

# Effect of Moderate Alcohol Consumption on Adiponectin, Tumor Necrosis Factor- $\alpha$ , and Insulin Sensitivity

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**OBJECTIVE** — Epidemiological studies suggest that moderate alcohol consumers have enhanced insulin sensitivity and a reduced risk of type 2 diabetes. Adiponectin, an adipocyte-derived plasma protein, has been found to be negatively associated with adiposity and positively associated with insulin sensitivity. Moderate alcohol consumption may increase adiponectin, which in turn causes a decrease of tumor necrosis factor (TNF)- $\alpha$ . A decreased TNF- $\alpha$  level may consequently increase insulin sensitivity.

**RESEARCH DESIGN AND METHODS** — To test this hypothesis, we performed a randomized crossover partially diet-controlled study. A total of 23 healthy middle-aged male subjects consumed daily four glasses of whisky (40 g ethanol) or tap water with dinner during two successive periods of 17 days.

**RESULTS** — Moderate alcohol consumption increased plasma adiponectin level (11%;  $P = 0.0002$ ) but did not affect plasma TNF- $\alpha$  level. An increase in insulin sensitivity index was observed in an insulin-resistant subgroup (21%;  $P = 0.11$ ), which positively correlated with the relative alcohol-induced increase in plasma adiponectin level ( $r = 0.73$ ,  $P = 0.02$ ).

**CONCLUSIONS** — The experimental results are in agreement with observational data. Moderate alcohol consumption improved insulin sensitivity in relatively insulin-resistant middle-aged men, an effect that may be mediated through alcohol-induced increases in adiponectin.

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Cross-sectional (1–7) and large prospective (8–13) studies have shown that moderate alcohol consumption is associated with enhanced insulin sensitivity and a reduced incidence of type 2 diabetes.

Recent hyperinsulinemic-euglycemic

clamp (14,15) and other (16) studies have revealed that a high level of adiponectin, an adipocyte-derived plasma protein, is closely related to increased insulin sensitivity. This suggests that dysregulation of adiponectin may be relevant to the development of insulin resistance (17). Plasma

adiponectin levels are indeed significantly lower in subjects with obesity (16,18) and type 2 diabetes (19).

A recent study showed that low fasting plasma adiponectin concentrations are associated with high basal and low insulin-stimulated skeletal muscle insulin receptor tyrosine phosphorylation, which is one possible cause of decreased insulin sensitivity (20). High levels of tumor necrosis factor (TNF)- $\alpha$  have long been implicated to cause high basal tyrosine phosphorylation of the skeletal insulin receptor (21) and in turn insulin resistance (22,23). In vitro studies showed that adiponectin reduced both TNF- $\alpha$  production and TNF- $\alpha$ -induced biological effects (17,24,25).

To date, the mechanism for increased insulin sensitivity in moderate alcohol consumers is not well understood. We hypothesized that moderate alcohol consumption affects circulating adiponectin and, via this pathway, the TNF- $\alpha$  level, which has direct effects on the tyrosine phosphorylation of the skeletal insulin receptor and thus insulin resistance. This hypothesis was tested by measuring plasma adiponectin and TNF- $\alpha$  levels and insulin sensitivity in a randomized crossover partially diet-controlled trial.

## RESEARCH DESIGN AND METHODS

A total of 24 male subjects aged 45–65 years, all apparently healthy and nonsmoking, were recruited from the Netherlands Organization for Applied Scientific Research BIBRA International (Carshalton, Surrey, U.K.) database of healthy human volunteers by advertising in local newspapers and by leaflet drops to local residential areas. A questionnaire (self-report) was used for information on alcohol intake, medical history, and family history of alcoholism. The questionnaire was checked by a medical investigator during an interview with the volunteer, and subsequently a physical examination was performed. Subjects were considered healthy based on the values of the prestudy laboratory tests, their medical history, and the physical exami-

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**Abbreviations:** ISI, insulin sensitivity index; TNF, tumor necrosis factor.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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nation. Subjects fulfilled the following inclusion criteria: consumption between 10 and 28 alcohol-containing beverages weekly, BMI between 20 and 35 kg/m<sup>2</sup>, and no family history of alcoholism. A wide range of BMIs was chosen to investigate whether the effect of moderate alcohol consumption on the outcome measures is modified by level of obesity (26). One volunteer was withdrawn from the study because of a cause unrelated to treatment. The remaining 23 subjects finished the experiment successfully.

