Alcohol consumption, the metabolic syndrome and insulin resistance in 58-year-old clinically healthy men (AIR study)

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ABSTRACT

It has been shown that a light-to-moderate intake of alcohol may enhance insulin sensitivity; a decrease in insulin sensitivity is a component of the clustering of risk factors known as the metabolic syndrome. However, previous studies have been limited to relatively small or heterogeneous study groups, or have used suboptimal methods of measuring insulin action. Hence the aim of the present study was to examine whether the metabolic syndrome (as recently defined), components of this syndrome and smoking are associated with alcohol consumption. The study was performed in a population-based sample of clinically healthy men (n = 391), all 58 years old and not undergoing any treatment with cardiovascular drugs. Insulin-mediated glucose uptake (euglycaemic hyperinsulinaemic clamp) was measured in a subgroup of these subjects (n = 104). Trend analysis showed no difference in alcohol intake across the groups of men with none of the criteria in the definition of the metabolic syndrome (n = 77), men with one or more of the criteria (n = 252) and men fulfilling all criteria (n = 62). However, in the whole group (n = 391), alcohol consumption was significantly positively associated with serum triacylglycerols (triglycerides), high-density lipoprotein (HDL) cholesterol and cigarette-years. Furthermore, alcohol consumption was positively associated with insulin-mediated glucose uptake (r = 0.20, P < 0.05). In multiple regression analyses, body mass index, alcohol consumption and serum triacylglycerols were independent co-variates to insulin-mediated glucose uptake. Thus, in 58-year-old healthy men recruited from the general population, there was a significant association between alcohol consumption, serum triacylglycerols, HDL cholesterol and cigarette-years. In a subgroup of 104 subjects, alcohol consumption was independently and positively associated with insulin-mediated glucose uptake. To our knowledge, this is the first study to show an independent relationship between insulin sensitivity, as measured by the clamp technique, and alcohol intake.

INTRODUCTION

A growing number of studies support the hypothesis that regular light-to-moderate alcohol consumption is beneficial in lowering the risk of coronary heart disease [1,2]. It has also been shown previously that chronic light-to-moderate alcohol intake may enhance insulin sensitivity; a decrease in insulin sensitivity is a component of the clustering of risk factors known as the metabolic syndrome. However, these studies have been limited to relatively small or heterogeneous study groups, or have involved indirect methods of measuring insulin action. To our knowledge, this is the first study to show an independent relationship between insulin sensitivity, as measured by the clamp technique, and alcohol intake.

Key words: alcohol consumption, insulin resistance, metabolic syndrome, smoking.
Abbreviations: BMI, body mass index; FFM, fat-free mass; GIR, glucose infusion rate; GIR_{FFM}, GIR adjusted for FFM; HDL, high-density lipoprotein; LDL, low-density lipoprotein; WHR, waist/hip ratio.
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The methods used to disclose insulin sensitivity have varied from quantification of surrogate variables such as fasting plasma insulin [3] and insulin levels before and after oral glucose tolerance tests [4,5], to the insulin suppression test [5]. Yet authorities concur that the ‘reference technique’ for measuring insulin sensitivity is by utilizing the euglycaemic hyperinsulinaemic clamp technique [6,7].

It has also been postulated that alcohol conveys its positive effects on coronary heart disease by producing an altered lipid and lipoprotein profile. Indeed, it has been shown that regular moderate alcohol intake may be linked to raised levels of high-density lipoprotein (HDL) [2,3,8,9]. Alcohol intake has, however, also been shown to be associated with raised triacylglycerol (triglyceride) levels [10]. Interestingly, both of these variables are included in the definition of the metabolic syndrome. These observations raise the question: is there an association between the aforementioned variables and alcohol intake?

