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ARTICLE

Moderate alcohol consumption is associated with improved insulin sensitivity, reduced basal insulin secretion rate and lower fasting glucagon concentration in healthy women

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Abstract
Aims/hypothesis Moderate alcohol consumption is associated with a reduced risk of type 2 diabetes with a stronger effect in women. As the underlying mechanisms remain poorly characterised, we investigated its relationship with insulin resistance, insulin secretion, clearance of insulin and glucagon concentration.

Methods One-thousand two-hundred and seventy-six non-diabetic individuals from the RISC (relationship between insulin sensitivity and cardiovascular disease) study without high alcohol consumption were studied; all had a euglycaemic–hyperinsulinaemic clamp and an OGTT with assessment of insulin sensitivity, secretion and clearance.

The list of RISC investigators is presented in the electronic supplementary material (ESM).

Electronic supplementary material The online version of this article (doi:10.1007/s00125-012-2701-3) contains peer-reviewed but unedited supplementary material, which is available to authorised users.

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Results Alcohol consumption was positively associated with insulin sensitivity in women (β=0.15, p_{trend}=0.005) and in men (β=0.07, p_{trend}=0.07) after controlling for age, centre, waist, smoking and physical activity. In women, this association persisted after adjustment for adiponectin but was attenuated after controlling for HDL-cholesterol, suggesting that part of the protection is related to a higher HDL-cholesterol concentration. Higher alcohol consumption was associated with lower basal insulin secretion in women only (β=−0.10, p_{trend}=0.004) and this association persisted after adjustment for insulin sensitivity. In men, increasing alcohol consumption was associated with enhanced insulin clearance and increased fasting NEFA concentrations, independently of insulin sensitivity. Fasting glucagon decreased with increasing alcohol in women only (abstainers 9.2±4.4; <28 g/week 8.6±4.0; 28–64 g/week 8.1±3.7; >64 g/week 7.5±3.1 pmol/l; p_{trend}=0.01).
Conclusions/interpretation Light-to-moderate alcohol consumption was associated in healthy women with enhanced insulin sensitivity, reduced basal insulin secretion rate and lower fasting plasma glucagon concentration, providing consistent mechanisms for the reduced risk of diabetes.

Keywords Alcohol · Glucagon · Insulin clearance · Insulin resistance · Insulin secretion

Abbreviations

GGT	Gamma glutamyl transferase
IGT	Impaired glucose tolerance
RISC	Relationship between insulin sensitivity and cardiovascular disease

Introduction

Epidemiological studies have shown that moderate alcohol consumption is associated with a reduced risk of type 2 diabetes [1–6]. This has been confirmed by a recent meta-analysis of observational studies that reported a U-shaped relationship for both sexes with a risk reduction for a moderate alcohol intake and an increased risk for an intake of over 50–60 g/day [7]. The relationship differed between men and women, with a lower RR in women than men for moderate alcohol intake, compared with alcohol abstainers.

The underlying mechanisms behind this reduction in risk with moderate alcohol intake remain poorly understood. It has been proposed that this risk reduction might be explained by improved insulin sensitivity but this has not been assessed by the method of reference, the hyperinsulinaemic–euglycaemic clamp. Furthermore, the relationship between alcohol consumption and both glucagon concentration and insulin secretion has not been previously addressed.

The aim of the present study is to determine the relationship between moderate alcohol intake and both insulin sensitivity (assessed by the hyperinsulinaemic–euglycaemic clamp) and insulin secretion in a large cohort of healthy men and women participating in the RISC study (Relationship between Insulin Sensitivity and Cardiovascular disease study) [8, 9]. We performed a sex-specific analysis, as mean alcohol intake was significantly lower in women and also to determine possible sex differences in the association between alcohol consumption and glucose metabolism, as seen in the meta-analysis [7]. Furthermore, we aimed to explore the potential confounding role of adiponectin and HDL-cholesterol in mediating the relationship between alcohol intake and glucose metabolism.

