Alterations in total and high–molecular-weight adiponectin after 3 weeks of moderate alcohol consumption in premenopausal women

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ABSTRACT

Moderate alcohol consumption is associated with increased concentrations of adiponectin. Whether this is the case for both total and high–molecular-weight (HMW) adiponectin is uncertain. Furthermore, the rate at which this increase occurs is unclear. Therefore, we examined the effect of moderate alcohol consumption on total and HMW adiponectin. In a randomized, crossover trial, 24 premenopausal women who were regular alcohol consumers received beer (~26 g alcohol) or alcohol-free beer daily for 3 weeks preceded by a 1-week washout. Blood samples were collected weekly after an overnight fast for measurement of total and HMW adiponectin and markers of glucose and lipid metabolism. There was a significant interaction ($P < .05$) between the 2 treatments over time for both plasma HMW and total adiponectin concentrations. Within 3 weeks, plasma total (8.2%, $P = .01$) and HMW (8.2%, $P = .02$) adiponectin levels were higher after moderate alcohol consumption compared with abstention. Changes over time in total adiponectin were positively associated with changes in HMW adiponectin during the nonalcoholic beer ($r = 0.80$; 95% confidence interval, 0.55-0.92) and beer ($r = 0.82$; 0.58-0.93) intervention. Alcohol consumption did not affect the ratio of HMW to total adiponectin or the serum glucose, insulin, hemoglobin A_{1c}, or triglyceride levels compared with abstention during the intervention periods. Both total and HMW adiponectin concentrations are higher after moderate alcohol consumption compared with abstention in premenopausal women. These effects were evident after at least 3 weeks of consumption and occurred concomitantly.

1. Introduction

Moderate alcohol consumption is associated with a reduced risk of type 2 diabetes mellitus [1,2]. This association could— in addition to improved insulin sensitivity and favorable changes in triglycerides [3,4]—partially be explained by increased circulating adiponectin levels [5]. Adiponectin is an adipose tissue-specific adipokine involved in glucose and

lipid metabolism [6]. It is associated positively with insulin sensitivity [7] and inversely with inflammatory markers [8] and the metabolic syndrome [9]. Moreover, higher adiponectin levels have been consistently associated with a lower risk of type 2 diabetes mellitus [10] and cardiovascular disease [11].

In observational studies, alcohol consumption has been positively associated with adiponectin concentrations [12-14], an effect confirmed in short-term (~3 weeks) randomized trials of alcohol administration [15-18].

Acute alcohol consumption, however, does not alter postprandial levels of adiponectin [19,20], suggesting that a longer period of alcohol consumption is needed to increase adiponectin levels. Furthermore, almost all of these short-term trials only included men, although adiponectin levels differ between men and women [21], and lower levels are observed in premenopausal compared with postmenopausal women [22,23]. Moreover, the majority of these trials investigated only total adiponectin levels, although the high–molecular-weight (HMW) isomer of adiponectin has been proposed as the most active metabolic form [24-26].

Hence, this trial was designed to investigate the weekly alterations in levels of both total and HMW adiponectin and associated markers of glucose and lipid metabolism by changes in alcohol consumption over a 3-week period in a group of premenopausal women.