Hence the aim of the present study was to assess the relationship between alcohol intake and the presence of factors that comprise the metabolic syndrome. Furthermore we aimed to investigate the relationship between alcohol consumption and insulin resistance, as measured by the euglycaemic clamp technique. The results were obtained in a population-based sample of healthy men of Swedish ancestry, all 58 years old, selected in order to minimize the effects of confounding factors such as race, sex, age and treatment with different drugs for cardiovascular disease.

**METHODS**

**Study subjects**

This study has been described in detail elsewhere [11]. The inclusion criteria were age 58 years, male sex, and Swedish ancestry. Exclusion criteria were cardiovascular or other clinically overt disease, treatment with cardiovascular drugs that might disturb the measurements performed in the study, or unwillingness to participate. The subjects were selected randomly from among men in the County Council register and were invited to a screening examination.

A power calculation indicated that it was necessary to recruit at least 390 men into the study, with the main objective being to examine the relationship between insulin sensitivity and ultrasound-assessed atherosclerosis. The present report is a substudy within that project. The screening protocol aimed at enriching the population sample with men with low or high insulin sensitivity. Thus, in connection with the screening examination, the subjects were divided into quintiles of a body mass index (BMI)/blood glucose score, which allowed immediate stratification and selection for further studies (n = 818). The following equation was used:

\[
\text{BMI/blood glucose score} = 46.22 - 2.17 \times \text{BMI} - 0.84 \times (\text{whole-body glucose})
\]

This algorithm was based on results from a previous study of clinically healthy men of similar age who had undergone a euglycaemic hyperinsulinaemic clamp examination. The correlation coefficient between the BMI/blood glucose score and the observed insulin sensitivity was 0.81. In the present population sample this score was correlated significantly with insulin sensitivity measured with the euglycaemic hyperinsulinaemic clamp method, when expressed as insulin-mediated glucose uptake adjusted either for body weight (r = 0.69, P < 0.001) or for fat-free mass (FFM) (r = 0.59, P < 0.001) (n = 104).

Following the screening examination (n = 818), every subject in quintile 1 (indicating low insulin sensitivity; n = 153; 39% of the final study population of 391) and quintile 5 (indicating high sensitivity; n = 144; 37%), and every fifth man in quintiles 2–4 (indicating intermediate sensitivity; n = 94; 24%), was invited to further examinations (n = 391). Among these 391 subjects, 22 were found to have a fasting blood glucose concentration of ≥ 6.1 mmol/l. However, none of the subjects had overt diabetes mellitus. From the main study group (n = 391), every fourth subject was randomly selected for a euglycaemic hyperinsulinaemic clamp examination (n = 104). Subjects in the clamp subgroup (n = 104) were distributed as follows: quintile 1, n = 36 (35% of the subpopulation of 104); quintile 2–4, n = 28 (27%); quintile 5, n = 40 (38%). Insulin-mediated glucose uptake was 4.9 ± 2.7, 9.7 ± 2.4 and 9.9 ± 2.8 mg/min⁻¹·kg⁻¹ FFM for those in score-based quintiles 1, 2–4 and 5 respectively (P < 0.001 for trend). This classification was only used in order to recruit subjects with a wide range of insulin sensitivities, and was not used when analysing study results.

The subjects received both written and oral information before they gave their consent to participate. The study was approved by the Ethics Committee at Sahlgrenska University Hospital.

**Definition of the metabolic syndrome**

The definition suggested by a working group consulted by WHO in 1998 [12] was used. The metabolic syndrome is defined as glucose intolerance and/or insulin resistance (see below), together with two or more of the following risk factors. (1) Raised arterial pressure ≥ 160/90 mmHg (either value). (2) Raised triacylglycerols (≥ 1.7 mmol/l) and/or low HDL cholesterol (< 0.9 mmol/l). (3) Abdominal obesity [waist/hip ratio (WHR) > 0.90] and/or BMI > 30. (4) Microalbuminuria (urinary albumin excretion rate ≥ 20 µg/min or albumin/creatinine ratio ≥ 20 mg/g). In the present study subjects with a dipstick
Glucose intolerance was defined as fasting blood glucose ≥5.6 mmol/l. Insulin resistance was defined as fasting plasma insulin ≥14.86 m-units/l. This definition was obtained by using the euglycaemic hyperinsulinaemic clamp method to define glucose uptake below the lowest quartile for the background population under investigation in a representative sample of 50 subjects from the present study. The plasma insulin level corresponding to the lowest quartile for glucose uptake was calculated and used as a cut-off point when defining insulin resistance. The positive and negative predictive values of this plasma insulin cut-off point were 0.88 and 0.86 respectively in 52 subjects from the same background population that had undergone a clamp examination and who were not included in the first calculation above.

