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SHORT COMMUNICATION

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

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Abstract
Aims/hypothesis The aim of this study was to investigate whether moderate alcohol consumption increases plasma high molecular weight (HMW) adiponectin and/or muscle oxidative capacity.
Materials and methods Eleven lean (BMI 18–25 kg/m^2) and eight overweight (BMI ≥27 kg/m^2) men consumed 100 ml whisky (~32 g alcohol) or water daily for 4 weeks in a randomised, controlled, crossover trial. After each treatment period, muscle biopsies and fasting blood samples were collected.
Results Adiponectin concentrations increased ($p<0.001$) by 12.5% after 4 weeks of moderate alcohol consumption. Moderate alcohol consumption tended to increase HMW adiponectin by 57% ($p=0.07$) and medium molecular weight adiponectin by 12.5% ($p=0.07$), but not low molecular weight (LMW) adiponectin. Skeletal muscle citrate synthase, cytochrome *c* oxidase and β-3-hydroxyacyl coenzyme A dehydrogenase (β-HAD) activity were not changed after moderate alcohol consumption, but an interaction between alcohol consumption and BMI was observed for cytochrome *c* oxidase ($p=0.072$) and citrate synthase ($p=0.102$) activity. Among lean men, moderate alcohol consumption tended to increase cytochrome *c* oxidase ($p=0.08$) and citrate synthase activity ($p=0.12$) by 23 and 26%, respectively, but not among overweight men. In particular, plasma HMW adiponectin correlated positively with activities of skeletal muscle citrate synthase ($r=0.64$, $p=0.009$), cytochrome *c* oxidase ($p=0.59$, $p=0.009$) and β-HAD ($r=0.46$, $p=0.056$), while such correlation was not present for LMW adiponectin. Whole-body insulin sensitivity and intramyocellular triacylglycerol content were not affected by moderate alcohol consumption.
Conclusions/interpretation Moderate alcohol consumption increases adiponectin concentrations, and in particular HMW adiponectin. Concentrations of HMW adiponectin in particular were positively associated with skeletal muscle oxidative capacity.

Keywords Adiponectin · High molecular weight adiponectin · Insulin sensitivity · Intramyocellular triacylglycerols · Moderate alcohol consumption · Muscle oxidative capacity

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Abbreviations

β-HAD	β-3-hydroxyacyl coenzyme A dehydrogenase
HMW	high molecular weight
IMTG	intramyocellular triacylglycerol
LMW	low molecular weight
MMW	medium molecular weight

Introduction

Moderate alcohol consumption is associated with a decreased risk of type 2 diabetes compared with abstention [1], which could be explained by improved insulin sensitivity [2]. The underlying mechanism for this is not clear, but several pathways may be involved. First, moderate alcohol consumption increases plasma adiponectin concentrations, which could precede changes in insulin sensitivity [2]. Adiponectin improves insulin sensitivity by increasing muscle fat oxidation and/or decreasing intramyocellular triacylglycerols (IMTGs) [3]. Adiponectin is present in plasma as a trimer, hexamer or high molecular weight (HMW) form, the latter possibly being the most relevant in the aetiology of insulin resistance [4]. Second, moderate alcohol consumption acutely affects energy expenditure, diet-induced thermogenesis, lipolysis and lipid oxidation [5]. These changes may result from acetate production from ethanol, which is converted to acetyl-CoA mainly in peripheral tissue such as muscle [5]. Such acute changes could cumulatively affect oxidative capacity and insulin sensitivity. This is the first human study to investigate whether chronic moderate alcohol consumption affects plasma adiponectin oligomers and muscle oxidative capacity.

