

Moderate alcohol consumption increases insulin sensitivity and *ADIPOQ* expression in postmenopausal women: a randomised, crossover trial

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Abstract

Aims/hypothesis To determine whether 6 weeks of daily, moderate alcohol consumption increases expression of the gene encoding adiponectin (*ADIPOQ*) and plasma levels of the protein, and improves insulin sensitivity in postmenopausal women.

Methods In a randomised, open-label, crossover trial conducted in the Netherlands, 36 apparently healthy postmenopausal women who were habitual alcohol consumers, received 250 ml white wine (~25 g alcohol/day) or 250 ml of white grape juice (control) daily during dinner for 6 weeks. Randomisation to treatment allocation occurred according to BMI. Insulin sensitivity and *ADIPOQ* mRNA and plasma adiponectin levels were measured at the end of both periods. Insulin sensitivity was estimated using the homeostasis model assessment of insulin resistance (HOMA-IR). Levels of *ADIPOQ* mRNA in subcutaneous adipose tissue were determined by RT-PCR.

Results All subjects completed the study. Six weeks of white wine consumption reduced fasting insulin (mean±SEM 40.0±3.4 vs 46.5±3.4 pmol/l; $p<0.01$) and HOMA-IR (1.42±0.13 vs 1.64±0.13; $p=0.02$) compared with 6 weeks of grape juice consumption. *ADIPOQ* mRNA levels (1.09±0.15 vs 0.98±0.15; $p=0.04$) and plasma levels of total (13.1

±0.8 vs 12.0±0.8 µg/ml; $p<0.001$) and high molecular weight (HMW) adiponectin (9.9±1.2 vs 8.8±1.2 µg/ml; $p=0.02$) significantly increased after alcohol compared with juice consumption. Changes in *ADIPOQ* mRNA levels correlated with changes in plasma levels of total adiponectin ($\rho=0.46$; $p<0.01$). Both fasting triacylglycerol (8.2%; $p=0.04$) and LDL-cholesterol levels (7.8%; $p<0.0001$) decreased, whereas HDL-cholesterol increased (7.0%; $p<0.0001$) after prolonged moderate alcohol intake. No notable adverse effects were reported.

Conclusions/interpretation Moderate alcohol consumption for 6 weeks improves insulin sensitivity, adiponectin levels and lipid profile in postmenopausal women. Furthermore, these data suggest a transcriptional mechanism leading to the alcohol-induced increase in adiponectin plasma levels.

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Keywords Adiponectin · Alcohol consumption · Gene expression · High molecular weight adiponectin · Insulin sensitivity · Lipid profile · Peroxisome proliferator-activated receptor gamma · Postmenopausal women

Abbreviations

EtG	ethyl glucuronide
HMW	high molecular weight
HOMA-IR	homeostasis model assessment of insulin resistance
PAI-1	plasminogen activator inhibitor 1
PPAR	peroxisome proliferator-activated receptor

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Introduction

Moderate alcohol consumption has consistently been associated with a decreased risk of type 2 diabetes compared with abstaining and excessive drinking [1, 2]. The reason for the lower risk of type 2 diabetes is not entirely clear but a plausible explanation seems to be improved insulin sensitivity [3]. However, so far only one randomised controlled trial among postmenopausal women has confirmed this notion [4]. Other studies, mainly performed among young male populations, failed to detect such effects [5–12].

Adiponectin, an adipose tissue-derived hormone, is thought to play an important role in the regulation of insulin sensitivity and glucose and lipid metabolism [13]. Its plasma levels are positively associated with insulin sensitivity [14] and are inversely associated with impaired glucose metabolism and type 2 diabetes [15–17]. Furthermore, observational studies revealed an association between moderate alcohol consumption, adiponectin concentrations and insulin sensitivity [18, 19]. Randomised controlled trials also showed a significant increase in the circulating levels of both total and high molecular weight (HMW) adiponectin [6–8] after a period of moderate alcohol consumption compared with abstention.

