background and aims:* To investigate the effect of moderate alcohol consumption on lipoprotein-associated phospholipase A2 activity, markers of inflammation and oxidative stress and whether these effects are modified by BMI.
Methods and results: Eleven lean (BMI: 18.5–25 kg/m^2) and 9 overweight (BMI > 27 kg/m^2) men participated in a randomized controlled crossover trial. After consuming 3 cans of beer (40 g ethanol) or alcohol-free beer daily during 3 weeks, fasting blood samples were taken. HDL cholesterol increased by 18.2% ($p < 0.001$) after beer compared to alcohol-free beer, while LDL cholesterol decreased by 7.8% ($p = 0.008$). Lipoprotein-associated phospholipase A2 activity was not different ($p = 0.23$) between beer (47.5 ± 0.8) and alcohol-free beer (48.9 ± 0.8). High-sensitive C-reactive protein was unaffected, but urinary isoprostanes tended to increase ($p = 0.09$) after beer (114.0 ± 6.9) compared to alcohol-free beer (96.9 ± 6.5). An interaction between BMI and treatment ($p < 0.05$) on liver enzymes was observed, indicating an increase of liver enzymes after moderate alcohol consumption in overweight men only.
Conclusion: Despite profound effects on HDL and LDL cholesterol, moderate alcohol consumption did not affect lipoprotein-associated phospholipase A2 activity. Liver enzymes increased after alcohol consumption in overweight men only, suggesting a less favorable response to moderate alcohol consumption in overweight people.

Introduction

Moderate alcohol consumption is associated with a decreased risk of cardiovascular disease (CVD) [1]. About 50% of this association is explained by an increase of HDL cholesterol [1]. Other factors such as a decrease of

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inflammatory [2] or fibrinolytic factors [3], or an increase of insulin sensitivity may also be involved [4,5]. In addition, moderate alcohol consumption increases functional properties of HDL cholesterol such as paraoxonase activity [6] and cholesterol efflux [7]. However, these effects have mainly been studied in middle-aged men or women [8] and a paucity of data exists for younger men.

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

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

Methods

Subjects and design

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

The crossover trial consisted of 2 treatment periods of 3 weeks, each preceded by 1-week washout in which subjects were instructed not to consume alcohol. The men were randomized based on BMI-group to receive the sequence beer (Amstel Bier, Amsterdam, The Netherlands; 5% vol ethanol) followed by alcohol-free beer (Amstel Malt Bier, Amsterdam, The Netherlands; $<$0.1% vol ethanol) or the other way around. They consumed 3 cans of beer or alcohol-free beer daily with the evening meal, equaling 40 g ethanol/day during beer treatment. In the last 10 days of each treatment period the diet was fully controlled. All food was supplied by TNO and subjects were not allowed to eat or drink anything except the foods supplied, tap water, tea or coffee. The composition of the diet was based on the Dutch Food Consumption Survey of 1998 [20] and consisted of 37% fat, 15% protein, and 48% carbohydrates, excluding energy from alcohol.

Blood and urine sampling and analysis

At the end of each treatment period (day 22) all subjects visited TNO after an overnight fast for blood sampling and blood pressure assessment. Blood was obtained from the antecubital vein and collected in tubes containing lithium-heparin or gel and clot activator (Becton Dickinson, Vacutainer Systems, Plymouth, UK). To obtain plasma or serum, blood was centrifuged for 15 min at 2000g at 4 °C, between 15 and 30 min after collection. Systolic and diastolic blood pressure were measured using a blood pressure monitor (Omron Healthcare Europe BV, The Netherlands) 3 times after a 15 min rest.

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

Urine was collected during 24 h on the last 2 days of the study and urine samples were stored at −80 °C until analysis. Ethyl glucuronide (EtG), a direct metabolite of ethanol, in 24-hour urine specimens was determined by LC–MS and was used as a marker of alcohol consumption during the previous days [22]. Urinary 8-iso-prostaglandin F2-alpha was determined as a marker of oxidative stress [23] using GC–MS according to the modified method of Morrow et al. [24]. Data are expressed as pg 8-iso-prostaglandin F2-alpha/mg creatinine.