Materials and methods

In a randomised, controlled, crossover trial, conducted at TNO Quality of Life, 19 healthy, moderate-drinking, lean (BMI 18–25 kg/m^2, n=11) and overweight (BMI ≥27 kg/m^2, n=8) men (aged 18–40 years), consumed 100 ml whisky (32 g alcohol per day; Famous Grouse Scotch Whisky, 40% vol, Perth, Scotland) or mineral water (Spa Reine, Spa, Belgium) daily for 4 weeks. For the last 7 days of each treatment period the diet was fully controlled, and treatments were separated by a 2 day wash-out period. This sample size was sufficient to detect ~15% difference of β-3-hydroxyacyl-CoA dehydrogenase (β-HAD) and citrate synthase activity in this crossover trial and correlation coefficients of 0.55 or higher with 80% power and accepting an alpha of 0.05. At the end of each treatment period, muscle biopsies from the vastus lateralis muscle were taken to assess β-HAD, cytochrome *c* oxidase and citrate synthase activity [6], IMTG [7] and succinate dehydrogenase activity [8]. Fasting blood samples were collected and an OGTT was performed to calculate whole-body insulin sensitivity. Subjects gave written informed consent and the University Medical Center Utrecht Medical Ethics Committee approved the protocol. The study was conducted according to the Declaration of Helsinki (2000) and the International Conference on Harmonisation Guidelines for Good Clinical Practice. Data were analysed using the SAS statistical software package (SAS/STAT version 8, SAS Institute, Cary, NC, USA). Treatment effects were assessed by ANOVA, using general linear modelling, with BMI, period, treatment and treatment order included in the model. Two-sided p values below 0.05 were considered statistically significant.

Results

Compliance to treatments was good as assessed by measurements of urinary ethyl glucuronide every 5 days, showing one negative sample during the whisky-drinking and no positive samples during the water-drinking period. Another indication was the 11% increase (p<0.001) of serum HDL-cholesterol after whisky compared with water consumption. Table 1 shows results on insulin sensitivity, adiponectin and muscle enzyme activities. Insulin sensitivity was not changed after moderate alcohol consumption, but plasma adiponectin concentrations increased from 7.9±0.2 mg/l after water to 9.0±0.2 mg/l (means±SEM) after whisky consumption. Moderate alcohol consumption tended to increase HMW and medium molecular weight (MMW) adiponectin, but not low molecular weight (LMW) adiponectin. Differences in insulin sensitivity and adiponectin and its oligomers tended to be more pronounced among lean than overweight men (Table 2). Muscle oxidative capacity measured by β-HAD, cytochrome *c* oxidase and citrate synthase activity did not differ between treatments. A borderline significant interaction between treatment and BMI was observed for cytochrome *c* oxidase (p=0.072) and citrate synthase (p=0.102) activity. Moderate alcohol consumption tended to increase cytochrome *c* oxidase (+23.7%; 95% CI −3.9 to +51.2%, p=0.08) and citrate synthase (+24.7%; 95% CI −7.7 to +57.2%, p=0.11) activity among lean, but not overweight men (Table 2). Succinate dehydrogenase activity in mixed muscle fibres decreased (p=0.03) by 15% after moderate alcohol consumption. IMTGs did not differ, despite a 15–20% difference between both treatments. These results were not different for lean and overweight men (Table 2). After whisky consumption, HMW correlated positively with activities of citrate synthase (r=0.64, p=0.004), cytochrome *c* oxidase (r=0.59, p=0.009) and β-HAD (r=0.46, p=0.056). Activities of citrate synthase (r=0.44, p=0.07),

Table 1 Insulin sensitivity index, adiponectin oligomer concentrations, HbA_{1c} and enzyme activities after 4 weeks of consumption of whisky or mineral water in 19 lean or overweight men