The study was conducted in accordance with the Declaration of Helsinki South Africa Revision 1996 and International Conference of Harmonisation Harmonized Tripartite Guideline for Good Clinical Practice. Approval to proceed with the study was given by an independent medical ethics committee, and all subjects provided written informed consent before participation.

The subjects entered a randomized crossover partially diet-controlled trial consisting of two periods of 17 days. A random sample of 12 men was allocated to the sequence of consuming whisky (Famous Grouse Scotch Whisky, 40% by volume alcohol) during dinner for 17 days followed by drinking tap water (control beverage) during dinner for the last 17 days. The other 12 men consumed water first, followed by whisky. The participants and staff administering the protocol were unblinded to the treatment sequence. Because of the time required to measure insulin sensitivity by the hyperinsulinemic isoglycemic glucose clamp technique, it was necessary to stagger the starting days. Three subjects commenced the study each day until day 8, at which time all 24 subjects had started the study.

Four glasses (125 ml in total) of each beverage were consumed daily during dinner at the Netherlands Organization for Applied Scientific Research BIBRA. One glass was taken before dinner, two glasses during dinner, and one glass after dinner. During the whisky period, alcohol intake equaled 40 g/day.

The daily dinner contained ~4,200 kJ and consisted of ~21% of energy from protein, 38% of energy from fat, and 41% of energy from carbohydrate. The menu comprised a 4-day rotation. Dinner was prepared each day by a local caterer using the same source of ingredients throughout.

There was a 3-day alcohol-free period before each treatment to prevent possible

carryover effects, and subjects were required to remain alcohol free (except that consumed for study purposes) for the duration of the experiment. Subjects were asked to continue their normal eating habits (except for the provided dinner) and carry on with their normal everyday activities. Compliance to the protocol was checked by a daily questionnaire.

At the end of each treatment period, fasting blood samples were collected in the morning before the hyperinsulinemic isoglycemic glucose clamp was carried out. Blood was taken from an antecubital vein and collected in a tube containing lithium-heparin and in a tube containing gel and a clot activator (Vacutainer Systems; Becton Dickinson, Plymouth, U.K.). To obtain plasma, the blood was centrifuged for 20 min at 2,000g and 20°C, between 15 and 30 min after collection. To obtain serum, the blood was centrifuged for 15 min at 2,000g and 4°C, between 15 and 30 min after collection. The plasma and serum samples were stored at -80°C until analysis. Body weight was measured on the first and last day of each treatment period with the subjects wearing indoor clothing, without shoes, wallet, and keys.

An alcohol breath test (Alcoholtest 7410; Dräger Nederland, Zoetermeer, the Netherlands) was carried out once during the whisky treatment at ~1 h after dinner.

#### Hyperinsulinemic isoglycemic glucose clamp

At the end of each treatment period after an overnight fast of at least 10 h, sensitivity to insulin-mediated glucose uptake was assessed by the hyperinsulinemic isoglycemic glucose clamp technique according to the principles described by DeFronzo et al. (27). Insulin and glucose solutions were administered through a cannulated vein. Arterialized (by warming at ~55°C) venous blood was sampled through a cannulated antecubital vein from the other arm. Insulin was constantly infused (1 mU · kg body wt<sup>-1</sup> · min<sup>-1</sup>). Blood glucose concentration was maintained at the basal concentration throughout the clamp by monitoring the glucose concentration at 5-min intervals using a blood glucose meter (Accutrend; Boehringer Mannheim, East Sussex, U.K.) and adjusting the infusion rate of a 20% wt/vol glucose solution. A steady-state plasma glucose level was reached between 60 and 110 min. Blood was col-

lected in a tube containing gel and a clot activator for plasma glucose determination and also at 0, 30, and 60 min for plasma insulin and C-peptide measurements. To obtain plasma, the blood was centrifuged for 20 min at 2,000g and 20°C, between 15 and 30 min after collection, and the plasma samples were stored at -80°C until analysis. The plasma glucose levels were used for the calculations of insulin sensitivity. A variation in plasma glucose levels <15% during the last hour of the clamp was accepted.