The study group (n = 391) was divided into three subgroups: (1) subjects fulfilling the criteria for the metabolic syndrome (n = 62); (2) subjects not fulfilling the criteria, but with one or several criteria used in the definition of the metabolic syndrome (n = 252); and (3) subjects with none of the risk factors constituting the metabolic syndrome (n = 77). Furthermore, a subgroup was randomly selected in which a euglycaemic hyperinsulinaemic clamp examination was carried out (n = 104).

**Measurements**

Established questionnaires were used to evaluate history of previous and current disease, smoking habits and alcohol consumption [13]. The subjects were asked whether they or any parent or sibling had suffered from cardiovascular diseases, diabetes mellitus, hypertension or obesity.

Body weight was measured on a balance scale with the subject dressed in underwear. Measurements of waist and hip circumferences were used to calculate WHR.

At the ultrasound examination, resting blood pressure was measured phonographically in the right arm after supine rest. Blood pressure was calculated to the nearest 1 mmHg, and the mean of two recordings was used in the statistical analyses. A 12-lead standard ECG was recorded. Heart rate was recorded from the ECG. Blood samples for serum cholesterol, serum triacylglycerols and lipoprotein fractions were drawn after a fasting period of at least 6 h and were frozen thereafter in aliquots at −70 °C within 4 h.

The total number of smoking years was multiplied by the number of cigarettes smoked daily, and the product was termed ‘cigarette-years’.

The subjects were asked to estimate their consumption of different types of alcoholic beverages. This information was then used in conjunction with data on percentual alcohol content in order to calculate the total daily intake of ethanol.

**Biochemical analysis**

Serum concentrations of total cholesterol and triacylglycerols were determined by fully enzymic techniques. HDL was determined after precipitation of apolipoprotein B-containing lipoproteins with MnCl₂ and dextran sulphate. Low-density lipoprotein (LDL) was calculated as described by Friedewald et al. [14]. Whole-blood glucose was measured with the glucose oxidase technique [15]. Total plasma insulin was determined in all subjects by RIA (Pharmacia Insulin RIA; Pharmacia Diagnostics, Uppsala, Sweden).

**Euglycaemic hyperinsulinaemic clamp examination**

Several weeks before each examination, the individual subject was asked not to change any habits and, during the 2 days preceding the day of the examination, to avoid unusual physical exercise, alcohol consumption or any major change in caloric intake. Instructions were given to avoid food intake, as well as any medication, smoking or snuff-taking, from midnight on the day preceding the clamp. The subject was allowed to drink water in the morning. Before the examination started, a questionnaire was completed in order to verify that the subject had followed the instructions regarding physical activity, food intake, alcohol intake and tobacco use. The subject was also asked if he had any disease symptoms, such as those indicating respiratory tract infection or fever. In unclear cases, the doctor responsible decided whether to cancel the examination. A euglycaemic hyperinsulinaemic clamp examination was then performed as described by DeFronzo et al. [16].

After the clamp examination, FFM was measured using the dual-energy X-ray absorptiometry body composition model [17] (Lunar DPX-L). Insulin sensitivity was calculated as the glucose infusion rate per min during the final 60 min of infusion, adjusted for FFM (GIRFFM) [18].