However, the mechanism by which moderate alcohol consumption increases adiponectin levels is unknown. Synthesis of adiponectin is controlled by peroxisome proliferator-activated receptor γ (PPAR- γ) activation, which functions as a transcriptional regulator [20]. Low doses of alcohol have been shown to alter the expression of the genes encoding tissue plasminogen activator (*PLAT*) and plasminogen activator inhibitor type-1 (*PAI-1* also known as *SERPINE1*) [21]. Like adiponectin, these markers are affected by moderate alcohol consumption and activated by PPAR- γ [22].

Consequently, we investigated whether moderate alcohol consumption affects expression of the gene encoding adiponectin (*ADIPOQ*) and levels of the protein, and whether it improves insulin sensitivity in postmenopausal women.

Methods

Study protocol The study used a randomised, open label, placebo-controlled, crossover design, consisting of two 6 week periods, each preceded by 1 week of washout. Participants consumed 250 ml of white wine (25 g alcohol; Chardonnay; Jean d'Alibert, Rieux, France) or alcohol-free white grape juice (Albert Heijn, Zaandam, the Netherlands) daily for 6 weeks during dinner. The study was conducted at TNO (a Dutch acronym for the Netherlands organisa-

tion for applied scientific research) Quality of Life, Zeist, the Netherlands. The primary objective of the study was to investigate the effect of moderate alcohol consumption on PPAR- γ activity and risk markers of metabolic disease, and it took place between March and June 2006. Participants were instructed to maintain their habitual body weight, food pattern and physical activity pattern and were told to refrain from any alcoholic products during the entire study (including washout periods) except for alcoholic beverages supplied by TNO. Of the 77 women who were screened, 40 were eligible to participate. A total of 36 women were randomly selected and initiated the study. Allocation to treatment order was randomised according to BMI. Both white wine and white grape juice contained 300 kJ/100 ml (~70 kcal/100 ml). Blood and adipose tissue sampling was done on the last day of each treatment period after an overnight fast. Compliance was monitored by weekly measurement of urinary ethyl glucuronide (EtG), a direct phase II metabolite of ethanol formed by the action of UDP-glucuronosyl transferase. EtG has been reported to be a superior marker, with 100% sensitivity as a biomarker of recent drinking [23]. It persists in the urine up to ~75–85 h after the last intake. Additional measures of compliance were an increase in HDL-cholesterol, daily questionnaires, and return of empty bottles.

Participants Postmenopausal women were recruited from a pool of volunteers from TNO Quality of Life and by advertisements in local newspapers. Eligible participants consumed between 5 and 21 units of alcohol per week, were apparently healthy and had an absence of menses for at least 2 years, a BMI between 18.5 and 35 kg/m² and no family history of alcoholism. Participants gave written informed consent and received compensation for their participation. An independent medical ethics committee (The Medical Ethics Committee of the University Medical Centre Utrecht) approved the research protocol.

Outcome measures Primary endpoints were markers of insulin sensitivity, such as homeostasis model assessment of insulin resistance (HOMA-IR), calculated as [fasting serum insulin (in μ U/l) \times fasting serum glucose (in mmol/l)]/22.5 [5], glucose, insulin, and several adiponectin-related measures, such as *ADIPOQ* mRNA levels and plasma levels of total and HMW adiponectin. Secondary variables included lipid profile (triacylglycerol and HDL- and LDL-cholesterol) HbA_{1c} and NEFA and correlations between specific outcome measures.

Analyses and assessment Ethyl glucuronide was determined in morning urine samples. Urinary samples were diluted about 20-fold using an internal standard solution. The resulting solution was analysed using a triple quadru-

Table 1 Characteristics of 36 postmenopausal women before intervention

Variable	Baseline value
Age (years)	56.5±4.2
Weight (kg)	71.4±10.0
BMI (kg/m ²)	25.4±3.3
Fasting glucose (mmol/l)	5.5 ±0.4
Fasting insulin (pmol/l)	37.4±12.6
Fasting triacylglycerol (mmol/l)	1.21±0.54
HDL-cholesterol (mmol/l)	1.67±0.27
LDL-cholesterol (mmol/l)	3.88±0.60
Total cholesterol (mmol/l)	6.11±0.72
Alkaline phosphatase (U/l)	76.1±19.3
Alanine aminotransferase (U/l)	15.3±8.2
Aspartate aminotransferase (U/l)	20.8±7.4
γ-Glutamyltranspeptidase (U/l)	21.3±11.4
Follicle-stimulating hormone (IU/l)	108.5±41.3