Statistical analysis

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

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Table 1 Mean ± SD baseline characteristics among lean and overweight men

Variable	Lean	Overweight
N	11	9
Age (years)	19 ± 2	21 ± 2
BMI (kg/m^2)	20.1 ± 1.0[a]	31.3 ± 3.9
Hemoglobin (mmol/l)	9.4 ± 0.5	9.4 ± 0.5
Total cholesterol (mmol/l)	4.1 ± 0.6[a]	5.0 ± 0.8
HDL cholesterol (mmol/l)	1.6 ± 0.4[a]	1.2 ± 0.2
LDL cholesterol (mmol/l)	1.9 ± 0.5[a]	3.2 ± 0.9
Triacylglycerols (mmol/l)	1.2 ± 0.6	1.5 ± 0.4
ALP (U/l)	100 ± 28	89 ± 27
AST (U/l)	21 ± 4[a]	32 ± 21
ALT (U/l)	15 ± 5[a]	60 ± 69
GGT (U/l)	18.0 ± 3.8[a]	43.3 ± 29.3

[a] $p < 0.05$ Lean compared to overweight group.

Results

Subjects

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

Overall effect of moderate alcohol consumption

Table 2 shows Lp-PLA2 activity, blood lipid profile, hs-CRP, F2-isoprostanes and blood pressure after beer and alcohol-free beer consumption. Moderate alcohol consumption had profound effects on blood lipid profile in this group of young men. Fasting serum HDL cholesterol level showed a large increase of 18% ($p < 0.001$), while LDL cholesterol decreased by ~8% ($p = 0.008$) after beer consumption as compared to alcohol-free beer. However, Lp-PLA2 activity was not different ($p = 0.23$) after beer (47.5 ± 0.8) compared to alcohol-free beer consumption (48.9 ± 0.8). Hs-CRP was not affected either, but F2-isoprostanes tended to increase ($p = 0.09$) after beer (114.0 ± 6.9) compared to alcohol-free beer consumption (96.9 ± 6.5). No effect of alcohol consumption on systolic blood pressure was found, while diastolic blood pressure tended to increase after beer consumption as compared to alcohol-free beer ($p = 0.10$). Liver enzymes were slightly elevated after beer consumption as compared to alcohol-free beer, although only the effect on GGT and AST was statistically significant.

Interaction of treatment with BMI

Table 3 shows Lp-PLA2 activity, blood lipid profile, hs-CRP, F2-isoprostanes and blood pressure after beer and alcohol-free beer consumption among lean and overweight subjects. The interaction between BMI and treatment was borderline significant for both GGT ($p = 0.046$) and AST ($p = 0.063$). Subgroup analysis indicated that the increase of liver enzymes, particularly GGT and AST, was larger among the overweight (26% and 20%, respectively) than the lean men (8% and 0%, respectively). No significant interactions between treatment and BMI were observed for other parameters. LDL cholesterol, however, decreased ($p = 0.010$) by 10% among lean subjects, but it did not change ($p = 0.23$) among overweight subjects. Similarly, Lp-PLA2 activity tended to decrease ($p = 0.21$) by 6% among lean subjects, but was unaffected among overweight subjects ($p = 0.90$).

BMI effects

Plasma Lp-PLA2 activity was higher ($p < 0.001$) among overweight (58.4 ± 0.9) than lean (38.0 ± 0.8) subjects. Hs-CRP

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

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

[a] Expressed as nmol/min/ml plasma.
[b] Expressed as pg 8-iso-prostaglandin F2-alpha/mg creatinine.

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

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

[a] Expressed as nmol/min/ml plasma.
[b] Expressed as pg 8-iso-prostaglandin F2-alpha/mg creatinine.
[c] Expressed as mmHg.

was also higher ($p = 0.015$) among overweight (2.2 ± 0.3) than lean (0.9 ± 0.2) subjects. As expected, diastolic blood pressure, total cholesterol, LDL cholesterol, triacylglycerol, ratio total to HDL cholesterol GGT and ALT were significantly higher and HDL cholesterol was significantly lower among overweight than lean subjects (data not shown).

Correlations

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

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

Discussion

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

The study was performed according to a randomized, controlled crossover design and the diet of the subjects was fully controlled during the last 10 days of each treatment period. It therefore seems unlikely that our results are confounded by diet or lifestyle.