**Statistical analysis**

All statistical computations were carried out using SPSS 10 for Windows (SPSS Inc., Chicago, IL, U.S.A.). Nonparametric Spearman’s rank correlation test was used in the correlation analysis. The Mann–Whitney test was used when comparing means in subjects with varying degrees of alcohol consumption, subjects with the metabolic syndrome and subjects with no risk factors.

Furthermore, a t-distributed variable was used to calculate 95% confidence intervals for differences. Mantel’s
test for linear association was used to test the relationship between alcohol intake and the presence of factors that constitute the metabolic syndrome.

A stepwise multiple regression model was used to study the determinants of the euglycaemic GIR. A two-tailed $P$ value of $< 0.05$ was considered as statistically significant. All results were calculated for the study group of 391 men unless otherwise noted.

**RESULTS**

**Characteristics of the subjects by tertiles of alcohol consumption**

The 391 subjects were divided into tertiles according to alcohol consumption (total median intake 9.77 g/day; range 0–129 g/day). The subjects in tertile III, with the highest intake (median 23.2 g/day; range 14.2–129 g/day), differed from those in tertile I (median 0 g/day; range 0–5.6 g/day) by having significantly higher mean values for HDL, serum triacylglycerols, serum cholesterol, pulse pressure, WHR and cigarette-years (Table 1). When tested for trend, HDL, serum triacylglycerols, serum cholesterol and pulse pressure turned out to be associated with alcohol intake. There were no significant differences between the two groups in mean BMI, systolic or diastolic blood pressure, heart rate, serum LDL cholesterol or whole-blood glucose.

**Alcohol consumption in subjects with the metabolic syndrome compared with subjects with no risk factors**

When the subjects were divided into groups according to the presence of risk factors for the metabolic syndrome, no significant differences in mean alcohol consumption were found between those subjects with the metabolic syndrome (as defined previously) and those with no risk factors (Table 2). A test for linear association on the same data did not give further evidence to indicate a statistical relationship ($P = 0.11$ for trend).

**Alcohol consumption in relation to metabolic variables, serum lipids, smoking and insulin sensitivity**

In the univariate correlation analyses, alcohol consumption was found to be significantly and positively correlated with HDL cholesterol, serum triacylglycerols and cigarette-years (Table 3). There was a borderline significant inverse relationship between fasting plasma insulin and alcohol consumption ($r = -0.09$, $P = 0.065$). There were no significant correlations between alcohol consumption and systolic or diastolic blood pressure, pulse pressure, heart rate, serum cholesterol, LDL cholesterol,
Table 3  Correlation analysis of alcohol consumption in relation to metabolic variables and insulin sensitivity
Spearman's correlation coefficients are given for the relationships between the listed variables and alcohol consumption (g/day); n = 391 except for GIR FFM (n = 104). Significance: *P < 0.05, **P < 0.01. Abbreviations: SBP/DBP, systolic/diastolic blood pressure; HR, heart rate.

<table>
<thead>
<tr>
<th>Variable</th>
<th>r</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.021</td>
</tr>
<tr>
<td>WHR</td>
<td>0.004</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>0.068</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>0.010</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>0.037</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>0.086</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>0.090</td>
</tr>
<tr>
<td>Serum triacylglycerols (mmol/l)</td>
<td>0.102 *</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>0.145 **</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>0.012</td>
</tr>
<tr>
<td>Blood glucose (mmol/l)</td>
<td>0.004</td>
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<tr>
<td>Plasma insulin (m-units/l)</td>
<td>0.094</td>
</tr>
<tr>
<td>Cigarette-years</td>
<td>0.158 **</td>
</tr>
<tr>
<td>GIR FFM</td>
<td>0.203 *</td>
</tr>
</tbody>
</table>

Multiple regression
In a multiple regression analysis of the subpopulation that underwent a euglycaemic hyperinsulinaemic clamp examination (n = 104), GIR_{FFM} was the dependent variable and the independent variables were continuous variables that showed a statistically significant univariate association with insulin-mediated glucose uptake. As presented in Table 4, BMI, alcohol intake and serum triacylglycerols, but not serum HDL cholesterol, WHR or cigarette-years, independently of each other explained 46% of the variation in glucose uptake.