	Mineral water	Whisky	% Change	*p* value
Insulin sensitivity index[a]	10.6±1.3	9.6±1.3	−9.4	0.61
Adiponectin (mg/l)	7.9±0.2	9.0±0.2	12.5	0.0008
HMW	0.7±0.1	1.1±0.1	57.1	0.074
MMW	4.0±0.2	4.5±0.2	12.5	0.065
LMW	3.2±0.3	3.5±0.3	9.4	0.442
HbA_{1c} (%)	4.9±0.02	4.8±0.02	−2.0	0.023
Muscle β-HAD (mol/μg protein)	4.22±0.48	4.37±0.49	3.6	0.827
Muscle citrate synthase (mol/μg protein)	0.66±0.05	0.75±0.05	13.6	0.262
Muscle cytochrome *c* oxidase (mol/μg protein)	1.48±0.12	1.67±0.12	12.8	0.262
Intramyocellular triacylglycerols (AU)				
Type 1	0.027±0.004	0.022±0.004	−18.5	0.339
Type 2	0.010±0.002	0.008±0.002	−20.0	0.489
Mixed	0.019±0.003	0.016±0.002	−15.8	0.429
Succinate dehydrogenase activity (AU)				
Type 1	0.084±0.005	0.075±0.004	−10.7	0.122
Type 2	0.057±0.003	0.052±0.003	−8.8	0.221
Mixed	0.073±0.003	0.062±0.003	−15.1	0.028

Data are presented as means±SEM or %
AU Arbitrary units
[a] Insulin sensitivity according to Matsuda and DeFronzo [15]

Table 2 Insulin sensitivity index, adiponectin oligomer concentrations, HbA_{1c} and enzyme activities (means±SEM) after 4 weeks of consumption of whisky or water in lean and overweight subjects

	Lean group				Overweight group			
	Water	Whisky	% Change	*p* value	Water	Whisky	% Change	*p* value
Insulin sensitivity index[a]	13.3±1.9	13.0±1.9	−2.3	0.93	7.8±1.6	4.6±1.6	−41.0	0.21
Adiponectin (mg/l)	8.1±0.3	9.4±0.3	16.1	0.01	7.7±0.2	8.5±0.2	10.4	0.08
HMW	0.8±1.2	1.2±0.2	50.0	0.12	0.8±0.1	0.8±0.1	0	0.62
MMW	4.2±0.3	4.8±0.3	14.3	0.13	3.9±0.2	4.1±0.2	5.1	0.52
LMW	3.2±0.4	3.4±0.4	6.3	0.69	3.1±0.4	3.5±0.4	12.9	0.50
HbA_{1c} (%)	4.9±0.02	4.8±0.02	−2.0	0.04	4.9±0.02	4.9±0.02	0	0.53
Muscle β-HAD (mol/μg protein)	4.67±0.50	5.10±0.45	9.2	0.55	3.46±0.90	3.69±1.01	6.7	0.87
Muscle citrate synthase (mol/μg protein)	0.73±0.08	0.92±0.07	26.0	0.11	0.60±0.05	0.54±0.06	−10.0	0.51
Muscle cytochrome *c* oxidase (mol/μg protein)	1.72±0.15	2.12±0.14	23.3	0.08	1.33±0.13	1.13±0.15	−15.0	0.36
Intramyocellular triacylglycerols (AU)								
Type 1	0.022±0.006	0.016±0.005	−27.3	0.50	0.032±0.006	0.028±0.006	−12.5	0.61
Type 2	0.006±0.002	0.008±0.002	33.3	0.41	0.013±0.003	0.007±0.003	−46.2	0.14
Mixed	0.014±0.004	0.013±0.003	−7.1	0.86	0.024±0.004	0.019±0.004	−20.8	0.40
Succinate dehydrogenase activity (AU)								
Type 1	0.086±0.005	0.080±0.005	−7.0	0.40	0.084±0.009	0.069±0.008	−17.9	0.29
Type 2	0.060±0.005	0.055±0.005	−8.3	0.50	0.054±0.005	0.048±0.004	−11.1	0.34
Mixed	0.076±0.005	0.066±0.005	−13.2	0.16	0.069±0.005	0.057±0.004	−17.4	0.15

Data are presented as means±SEM or %
AU Arbitrary units
[a] Insulin sensitivity according to Matsuda and DeFronzo [15]

cytochrome *c* oxidase (r=0.32, p=0.20) and β-HAD (r=0.44, p=0.07) also tended to correlate with MMW adiponectin, but not LMW adiponectin. A similar pattern was observed for correlations of adiponectin oligomers with HDL-cholesterol (HMW: r=0.55, p=0.014; MMW: r=0.55, p=0.014; LMW: r=0.21, p=0.38).