Because the rate of glucose utilization is related to the blood glucose concentration, the metabolic clearance rate was calculated by dividing the average glucose infusion during the last hour of the clamp by the average plasma glucose concentration and multiplying by 5. To correct for differences in the steady-state plasma insulin achieved during the clamp, the metabolic clearance rate was divided by the steady-state plasma insulin, which gives the insulin sensitivity index (ISI). The ISI, corrected for plasma glucose during steady state  $\{[(\text{glucose infusion rate}/\text{steady-state plasma glucose}) \times 5]/\text{steady-state plasma insulin (mg glucose}^{-1} \cdot \text{kg body wt}^{-1} \cdot \text{min} \cdot \mu\text{U insulin}^{-1} \cdot \text{ml}^{-1})\}$ , was taken as the measure of insulin sensitivity.

#### Adiponectin, TNF- $\alpha$ , HDL cholesterol, triglycerides, free fatty acids, glucose, insulin, and C-peptide

Plasma adiponectin concentrations were determined using a validated sandwich enzyme-linked immunosorbent assay using an adiponectin-specific antibody (ANOC9108) (intra- and interassay coefficients of variation of 3.3 and 7.4%, respectively) (18,28,29). Plasma TNF- $\alpha$  levels were determined using a high-sensitivity enzyme-linked immunosorbent assay [Quantikine (R) HS; R&D Systems, Abingdon, Oxon, U.K.]. The lower limit of detection for plasma TNF- $\alpha$  was 0.25 pg/ml, and the intra- and interassay coefficients of variation were <13%. Serum HDL cholesterol and free fatty acid levels were determined with an enzymatic method using commercially available test kits (Roche Diagnostics, Mannheim, Germany, and Randox, Crumlin, Antrim, U.K., respectively). Serum triglyceride levels were determined with the GPO-PAP (glycerolphosphate-

oxidase-phenolaminoantipyrin) method using a commercially available test kit from Roche Diagnostics.

Levels of plasma glucose, insulin, and C-peptide during the last hour of the clamp were determined using commercially available test kits (Roche Diagnostics; Tosoh Corporation, Tokyo; and Euro-Diagnostica, Malmö, Sweden, respectively). The intra- and interassay coefficients of variation of these analyses varied between 1.1 and 5.5%.

All samples were analyzed in one run after the finish of the study. Staff members who conducted the laboratory analyses were blinded to the group assignments.

### Power calculation

A power calculation was based on the assumption that the variance of insulin sensitivity within subjects is ~10%. A sample size of 24 subjects would enable us to observe an increase in insulin sensitivity of 10% with a power of 80%.

### Data analysis

Data were analyzed using the SAS statistical software package (SAS/STAT version 6.12; SAS Institute, Cary, NC). Carryover effects were tested by ANOVA. Treatment effects were assessed by the mixed procedure. A model was built including the following factors: insulin sensitivity subgroup (insulin-sensitive subgroup: BMI <25 kg/m<sup>2</sup> and ISI >5 mg glucose<sup>-1</sup> · kg body wt<sup>-1</sup> · min · μU insulin<sup>-1</sup> · ml<sup>-1</sup>; insulin-resistant subgroup: BMI ≥25 kg/m<sup>2</sup> and ISI ≤5 mg glucose<sup>-1</sup> · kg body wt<sup>-1</sup> · min · μU insulin<sup>-1</sup> · ml<sup>-1</sup>, period, treatment, and insulin sensitivity subgroup in combination with treatment. The effects within insulin-sensitive and -resistant subgroups were also analyzed in this model.