2. Methods

2.1. Study design

The study used a randomized, open-label, crossover design consisting of two 3-week periods, each preceded by 1 week of washout. Allocation to treatment order (alcohol-free beer–beer vs beer–alcohol-free beer) was randomized according to age and body mass index (BMI). Subjects daily consumed 2 cans (66 cL) of beer (~26 g alcohol) or 2 cans of alcohol-free beer (<0.2 g alcohol) (both Amstel, the Netherlands) for 3 weeks during dinner. The beer contained 40 kcal/100 mL (170 kJ/100 mL), of which 28 kcal/100 mL was from alcohol and 12 kcal/100 mL was from carbohydrates. The alcohol-free beer contained 24 kcal/100 mL (100 kJ/mL), of which 24 kcal/100 mL was from carbohydrates. The study was conducted at TNO (Dutch acronym for Applied Scientific Knowledge) Zeist, the Netherlands. Subjects were instructed to maintain their habitual body weight, food ,and physical activity pattern and were told not to consume any additional alcohol during the entire study (including washout periods). Body weight was measured weekly wearing indoor clothing, without shoes, wallet, and keys, using a digital weight scale with a precision of 0.1 kg (model 701; SECA, Hamburg, Germany). Blood and urine sampling was done weekly for 8 weeks after an overnight fast. Compliance was monitored by weekly measurement of ethyl glucuronide, a direct phase II metabolite of alcohol consumption, in the urine and increase in serum high-density lipoprotein (HDL) cholesterol. Cutoff limit to assess compliance during alcohol-free intervention for urinary ethyl glucuronide values was set at greater than 0.25 mg/mL (positive sample) to obtain a high sensitivity but avoid positive results due to unintentional ethanol exposure. Cutoff limit during the beer intervention was set at ethyl glucuronide values less than 0.5 mg/mL (2.2 μmol/L) (negative sample) [4].

2.2. Study subjects

Twenty-four subjects were recruited from a pool of volunteers of TNO. Eligible premenopausal women consumed between 5 and 21 units of alcohol per week, were apparently healthy, were between 20 and 40 years old, used phase I or II oral contraceptives, had a BMI between 19 and 25 kg/m^2, refrained from smoking, and had no family history of alcoholism. They gave written informed consent and received compensation for their participation. The study was approved by an independent centralized ethics committee (METOPP, Tilburg, the Netherlands). This trial is registered at Clinicaltrials.gov, no. NCT00524550.

2.3. Handling and analysis of blood and urine samples

Ethyl glucuronide samples in morning urine were sampled and treated as described previously [4]. In short, urinary samples were diluted about 20 times using an internal standard solution. The resulting solution was analyzed using a triple quadrupole Ultra Performance LC/MS in MRM mode (Waters, Saint-Quentin En Yvelines Cedex, France) with a detection limit of 50 ng/mL.

Blood samples were obtained weekly from the antecubital vein of the forearm, collected in tubes containing clot activator for serum and in ice-chilled tubes containing potassium ethylene diamine tetraacid (K$_3$EDTA) for plasma (Vacutainer Systems; Becton Dickinson, Plymouth, United Kingdom), centrifuged for 15 minutes at 2000g at 4°C within 15 to 30 minutes after collection, and stored at –70°C. Biochemical determinations in blood were performed at TNO using Olympus analytical equipment and reagents except for adiponectin. Plasma total and HMW adiponectin concentrations were determined by an enzyme-linked immunosorbent assay (ELISA) (catalogue no. 47-ADPHU-E01; Alpco Diagnostics, Salem, NH). The intraassay coefficients of variation for the 2 forms of adiponectin were 5.4% for total and 5.0% for HMW adiponectin.

2.4. Statistics

Variables over time were compared between treatments using a mixed analysis of variance model that included fixed terms for treatment, time, treatment order (indicating possible carryover effects), and the interaction between treatment and time and random terms for subject and period. Body weight was included in the model as a random factor to correct for potential fluctuations in body weight. Orthogonal polynomials were used to test for linear or quadratic trends. Regression analyses were performed to describe the slopes of the time trend curve for both beverages. For the correlation between changes over time in adiponectin and HMW adiponectin, a Fisher z transformation was applied on individual correlations to correct for deviations from the normal distribution; and 95% confidence intervals were calculated. Statistical analyses were performed using the SAS statistical software package (SAS version 8; SAS Institute, Cary, NC). Data are

Table 1 – Baseline characteristics of 24 premenopausal women after an overnight fast