Data are mean±SD

pole Ultra Performance LC/MS in MRM mode (Waters, Saint-Quentin en Yvelines, France) with a detection limit of 50 ng/ml. The cut-off limit to assess compliance during juice intervention for urinary EtG values was set at >0.25 mg/ml (positive sample), to obtain high sensitivity but avoid positive results due to unintentional ethanol exposure. The cut-off limit during wine intervention was set at EtG <0.5 mg/ml (2.2 μmol/l) (negative sample), as proposed by Bottcher et al. [24]. Blood samples were obtained from the antecubital vein of the forearm and collected in tubes containing clot activator for serum and in ice-chilled tubes containing or potassium ethylenediamine tetraacetic acid (K₃EDTA) for plasma (Vacutainer Systems, Becton Dickinson, Plymouth, UK). Blood was centrifuged

for 15 min at 2,000×g at 4°C within 15–30 min after collection and stored at −80°C. All biochemical determinations in blood were performed at TNO Quality of Life using Olympus analytical equipment and reagents except for adiponectin. Plasma total adiponectin concentrations were determined by RIA (Linco Research, St Charles, MO, USA) with a mean intra-assay coefficient of variation of 5.0%, and plasma HMW adiponectin levels were determined by a novel ELISA (Linco Research) with a mean intra-assay coefficient of variation of 14.1%. LDL-cholesterol was calculated using the Friedewald formula. Subcutaneous adipose tissue was sampled from the buttock by means of a Strauss cannula, outer diameter 1.5 mm, and stored at −70°C until further analysis. Total RNA was extracted from adipose tissue with TRIzol reagent (Invitrogen, Breda, the Netherlands); 1 mg total RNA was then reverse-transcribed with iScript (Bio-Rad, Veenendaal, the Netherlands). cDNA was PCR-amplified with Platinum Taq DNA polymerase (Invitrogen) on an iCycler (Bio-Rad) or MyIQ (Bio-Rad) PCR machine. Expression was standardised to the level of acidic ribosomal protein *ARBP* [25]. Primer sequences used were as follows: *ADIPOQ* forward, TATCCCAACATG CCCATTCG; *ADIPOQ* reverse, TGGTAGGCAAAGTAG TACAGCC; *PPARG* forward, TCCATGCTGTTATGGGT GAA; *PPARG* reverse, TCAAAGGAGTGGGAGTGGTC; *ARBP* forward, CGGGAAGGCTGTGGTGCTG; *ARBP* reverse, GTGAACACAAAGCCCACATTC.

Statistical analyses Statistical analyses were performed using the SAS statistical software package (SAS version 8; SAS Institute, Cary, NC, USA). All variables were compared between treatments with a mixed analysis of variance model that included terms for treatment, period and the interaction between period and treatment (indicat-

Table 2 Markers of insulin sensitivity, adiponectin and lipid profile in 36 postmenopausal women measured at the end of a 6 week period of consuming white grape juice or white wine

Variable	Grape juice	White wine	<i>p</i> value
Insulin sensitivity			
HOMA-IR	1.64±0.13	1.42±0.13	0.02
Fasting insulin (pmol/l)	46.5±3.4	40.0±3.4	<0.01
Fasting glucose (mmol/l)	5.4±0.1	5.4±0.1	0.72
HbA _{1c} (%)	6.0±0.04	5.9±0.04	0.09
Fasting NEFA (mmol/l)	0.43± 0.04	0.44±0.04	0.67
Body weight (kg)	70.4±1.7	71.1±1.7	<0.001
Adiponectin			
<i>ADIPOQ/ARBP</i> mRNA (arbitrary units)	0.98±0.15	1.09±0.15	0.04
<i>PPARG/ARBP</i> mRNA (arbitrary units)	0.67±0.09	0.73±0.09	0.13
Fasting total adiponectin (μg/ml)	12.0±0.8	13.1±0.8	<0.001
Fasting HMW adiponectin (μg/ml)	8.8±1.2	9.9±1.2	0.02
Lipid profile			
Fasting triacylglycerol (mmol/l)	1.18±0.08	1.03±0.08	0.04
HDL-cholesterol (mmol/l)	1.57±0.04	1.68±0.04	<0.0001
LDL-cholesterol (mmol/l)	3.84±0.12	3.51±0.12	<0.0001