Correlation coefficients were computed to assess associations between rel-

**Table 1—Characteristics of the volunteers included in the data analysis**

Age (years)	52 ± 5 (45–65)
Body weight (kg)	81.4 ± 11.5 (61.2–100.9)
BMI (kg/m <sup>2</sup> )	26.7 ± 3.0 (21.4–33.3)
Hemoglobin (mmol/l)	8.8 ± 0.6 (7.5–10.0)
Triglycerides (mmol/l)	1.2 ± 0.7 (0.3–3.0)
Total cholesterol (mmol/l)	5.6 ± 0.9 (3.9–7.3)
HDL cholesterol (mmol/l)	1.4 ± 0.4 (0.9–2.5)
LDL cholesterol (mmol/l)	3.6 ± 0.7 (2.2–5.5)
Alkaline phosphatase (units/l)	56 ± 13 (39–88)
Asparagine aminotransferase (units/l)	25 ± 5 (18–41)
Alanine aminotransferase (units/l)	28 ± 10 (14–51)
γ-Glutamyltransferase (units/l)	26 ± 12 (13–49)
Glucose (mmol/l)	4.6 ± 0.6 (3.7–5.7)
Insulin (mU/l)	8.9 ± 8.8 (1.0–40.0)

Data are means ± SD (range). n = 23.

ative changes in outcome measures. Two-sided *P* values were considered statistically significant at *P* ≤ 0.10.

**RESULTS**— Characteristics of the 23 subjects in the data analysis are given in Table 1. No important deviations in consumption of the supplied foods and drinks occurred during the study, average body weight did not differ between whisky and water treatment periods, and no carryover effects in outcome measures were seen (data not shown). Physical adverse events included some minor headaches and common colds mainly (data not shown). Medicine use was restricted to paracetamol and medication against common colds (cough mixture, ear drops). One subject used salbutamol, a β-specific bronchodilator, which did not affect the outcome, because exclusion of this subject in the data analysis did not change the results. The mean breath alcohol concentration at 1 h after dinner with whisky was 0.43 g/l (range 0.26–0.68).

### Adiponectin, TNF-α, HDL cholesterol, triglycerides, and free fatty acids

Plasma adiponectin concentration increased by 11% after 17 days' consumption of whisky compared with water consumption (*P* = 0.0002; Table 2). Moderate alcohol consumption did not affect plasma TNF-α level (*P* = 0.62; Table 2). Serum HDL cholesterol level increased with whisky consumption compared with water consumption (1.30 vs. 1.22 mmol/l, *P* = 0.0009), whereas serum triglyceride and free fatty acid levels did not change (1.36 vs. 1.24 mmol/l, *P* = 0.16, and 0.33 vs. 0.37 mmol/l, *P* = 0.18, respectively).

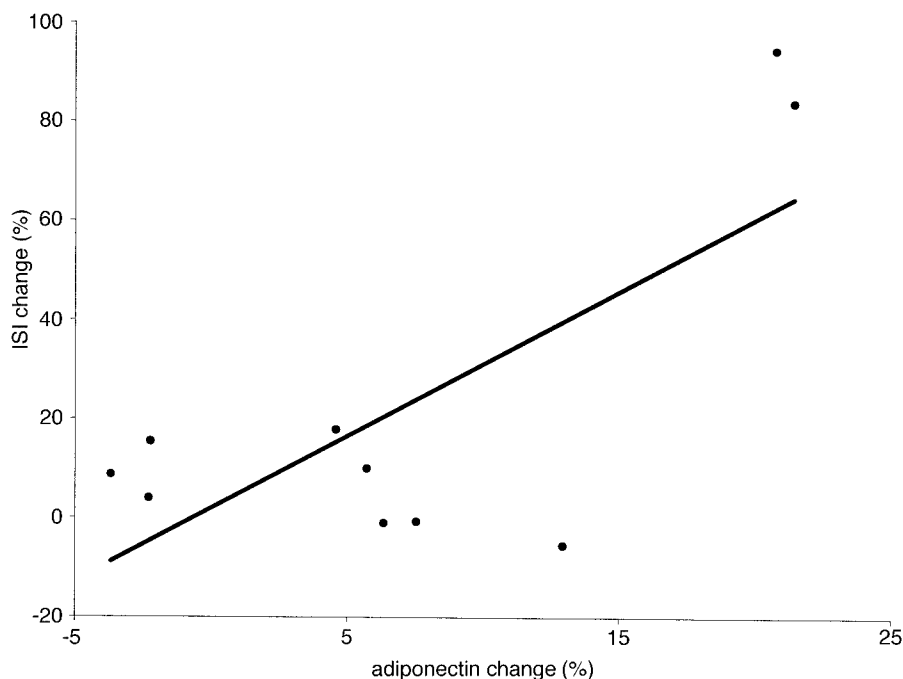
### ISI

One subject had a variation in plasma glucose levels >15% during the last hour of the clamp, and therefore that measurement was excluded from the data analysis. Insulin infusion during the clamp resulted in an average rise in plasma insulin from 8.4 ± 4.8 to 90.9 ± 8.8 mU/l (means ± SD) during the whisky treat-