Variable	Mean ± SD	Range
Body weight (kg)	67.6 ± 6.5	54.4-79.4
Glucose (mmol/L)	4.95 ± 0.28	4.39-5.39
Insulin (pmol/L)	41.7 ± 16.0	20.1-81.3
HDL cholesterol (mmol/L)	1.61 ± 0.33	1.06-2.17
LDL cholesterol (mmol/L)	2.62 ± 0.46	1.70-3.40
Triglycerides (mmol/L)	1.11 ± 0.40	0.42-1.98
Alanine aminotransferase (U/L)	16.9 ± 6.4	9-30
Aspartate aminotransferase (U/L)	21.0 ± 5.7	13-41
Alkaline phosphatase (U/L)	62.6 ± 15.6	33-86
γ-Glutamyltranspeptidase (U/L)	19.0 ± 9.1	7.6-39.2

Data are expressed as mean ± SD. LDL indicates low-density lipoprotein.

presented as means ± SEM unless specified otherwise. Statistical significance was defined as $P < .05$.

3. Results

All 24 enrolled women completed the study (Table 1). Mean age and BMI of the women were 23.9 ± 4.3 years and 22.2 ± 1.6 kg/m^2, respectively. No carryover effects were seen in any outcome measure. Analysis of urinary ethyl glucuronide revealed 2 negative samples during the beer intervention and no positive samples during the alcohol-free beer intervention or washout period out of the 191 samples analyzed (overall compliance of 99.0%). Another measure of compliance was the 8.5% increase ($P < .01$) in HDL cholesterol after 3 weeks

Table 2 – Characteristics of 24 premenopausal women after 3 weeks of consuming beer or alcohol-free beer after an overnight fast

	Alcohol-free beer	Beer	P value
Adiponectin			
Total adiponectin (μg/mL)	6.77 ± 0.41	7.24 ± 0.41	.01
HMW adiponectin (μg/mL)	3.47 ± 0.30	3.73 ± 0.30	.02
Ratio of HMW to total adiponectin (arbitrary units)	0.49 ± 0.02	0.49 ± 0.02	.90
Glycemic markers			
Glucose (mmol/L)	4.79 ± 0.11	4.84 ± 0.11	.36
Insulin (pmol/L)	45.7 ± 4.0	46.1 ± 4.0	.90
Hemoglobin A_{1c} (%)	5.0 ± 0.04	4.9 ± 0.04	.16
Lipid profile			
HDL cholesterol (mmol/L)	1.52 ± 0.07	1.62 ± 0.07	<.01
LDL cholesterol (mmol/L)	2.40 ± 0.07	2.37 ± 0.07	.77
Triglycerides (mmol/L)	1.27 ± 0.08	1.25 ± 0.08	.61
Free fatty acids (mmol/L)	0.34 ± 0.03	0.29 ± 0.03	.26
Liver enzymes			
Alanine aminotransferase (U/L)	10.8 ± 1.8	10.0 ± 1.8	.21
Aspartate aminotransferase (U/L)	17.8 ± 2.0	17.8 ± 2.0	.95
Alkaline phosphatase (U/L)	56.9 ± 6.5	57.8 ± 6.5	.68
γ-Glutamyltranspeptidase (U/L)	16.5 ± 2.2	18.5 ± 2.2	.01

Data are expressed as mean ± SEM.
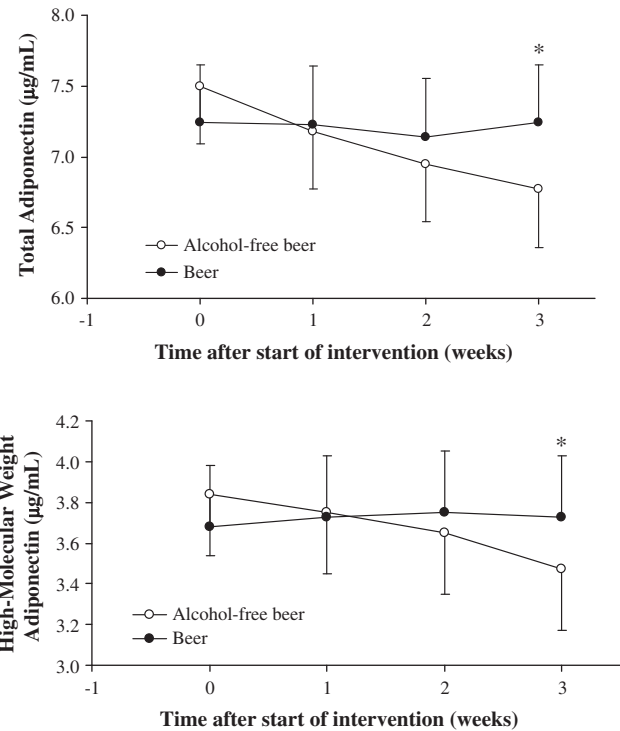
Fig. 1 – Mean ± SEM weekly plasma concentrations of total adiponectin and HMW adiponectin during a 3-week intervention with daily beer (•) or alcohol-free beer consumption (○) each preceded by 1 week of washout among 24 normal-weight premenopausal women. *$P < .05$ between beer and alcohol-free beer consumption at time point during the intervention.