Data are mean±SEM

ing possible carryover effects). Body weight was included in the model as a random factor to adjust for changes in body weight. Correlation coefficients were computed according to Spearman rank order to assess associations between intervention-induced changes in outcome measures. Based on a study by Arvidsson et al. [26], a sample size of 36 was considered sufficient to detect a 20% difference in *ADIPOQ* mRNA levels with a power of 80%, accepting a two-sided α of 0.05. Data are presented as mean \pm SEM unless otherwise specified. Statistical significance was defined as $p < 0.05$.

Results

General results All 36 postmenopausal women completed the study. Participants were slightly overweight (BMI 25.4 \pm 3.3 kg/m²). Mean glucose, insulin, triacylglycerol and cholesterol concentrations before the intervention indicated that participants were euglycaemic and normolipidaemic (Table 1).

Compliance with treatments was assessed by weekly urinary EtG, revealing two positive samples during the juice-drinking period and six negative samples during the wine-drinking period out of the 468 samples analysed (overall compliance 98.3%). Another indicator of compliance was the 7.0% higher level of HDL-cholesterol after wine consumption compared with the value after juice consumption (Table 2).

No significant ($p > 0.05$) carryover effects, as assessed by interaction between treatment and period effects, were observed for any outcome measures. Treatment means of variables and p values estimated from models with period effects were very similar to those obtained from models without a period effect, and we report the results of the latter. Mean body weight was 0.7 kg higher ($p < 0.001$) at the end of the wine-drinking period than at the end of the juice-drinking period (Table 2). Results were adjusted for this difference in body weight but did not essentially change them. Therefore, unadjusted results are presented here.

Insulin sensitivity Fasting serum insulin concentration was lower (by 12.3%; $p < 0.01$) and the HbA_{1c} percentage tended to be lower ($p = 0.09$) after 6 weeks of moderate alcohol consumption than after 6 weeks of abstinence. Insulin sensitivity, as expressed by the HOMA-IR index, improved by 11.9% ($p = 0.02$) after white wine consumption compared with that following juice consumption. However, serum glucose and NEFA concentrations did not differ significantly between the two interventions at the end of the intervention period (Table 2).

Adiponectin There was a significant increase in the relative *ADIPOQ* mRNA levels in subcutaneous adipose tissue (27.7%; $p = 0.04$) of women who consumed alcohol in moderation for 6 weeks compared with those who consumed juice. Consistently, the ratio of *PPARG/ARBP* mRNA levels also tended to increase, although not significantly ($p = 0.13$), after the alcohol treatment. Plasma protein levels of total adiponectin (10.6%; $p < 0.001$) and HMW adiponectin (15.8%; $p = 0.02$) increased significantly after consuming white wine, rather than juice, for 6 weeks (Table 2).

Lipid profile Besides a significant increase in HDL-cholesterol, changes in lipid profile were further characterised by significantly lower levels of LDL-cholesterol (7.8%; $p < 0.001$) and triacylglycerol (8.2%; $p = 0.04$) after the wine-drinking period compared with the juice-drinking period (Table 2).

Correlations Changes in *ADIPOQ* mRNA levels due to alcohol consumption correlated positively with changes in *PPARG* mRNA levels ($\rho = 0.76$; $p < 0.001$) and with changes in the plasma protein level of total adiponectin ($\rho = 0.46$; $p < 0.01$), but not with changes in plasma levels of HMW adiponectin ($\rho = -0.25$; $p = 0.15$). No significant correlations were found between changes in HOMA-IR and changes in plasma levels of total adiponectin ($\rho = 0.27$; $p = 0.11$) or HMW adiponectin ($\rho = -0.18$; $p = 0.29$). Furthermore, changes in HDL-cholesterol correlated significantly with changes in total adiponectin ($\rho = 0.47$; $p < 0.01$) but not with HMW adiponectin levels ($\rho = 0.13$; $p = 0.44$).