**Table 2—Plasma adiponectin and TNF-α levels and ISI after 17 days' consumption of water and whisky with dinner**

	All subjects (n = 23)			Insulin-sensitive subgroup (n = 13)			Insulin-resistant subgroup (n = 10)		
	Water	Whisky	<i>P</i>	Water	Whisky	<i>P</i>	Water	Whisky	<i>P</i>
Adiponectin (μg/ml)	7.94	8.78	0.0002	8.65	9.79	0.0002	7.23	7.76	0.07
TNF-α (pg/ml)	0.69	0.67	0.62	0.67	0.60	0.25	0.72	0.75	0.73
ISI (mg glucose <sup>-1</sup> · kg body wt <sup>-1</sup> · min · μU insulin <sup>-1</sup> · ml <sup>-1</sup> )	5.91	6.21	0.36	8.10	7.92	0.67	3.73	4.50	0.11

Insulin-sensitive subgroup: BMI <25 kg/m<sup>2</sup> and ISI >5 mg glucose<sup>-1</sup> · kg body wt<sup>-1</sup> · min · μU insulin<sup>-1</sup> · ml<sup>-1</sup>. Insulin-resistant subgroup: BMI ≥25 kg/m<sup>2</sup> and ISI ≤5 mg glucose<sup>-1</sup> · kg body wt<sup>-1</sup> · min · μU insulin<sup>-1</sup> · ml<sup>-1</sup>.



**Figure 1**—Relationship between relative alcohol-induced changes in adiponectin and ISI within the insulin-resistant subgroup. Pearson:  $r = 0.73$  (95% CI 0.19–0.93),  $P = 0.02$ .

ment and from  $8.9 \pm 4.9$  to  $95.0 \pm 23.1$  mU/l during the water treatment. Plasma C-peptide concentrations decreased during the clamp after both the whisky (from  $827 \pm 59$  to  $736 \pm 71$  pmol/l) and water (from  $838 \pm 59$  to  $794 \pm 80$  pmol/l) treatment. The stability of the plasma glucose level in the last hour of the clamp, indicated by the coefficients of variation, were  $5.7 \pm 2.2$  and  $6.8 \pm 3.3\%$  during the whisky and water treatment, respectively. Moderate alcohol consumption did not affect ISI in the whole group of subjects ( $P = 0.36$ ; Table 2); a borderline significant increase in insulin sensitivity was observed in the insulin-resistant subgroup ( $P = 0.11$ ; Table 2).

### Correlations

The data tended to show a positive relationship between the relative alcohol-induced increase in adiponectin and ISI within the insulin-resistant subgroup, but CIs of the correlation coefficient were large (Fig. 1). The correlation coefficients between plasma adiponectin and TNF- $\alpha$  levels and between plasma TNF- $\alpha$  level and ISI were not significant (Pearson:  $r = -0.42$ ,  $P = 0.22$ ; Pearson:  $r = -0.39$ ,  $P = 0.27$ , respectively).

In the whole group, the relative alcohol-induced changes in serum HDL cho-

lesterol, triglycerides, and free fatty acid levels did not correlate with the relative alcohol-induced increase in plasma adiponectin level (Pearson:  $r = 0.26$ ,  $P = 0.23$ ; Pearson:  $r = 0.13$ ,  $P = 0.56$ ; Pearson:  $r = -0.16$ ,  $P = 0.46$ , respectively).