of beer compared with nonalcoholic beer consumption (Table 2).

Mean body weight was slightly higher during the 3-week beer drinking period (67.9 ± 1.3 kg vs 67.6 ± 1.3 kg, $P < .01$). Results were adjusted for this difference in body weight, but did not materially change. Therefore, unadjusted results are presented. Plasma levels of total and HMW adiponectin over time differed between the 2 treatments (P for interaction < .05, Fig. 1). After the first 2 weeks, adiponectin levels did not differ between treatments. However, after 3 weeks, both total (7.24 ± 0.41 μg/mL vs 6.77 ± 0.41 μg/mL, $P = .01$) and HMW (3.73 ± 0.30 μg/mL vs 3.47 ± 0.30 μg/mL, $P = .02$) adiponectin levels were 8.2% higher after consuming beer compared with consuming nonalcoholic beer. No differences between treatments over time or between treatments were observed in the ratio of HMW to total adiponectin or levels of serum glucose, insulin, triglycerides, or free fatty acids (data not shown).

The orthogonal polynomial describing the linear time trend for each adiponectin form was significantly different between the 2 interventions (P for interaction < .01). During the 3-week alcohol-free beer period, the slope for HMW was −0.27 ± 0.09 μg/mL ($P < .01$) and that for total adiponectin levels was −0.54 ± 0.13 μg/mL ($P < .001$), whereas the slopes of the 2 adiponectin forms during the beer period did not differ from zero. Changes over the weeks in total adiponectin were positively associated with changes in HMW adiponectin during the

alcohol-free beer ($r = 0.80$; 95% confidence interval, 0.55-0.92) and beer ($r = 0.82$; 0.58-0.93) intervention. Changes over time in the 2 adiponectin forms were not associated with changes in serum glucose, insulin, triglycerides, and free fatty acids.

4.   Discussion

The present study showed that both total and HMW adiponectin concentrations are elevated after moderate alcohol consumption compared with abstention. The changes in adiponectin levels of these young, normal-weight women were evident after at least 3 weeks of consumption. Furthermore, the changes over time in total and HMW adiponectin levels during each treatment were correlated, which suggest that these changes occurred concomitantly. No alterations were observed in weekly levels of glucose, insulin, free fatty acids, or triglyceride in these women.

A trial by Imhof and colleagues [15] investigated the effects on total adiponectin levels after 3 weeks of daily alcohol consumption among women (20 g alcohol/day) and men (30 g/day). They reported increased adiponectin levels after red wine but not after beer consumption in women and higher levels after beer and ethanol but not after red wine consumption in men. The authors hypothesized that drinking preferences of participants might substantially have affected the findings by incomplete adherence to the study protocol, as women preferred to drink wine, whereas men preferred to drink beer. Other experimental studies have consistently reported comparable increases in circulating total adiponectin levels of approximately 10% in young [18] and middle-aged men [16,17] and postmenopausal women [4], irrespective of beverage type. Moreover, we have previously reported that alcohol-intervention–associated changes in messenger RNA levels of the gene encoding for adiponectin in adipose tissue correlated with changes in plasma protein levels of total adiponectin [4]. This implies that the alcohol-induced increase in plasma adiponectin levels might be mediated by de novo synthesis, providing further support that the alcohol itself is responsible for increases in circulating adiponectin levels rather than an alcoholic-beverage–specific constituent.