Table 4  Stepwise multiple regression analysis showing contributions to the variance in GIR_{FFM}
The model also included serum HDL cholesterol, WHR and cigarette-years. Significance: *P < 0.001.

<table>
<thead>
<tr>
<th>Variable</th>
<th>β-coefficient (standard error)</th>
<th>P</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIR_{FFM}</td>
<td></td>
<td></td>
<td>0.458*</td>
</tr>
<tr>
<td>BMI</td>
<td>0.469 (0.059)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>Alcohol intake</td>
<td>0.326 (0.019)</td>
<td>0.000</td>
<td></td>
</tr>
<tr>
<td>log(Triacylglycerols)</td>
<td>-0.220 (1.302)</td>
<td>0.008</td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION
The results of the present study showed that, in a group of clinically healthy 58-year-old men, the subjects with the highest alcohol intake (tertile III) had higher mean values for HDL cholesterol, serum triacylglycerols, total cholesterol, pulse pressure, WHR and cigarette-years compared with subjects with a low alcohol intake (tertile I). When comparing subjects with the clustering of risk factors typical for the metabolic syndrome with subjects with no risk factors for this syndrome, no significant difference in alcohol consumption was found. In a sub-group of subjects that underwent a clamp examination, BMI, alcohol intake and serum triacylglycerols, but not HDL cholesterol, WHR or cigarette-years, independently of each other explained 46% of the variation in GIR_{FFM}.

In the present study of healthy 58-year-old men, a suggested operative definition of the metabolic syndrome was used, based on the combination of impaired fasting glucose or insulin resistance and the presence of at least two of six factors that are typical characteristics of the metabolic syndrome [11,12]. When using this definition, no significant differences between subject groups could be found when comparing mean alcohol intake.

As expected, we found that subjects with the highest alcohol intake had higher serum HDL cholesterol concentrations compared with low-level consumers, but also a mainly negative metabolic profile, with higher mean values for serum triacylglycerols, total cholesterol, pulse pressure and WHR. An improvement in insulin sen-
sitivity has been found to be associated with higher HDL-cholesterol levels [19]. It has been suggested that this is one of the routes by which alcohol acts upon HDL metabolism, although the exact mechanism remains to be elucidated [20]. In accordance with this, the serum HDL-cholesterol concentration has been shown to increase with alcohol consumption [21]. The present study corroborates these findings.

Moderate regular alcohol consumption has been found to be associated with increased insulin sensitivity in several studies [22]. Women consuming one or two drinks (10–20 g of alcohol) daily had a 15% lower BMI than non-drinkers, despite higher energy consumption, which may have reflected enhanced insulin sensitivity [23,24]. Several measures of insulin sensitivity have been used when investigating the relationship between alcohol intake and insulin sensitivity. For example, in the Bruneck study, comprising 820 healthy, non-diabetic men and women aged 40–79 years, low-to-moderate amounts of alcohol (defined as 0–100 g/day) taken on a regular basis were associated with significant decreases in fasting insulin concentrations, post-glucose insulin concentrations and estimates for insulin resistance obtained using the homoeostasis model [4]. Two further studies have also shown that an increased alcohol intake was associated with lower post-glucose insulin concentrations [20,25]. Other studies have demonstrated that an increased alcohol intake is associated with lower fasting insulin concentrations [3,5,26]. However, no previous study has examined the relationship between insulin sensitivity, as measured by the clamp technique, and chronic light-to-moderate alcohol intake. Although it has a high variability, the euglycaemic hyperinsulinaemic clamp technique is regarded as the ‘reference technique’ when measuring insulin resistance [6,7].