Discussion

The primary findings of this study are that daily, moderate alcohol intake improves insulin sensitivity and lipid profile in postmenopausal women. Furthermore, *ADIPOQ* mRNA levels in subcutaneous adipose tissue along with plasma levels of total and HMW adiponectin increased after moderate alcohol consumption. Moreover, changes in *ADIPOQ* mRNA levels correlated with changes in plasma total adiponectin, indicating that prolonged moderate alcohol intake increases circulating adiponectin through increased gene expression. However, improvements in insulin sensitivity were not associated with increased plasma adiponectin levels (HMW and total) after moderate alcohol consumption in these women.

A strength of this study is its randomised crossover design. Another strength is that we assessed compliance to study treatment several times in different ways throughout the study and observed no significant deviations. Several

limitations warrant consideration. A slightly lower body weight was observed after the juice intervention compared with the alcohol intervention. However, lower body weight is associated with improved insulin sensitivity and thus could have only affected the results towards no alcohol-induced improvement in insulin sensitivity. In the present study, only subcutaneous adipose tissue samples were used to determine *ADIPOQ* mRNA levels. Studies demonstrated that *ADIPOQ* mRNA levels were higher [27, 28] or not different [29] in subcutaneous compared with visceral adipose tissue in lean and obese participants. Furthermore, studies in human adipose tissue showed increased [30] as well as similar [31] *ADIPOQ* mRNA and in vitro adiponectin secretion levels from subcutaneous compared with omental adipocytes. Subcutaneous adipose tissue thus appears to be at least as important as omental adipose tissue for circulating plasma adiponectin levels.

The observed changes in insulin sensitivity after prolonged moderate alcohol intake are in accordance with a study by Davies et al. [4] in which moderate alcohol consumption in postmenopausal women for 8 weeks decreased fasting insulin and triacylglycerol levels—both independent risk factors for type 2 diabetes—and improved insulin sensitivity. However, other clinical trials did not find a change in fasting insulin levels [9] and triacylglycerol levels [32, 33] or in insulin sensitivity after moderate alcohol consumption [5–8, 10, 11], whereas levels of total adiponectin [6, 7] and HMW adiponectin [8] did increase. Differences in the designs used in these clinical trials are numerous. However, studies that did observe an effect on markers of insulin sensitivity after moderate alcohol consumption were all performed in middle-aged [4], relatively insulin-resistant [6] or diabetic people [34, 35]. This suggests that the effect of moderate alcohol intake on insulin sensitivity is more pronounced in people with (slightly) impaired glucose tolerance rather than young and glucose-tolerant people. Moreover, most interventions that did not observe a link between alcohol and insulin sensitivity involved only 30 [5, 12] or even fewer [6–8, 11] days of consecutive alcohol consumption, while this study and the studies of Davies et al. [4] and Shai et al. [34] lasted 6, 8 and 12 weeks respectively. This indicates that a longer duration of alcohol consumption may be needed to exert an effect on insulin sensitivity. Finally, the relation between moderate alcohol intake and insulin sensitivity might be influenced by sex, since the association between alcohol consumption and type 2 diabetes appears to be stronger in women than in men, as observed in some observational studies [1]. This sex difference may be explained partly by changes associated with menopause, which may mediate the relation between alcohol and triacylglycerol levels, the latter being an independent risk factor for type 2 diabetes [36]. Compared

with postmenopausal non-drinkers, postmenopausal drinkers have lower triacylglycerol levels, whereas male drinkers have higher triacylglycerol levels compared with male abstainers [37].