The data of our crossover study revealed that the difference in adiponectin levels between the 2 treatments are mainly due to a decrease in adiponectin levels during abstention rather than an increase during alcohol consumption. This, and the fairly similar mean HDL cholesterol levels before the interventions compared with after the alcohol intervention, might confirm that the women were indeed habitual drinkers (which was an inclusion criterion for the present study). Furthermore, the ratio of HMW to total adiponectin over the weeks did not differ between treatments. This, also reflected by the high correlations between changes in HMW and total adiponectin, points out that the intervention-induced changes in HMW and total adiponectin levels occur concurrently. Despite different physiological mechanisms, the observed adiponectin-kinetics resemble in part the alcohol-induced increase in apolipoprotein A-I and HDL cholesterol, although in these cases, changes were already seen after 5 and 10 days of alcohol consumption, respectively, compared with abstention [27].

In line with previous clinical trials with other groups of young and relatively healthy subjects, we did not observe changes in markers of insulin sensitivity [28,29]. Studies that did find an effect on insulin levels after alcohol consumption were performed in middle-aged, relatively less insulin-sensitive [3,4,30] or diabetic subjects [31-34] and lasted between 4 weeks to up to a year. This suggests that the effect of moderate alcohol intake on markers of glycemia occurs after a longer period of alcohol administration and/or in subjects with (slightly) impaired glucose tolerance rather than in young and insulin-sensitive subjects.

In observational studies, alcohol consumption has been positively associated with triglyceride levels in middle-aged men [35,36] but inversely associated with triglyceride levels in older women [35]. The latter has been confirmed in randomized trials of alcohol administration in postmenopausal women [3,4]. However, moderate alcohol consumption with a meal is known to increase postprandial triglyceride levels in both pre- and postmenopausal women [37]. We found that prolonged moderate alcohol consumption does not appreciably elevate or decrease serum fasting levels of triglycerides in these women. This is in accordance with a previous trial in premenopausal women with a slightly higher alcohol dose (30 g of alcohol/day) and longer intervention period (6 weeks) [38]. Similar to other studies among premenopausal women [38,39], we did observe increased serum HDL cholesterol levels after prolonged moderate alcohol consumption compared with abstention in these women.

Strong points of the study are its randomized, crossover design; the high compliance to the treatments throughout the study; and the measurement of HMW and total adiponectin with a more sensitive and precise method (ELISA) instead of quantitative Western blotting [18] and within the same ELISA [4]. Some limitations warrant consideration. The study duration of 3 weeks of alcohol consumption and a washout of 1 week before each intervention was relatively short. Maybe more profound differences would have appeared in adiponectin levels or markers of glycemia if the study persisted longer. However, previous studies that reported significant increases after moderate alcohol consumption in adiponectin also lasted approximately 3 weeks [15-18]. Second, the women investigated were relatively young and healthy and thus at low risk for type 2 diabetes mellitus. However, studies have shown that the association between moderate alcohol consumption and a lower risk of type 2 diabetes mellitus also holds in younger women [40] and among subjects already at low risk for diabetes on the basis of multiple combined low-risk lifestyle behaviors [41]. Third, the premenopausal women in the current study used oral contraceptives. Therefore, these results may not be readily generalizable to all premenopausal women, especially because there is no consensus whether adiponectin (total, HMW, or the ratio of HMW to total adiponectin) might be associated with sex steroids in premenopausal women [23,42,43].

In conclusion, both total and HMW adiponectin concentrations are higher after at least 3 weeks of moderate alcohol consumption compared with abstention in premenopausal women. Elevated circulating adiponectin levels in moderate drinkers may contribute to a reduced risk for type 2 diabetes mellitus not only in young and middle-aged

men and middle-aged women but also among premeno-pausal, normal-weight women.

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