Using urinary EtG as a marker for alcohol consumption we were perfectly able to discriminate between the alcohol and alcohol-free period of the study, in line with Sarkola et al. [22]. Variation in our results was smaller, possibly because we assessed EtG in 24-hour urine, while spot morning urine was used by Sarkola et al. [22]. Moreover, only negative results of EtG were found during the alcohol-free beer treatment, while Sarkola et al. found 2 positive samples during the placebo period suggesting incidental consumption of alcoholic beverages. Our results confirm both that urinary EtG may be a valuable marker of recent alcohol consumption and that compliance to study treatment was good.

The findings from our study are in line with the well-known effects of moderate alcohol consumption on blood lipid profile. The magnitude of effects observed in this population of young men is remarkable. We found an 18% increase of HDL cholesterol, which is almost twice the increase reported in previous studies [8]. HDL cholesterol is considered as the only lipoprotein mediating the association of moderate alcohol consumption with CVD. However, in our study LDL cholesterol also decreased significantly by 8%. This has only been reported previously for women [25–28]. Possibly a decrease of LDL cholesterol may be involved in the cardio-protective effect of moderate alcohol

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consumption in addition to HDL cholesterol, at least in specific populations such as females and young men.

Despite these large effects on HDL and LDL cholesterol, Lp-PLA2 activity did not change after moderate alcohol consumption. To our knowledge, this study is the first investigating the effect of a nutritional intervention (moderate alcohol consumption) on Lp-PLA2 activity. Only in an observational study of Oei et al. [12] a small but significant correlation between alcohol consumption and Lp-PLA2 activity was observed. Probably effects of moderate alcohol consumption on Lp-PLA2 activity are relatively small and only apparent in observational studies using larger populations and reflecting longer time-periods. As expected from the association of obesity with inflammation, Lp-PLA2 activity was much higher among overweight than lean subjects. This is in line with a positive correlation between Lp-PLA2 and BMI observed by Oei et al. [12]. The correlations between changes of Lp-PLA2 activity and LDL and HDL cholesterol were more modest than those demonstrated by Schaefer et al. [29] investigating the effect of statins on Lp-PLA2 concentration and activity. This difference can be explained by the relatively small change of Lp-PLA2 activity after moderate alcohol consumption compared to the larger effect of statins [29].

In this study a significant interaction between BMI and alcohol consumption for liver enzymes was observed, indicating a larger increase of liver enzymes after moderate alcohol consumption among overweight than lean subjects. This is in line with the study of Poikolainen and Vartiainen (1997) [19], showing that GGT increased with increasing alcohol intake in overweight subjects ($BMI > 27\ kg/m^2$), whereas in lean subjects an increase of GGT was only found at an intake of alcohol exceeding 300 g/week. Consistent with previous studies [18], we showed that BMI is an important predictor of elevation of liver enzymes.

Inflammation and oxidative stress are both involved in the development of NASH. We therefore hypothesized that BMI could modify effects on markers of inflammation and oxidative stress. Indeed, higher concentrations of inflammatory markers were observed in overweight than lean men, but in contrast to previous reports [2,30], we did not observe an effect of moderate alcohol consumption on hs-CRP. This may be due to the relatively large variation in these results. Some subjects had a mild form of common cold during the course of the study that may have confounded these results. However, moderate alcohol consumption tended to increase F2-isoprostanes, which is in line with the study of Hartman et al. [31].

Unfortunately, we could not demonstrate significant interactions between BMI and treatment for markers of inflammation and oxidative stress. However, the decrease of Lp-PLA2 activity after moderate alcohol consumption was larger among lean than overweight subjects. Furthermore, changes of Lp-PLA2 activity correlated strongly with changes of GGT and AST indicating a larger decrease of Lp-PLA2 with a smaller increase of liver enzymes. Also, LDL cholesterol decreased more strongly among lean than overweight subjects. Altogether, these findings suggest a less favorable effect of moderate alcohol consumption among overweight than lean subjects. This could be attributed to the presence of symptoms of liver damage due to obesity that may already be present at a young age [32].

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

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