Intervention-associated changes in *ADIPOQ* mRNA in subcutaneous adipose tissue correlated with changes in plasma protein levels of total adiponectin. In line with this are cross-sectional studies [38, 39] showing associations between subcutaneous *ADIPOQ* mRNA levels and plasma levels of adiponectin. This finding suggests that the alcohol-induced increase in plasma adiponectin levels is mediated by de novo synthesis rather than decreased renal function [40] or post-transcriptional regulation [41]. Changes in plasma HMW adiponectin did not correlate with changes in *ADIPOQ* mRNA levels. However, the formation of multimer oligomers of HMW adiponectin is influenced by several post-translational modifications [42], which can account for the absence of such a correlation.

Adiponectin synthesis is under the control of the transcription factor PPAR- γ [20]. PPAR- γ ligands induce expression of *ADIPOQ* via direct binding of the PPAR- γ /retinoid X receptor heterodimer to the PPAR-responsive element in the human *ADIPOQ* promoter. We indeed observed a trend for increased *PPARG* mRNA levels after the alcohol intervention. However, our sample size of 36 participants was anticipated to detect a 20% change in mRNA levels. As the intervention gave rise to a change of only 9% in *PPARG* mRNA, our study was underpowered to detect a significant difference. Besides this trend for increased PPAR levels, we also found a strong correlation between intervention-associated changes in levels of *PPARG* and *ADIPOQ* mRNAs. Furthermore, previous research has shown that *PAI-1* expression and PAI-1 protein levels increase after alcohol consumption [21]. *PAI-1* expression is also regulated by PPAR- γ [22]. It thus seems plausible that moderate alcohol use might alter *ADIPOQ* expression and, consequently, adiponectin protein levels, also in a PPAR- γ -mediated manner.

Consistent with previous findings in young and middle-aged men [6, 7], we now report that total adiponectin plasma levels also increase in postmenopausal women after moderate alcohol consumption. Moreover, we found that the HMW form of plasma adiponectin also increased after 6 weeks of alcohol consumption. The increase in HMW adiponectin after moderate alcohol consumption is in line with a previous study in which a trend was observed [8]. In the present study, however, we used a more sensitive and precise method to quantify HMW adiponectin—ELISA instead of quantitative western blotting.

The observed changes in insulin sensitivity as measured by HOMA-IR did not correlate with the alcohol-induced changes in total or HMW adiponectin, the latter of which is proposed to be a better predictor of insulin sensitivity [43].

These findings are consistent with other lifestyle interventions, such as weight loss [44] and exercise interventions [45], and with previous observations of our group [7, 8] which showed increased adiponectin plasma levels without changes in insulin sensitivity. In this study we did find an effect of alcohol on insulin sensitivity irrespective of changes in adiponectin levels. The present results may suggest a possible independent effect of alcohol on insulin sensitivity in addition to an adiponectin-mediated effect on insulin sensitivity.

Looking at our cross-sectional data, we did observe correlations between HOMA-IR and adiponectin levels after both alcohol and placebo treatment (data not shown). This is in line with published literature in which associations between adiponectin and insulin sensitivity have been shown consistently [14–17]. Possibly, increased adiponectin levels partly explain the improved insulin sensitivity seen among moderate alcohol consumers [18, 19]. Studies have revealed that circulating adiponectin levels are inversely associated with markers of inflammation [39, 46, 47], whereas low-grade inflammation is thought to contribute to insulin resistance [48]. Therefore, adiponectin may lead to improved insulin sensitivity through effects on inflammatory markers.

In conclusion, the results of the present study demonstrate that 6 weeks of moderate alcohol consumption improves insulin sensitivity and the lipid profile in postmenopausal women. Furthermore, moderate alcohol consumption increases subcutaneous adipose *ADIPOQ* mRNA levels and plasma levels of adiponectin. This suggests that the alcohol-induced increase in adiponectin is attributable, at least in part, to transcriptional alterations in adipose tissue. The observed improvements in insulin sensitivity, lipid profile and adiponectin levels when consuming 2.5 standard drinks per day may reduce the risk of type 2 diabetes and cardiovascular disease in postmenopausal women. Despite these potentially beneficial findings, the scientific literature indicates that alcohol drinking may also have detrimental effects on health [49]. Both potential benefits and risks should be taken into consideration when counselling people about consumption.

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Duality of interest The authors declare that there is no duality of interest associated with this manuscript.

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