In the present study a subgroup of subjects \( n = 104 \) underwent a euglycaemic hyperinsulinaemic clamp examination. We chose to correct the GIR for FFM, as it has been demonstrated that the severity of insulin-mediated glucose uptake is overestimated when adjusted for total body weight [6]. GIR_{FFM} showed a statistically significant positive correlation with alcohol intake. In a multivariate analysis, BMI, alcohol intake and serum triacylglycerols together and independently accounted for 46% of the variability in glucose uptake.

As mentioned above, we found an independent association between GIR_{FFM} and alcohol intake even after adjustment for metabolic variables such as BMI, triacylglycerols and HDL cholesterol.

Previous experimental studies dealing with acute and chronic ethanol intake have shown divergent results. After acute and relatively high alcohol intake, a decreased insulin sensitivity has been observed in metabolic studies [27,28]. An increased plasma insulin response to glucose after acute alcohol administration has been shown [29], but a biphasic effect has also been demonstrated, with hypoinsulinaemia following initial hyperinsulinaemia [30]. In animal models, inhibition by alcohol of insulin secretion by pancreatic \( \beta \)-cells has been seen [31–33]; a reduced hepatic uptake of insulin has also been reported [34].

Evidence of enhanced insulin sensitivity after chronic light-to-moderate alcohol intake is, on the other hand, relatively consistent [3,5,20,25]. Similar findings have been obtained in animal models [35]. Possible explanations for this phenomenon are as yet scarce. However, the observed relationship between GIR_{FFM} and alcohol intake in the present study localizes the site of alcohol action on insulin sensitivity to the cellular level, since the clamp will tend to eliminate variation in insulin sensitivity due to vascular effects. There are some limitations to the present study. Women were not included, and the results can only be applied for the population of clinically healthy 58-year-old men. Our model does not take into consideration different drinking patterns, or previous alcohol abuse. Also, alcohol intake is a skewed variable, and total abstainers are prevalent in the population. Even though the distribution may be normalized by logarithmic transformation of the alcohol intake or, alternatively, by treating drinkers and non-drinkers as separate populations, there is no agreement on the correct method of analysing this type of data [36]. No consensus exists for defining a moderate alcohol intake, although previous studies have generally regarded this as 20–40 g/day [1], which in our present study corresponds most closely to the tertile with the highest consumption (median 23.2 g/day).

Apart from cigarette smoking, which may have negative effects on insulin sensitivity [37], only components of the metabolic syndrome were considered in the present analyses. Of course, many other factors may affect insulin resistance, including physical activity [38,39], diet [40] and infection [41].

The use of alcohol is a habit that is prevalent in the general population and also potentially modifiable. Hence beneficial effects conferred by its consumption are of interest. One must, however, be cautious in issuing recommendations, given the drug’s well known adverse effects, including social and health risks such as hypertension, liver damage, impairment of \( \beta \)-cell function, diabetes due to pancreatitis, hypercortisolism, neurological damage and cardiomyopathy.

To summarize, in 58-year-old subjectively clinically healthy men recruited from the general population, there was a positive association between alcohol intake and several metabolic variables, such as HDL cholesterol and serum triacylglycerols. No significant difference in alcohol consumption was observed between subjects with the metabolic syndrome and subjects with no risk factors for this syndrome. To our knowledge this is the first study to show an independent relationship between insulin sensitivity, as measured by the clamp technique,
and alcohol intake. Insulin sensitivity was also independently associated with BMI and serum triacylglycerols. As this was a cross-sectional study, no conclusions can be drawn about causality.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Heart-Lung Foundation, the Swedish Medical Research Council (12270, 10880), King Gustav V and Queen Viktoria Foundation, and AstraZeneca (Mönåld, Sweden). We thank laboratory technicians Eva-Lena Alenåg, Anna Fröden and Caroline Schmidt for excellent research assistance. Other members of the AIR study group are John Wikstrand and Lena Bokemark.

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Received 8 June 2001/17 September 2001; accepted 13 November 2001