Discussion

This study showed that moderate alcohol consumption increases adiponectin concentrations, consistent with previous reports [2]. We have now observed that the alcohol-induced increase of adiponectin may be oligomer specific. Moderate alcohol consumption increased particularly HMW adiponectin, MMW adiponectin to a lesser extent, but not LMW adiponectin. These results are in line with those of Bobbert et al. [9] showing a similar pattern of changes in adiponectin oligomers after moderate weight reduction. Studies with thiazolidinedione or rigorous weight reduction show similar, but more pronounced results [10]. HDL-cholesterol concentrations correlated particularly with HMW adiponectin, as previously shown [9]. We have now also observed that HMW and MMW adiponectin are correlated with markers of muscle oxidative capacity, in line with reports of increased fat oxidation after adiponectin infusion [3] and a recent study showing that adiponectin increases muscle oxidative capacity [11]. Our results tend to confirm these findings and show that these relationships may be specific to HMW adiponectin. Altogether, this could provide an underlying mechanism for the proposed importance of HMW adiponectin in the aetiology of insulin resistance [4].

Despite this, the alcohol-induced increase of adiponectin and specifically HMW adiponectin did not affect muscle oxidative capacity, IMTG content and insulin sensitivity. As some subtle differences were present, we cannot completely rule out the hypothesis that changes in IMTG content and/or oxidative capacity could occur. Although it did not reach significance, we observed a 15–20% lower IMTG content after whisky than water consumption, which is of similar magnitude to that observed for a weight loss and physical activity intervention [12]. Furthermore, moderate alcohol consumption tended to increase muscle citrate synthase activity among lean men, but succinate dehydrogenase activity declined after moderate alcohol consumption in mixed muscle fibres. All citric acid cycle and respiratory chain enzymes are thought to change in parallel to perturbation [13]. Our findings with citrate synthase and succinate dehydrogenase, however, are contradictory to this notion. This could simply be due to the measurement of ex vivo oxidative capacity or to chance. Alternatively, moderate alcohol consumption could differentially affect various enzymes in oxidative pathways such as the citric acid or glyoxylate cycle as suggested by Kokavec and Crowe [14]. Because acetyl-CoA from ethanol oxidation is generated independently from activation of pyruvate dehydrogenase complex, it may not affect the citric acid cycle. Alcohol may instead affect the glyoxylate cycle that bypasses part of the citric acid cycle, including succinate dehydrogenase [13, 14].

Strengths of this study are its randomised, controlled crossover design. We assessed compliance to study treatments several times throughout the study and observed no significant deviations. It is therefore unlikely that our results are confounded by diet or lifestyle. Our study was, however, limited by a slightly small sample size for certain contrasts such as analyses in subgroups of lean and overweight men or of IMTG content. Therefore these results may be somewhat preliminary and need to be confirmed with larger sample sizes. Second, because insulin sensitivity was not the primary endpoint of this study, an OGTT was used to assess insulin sensitivity. However, we used an insulin sensitivity index that correlates well with the gold standard, the hyperinsulinaemic–euglycaemic clamp technique [15]. In addition, our results on insulin sensitivity do not differ from our previous studies using the clamp technique [2]. We therefore believe that using this insulin sensitivity index has not influenced our results to a large extent.

In conclusion, this study shows that 4 weeks of daily moderate alcohol consumption increases adiponectin concentrations and particularly HMW adiponectin concentrations, but does not affect insulin sensitivity and oxidative capacity. Concentrations of HMW and MMW adiponectin were positively associated with muscle oxidative capacity.

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Duality of interest The authors have no duality of interest.

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