**CONCLUSIONS**— To our knowledge, this is the first study investigating the effect of a nutritional compound on plasma adiponectin level. We tried to better understand the mechanism for moderate alcohol consumption to increase insulin sensitivity. Insulin sensitivity was measured with the clamp technique, which is regarded as the reference method for quantifying insulin resistance. Despite the strong design of the study (randomized, crossover, and partially diet-controlled), it may have been underpowered in some aspects.

A moderate dose of alcohol with dinner was associated with a significant increase in plasma adiponectin level in healthy middle-aged men. Moderate alcohol consumption did not affect plasma TNF- $\alpha$  level. In the insulin-resistant subgroup, a borderline significant increase in insulin sensitivity was observed after moderate alcohol consumption ( $P = 0.11$ ). To date, seven studies have investigated the effect of an intervention on

plasma adiponectin levels. Reductions in BMI of 10–40% caused an increase in plasma adiponectin levels up to  $\sim 300\%$  (from 4.4–8 to 6.6–13.6  $\mu\text{g/ml}$ ) (19,30,31). Plasma adiponectin level did not change after 6 months of exercise training (31). Administration of insulin-sensitizing agents in subjects with glucose intolerance or type 2 diabetes increased adiponectin levels from 30% up to 300% (from 3–9 to 9–16  $\mu\text{g/ml}$ ) (32–35). As early as 17 days of whisky consumption increased plasma adiponectin level by 11% compared with water consumption (from 7.94 to 8.78  $\mu\text{g/ml}$ ). The effect may be relatively small because our intervention period was short, the subjects were neither glucose intolerant nor were patients with type 2 diabetes, and nutritional interventions are usually less efficacious than pharmaceutical interventions or weight reduction.

Only three experimental studies investigated the effect of chronic alcohol consumption on insulin sensitivity. One study with 51 postmenopausal women showed a significant increase of insulin sensitivity of 7.2% after 8 weeks of moderate alcohol consumption (36), whereas the other two intervention trials did not find an effect (37,38). This lack of effect could be due to low alcohol dose, short length of the study period, subjects not at risk, or a more variable measure for insulin sensitivity. In our study, ISI increased notably (21%) after moderate alcohol consumption in the insulin-resistant subgroup.

We hypothesized that the increase in adiponectin after moderate alcohol consumption would decrease TNF- $\alpha$ , which consequently would cause an increase in insulin sensitivity. Within the insulin-resistant subgroup, adiponectin and insulin sensitivity increased, and the data suggest a positive relationship between relative changes in those parameters. However, the correlation coefficients between plasma adiponectin and TNF- $\alpha$  levels and between plasma TNF- $\alpha$  level and ISI were not statistically significant. This finding is inconsistent with our hypothesis. But measuring circulating TNF- $\alpha$  might not represent activity at the tissue level. Other potential mechanisms for the actions of adiponectin to improve insulin sensitivity are decreasing hepatic glucose production and increasing muscle fat oxidation (39,40).

The increase in adiponectin was more pronounced in the insulin-sensitive sub-

group, but an increase in ISI was observed in the insulin-resistant subgroup only. Therefore, these first findings in a relatively small group of subjects suggest that adiponectin may not be a direct marker for insulin sensitivity.

In addition to its potential role in the regulation of insulin sensitivity, low levels of adiponectin have also been related to a higher risk for cardiovascular disease (39,41). Recent studies in humans suggest that adiponectin is highly positively correlated with circulating HDL (16,42). Also in the current study, the correlation between the absolute levels of HDL cholesterol and adiponectin were positive ( $r = 0.45$  and  $P \leq 0.05$ ). The alcohol-induced increase in HDL cholesterol did not correlate with the alcohol-induced increase in plasma adiponectin, indicating that the adiponectin effect seems not to be just a marker of alcohol and HDL. A dissociation in alcohol effects on HDL cholesterol and adiponectin correspond with the findings of Furuhashi et al. (43), who showed an increase in adiponectin concentration without affecting HDL cholesterol. This suggests that HDL cholesterol and adiponectin might change independently of each other.

In conclusion, the results of this randomized trial are in agreement with epidemiological data. Moderate alcohol consumption improved insulin sensitivity in relatively insulin-resistant middle-aged men, an effect that may be mediated through alcohol-induced increases in adiponectin.

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