Methods

Study population RISC is a prospective observational cohort study whose rationale and methodology have been published, as well as the characteristics of the individuals recruited [8, 9]. Clinically healthy men and women, aged 30–60 years, were recruited by advertisement, from the local populations of 19 centres in 14 European countries. Initial exclusion criteria were: treatment for obesity, hypertension, lipid disorders or diabetes, pregnancy, cardiovascular or chronic lung disease, weight change ≥5 kg in the last 6 months, cancer (in the last 5 years) and renal failure. Exclusion criteria after screening were: arterial BP ≥140/90 mmHg, fasting plasma glucose ≥7.0 mmol/l, 2 h plasma glucose (following a 75 g OGTT) ≥11.0 mmol/l, total serum cholesterol ≥7.8 mmol/l, serum triacylglycerol ≥4.6 mmol/l and ECG abnormalities. In the present analysis, we included all the participants who were not ruled out by these criteria, whose clamp study passed the quality control check and for whom data on HDL-cholesterol and alcohol intake were available (n=1,289). We also excluded those (nine men and four women) with a high alcohol consumption (defined as >350 g/week for men and >280 g/week for women), as the purpose of the study was to investigate the metabolic effects of light-to-moderate alcohol consumption. Therefore, we studied a total of 1,276 individuals.

At 3 years 563 women and 461 men attended a second OGTT. Impaired glucose tolerance (IGT), defined as fasting glucose <7.0 mmol/l and 2 h glucose ≥7.8 mmol/l but <11 mmol/l or type 2 diabetes, was present in 58 men and 78 women.

Height was measured, and body weight and per cent body fat were evaluated by the TANITA bioimpedance balance (Tanita International Division, Arlington Heights, IL, USA). BMI was calculated and obesity was defined as BMI ≥30 kg/m^2.

Ethics Committee approval was obtained by each recruiting centre. Participants were given detailed written information on the study as well as an oral explanation, and they all signed a consent form.

Lifestyle and alcohol consumption Alcohol consumption was assessed using a standardised semiquantitative food-frequency questionnaire. For each beverage type (beer, red and white wine, port, vermouth, hard liquor), participants reported their usual number of glasses and the glass size (small, medium or large, according to photographs on the questionnaire). The ten frequency response categories ranged from 'never' to '40 or more times per week'. The consumption was quantified in grams of pure alcohol per week and then stratified into four categories: abstainers; by tertiles of alcohol intake, for men, <57, 57–130, >130 g/week and for women, <28, 28–64, >64 g/week.

Information on physical activity was collected with the 7-day International Physical Activity Questionnaire (IPAQ), a previously validated assessment tool for international studies, that provides a comprehensive evaluation of daily physical-activity habits [10].

OGTT OGTT was performed after a fasting period of at least 10 h. Blood samples were taken at fasting and 30, 60, 90 and 120 min into the OGTT, together with samples for central analysis of routine blood chemistry. Blood collected during the studies was separated into plasma and serum, portioned and stored at −20°C for glucose and insulin and −80°C for lipid analysis.

Glucose concentrations were measured by the glucose oxidase technique. Plasma insulin and C-peptide were measured by a two-site time-resolved fluoroimmunoassay (AutoDELFIA Insulin kit; Wallac, Turku, Finland) using monoclonal antibodies, with the following assay characteristics (for insulin and C-peptide, respectively): sensitivity 1–2 and 5 pmol/l, within-assay variation 5 and 5% and between-assay variation 5 and 3.5%. Liver enzymes were centrally assayed on a Dade-Behring Dimension RXL Autoanalyser (Cambridge, UK). Measurement of plasma NEFA concentration was carried out using a Randox enzymatic kit (Randox Laboratories Limited, Crumlin, UK). The CV was less than 5%. Serum adiponectin was determined by an in-house time-resolved immunofluorometric assay (TR-IFMA), based on two antibodies and recombinant human adiponectin, that measures total circulating adiponectin (including high- and low-molecular-mass isoforms) (R & D Systems, Abingdon, UK) [11]. All standards and unknown samples were analysed in duplicate, with the exception of non-specific binding, which was analysed in quadruplicate. The intra-assay CV was <5% and the inter-assay CV was <10%. The samples for glucagon assessment were collected in a tube containing EDTA plus a protease inhibitor (aprotinin) and repeated freeze–thaw of these samples was avoided. Glucagon concentration was measured in a centralised laboratory in Odense, Denmark by an immunoassay method that is highly specific for the free C-terminus of the molecule, and therefore specific for pancreatic glucagon, with the following assay characteristics: normal range (5–20 pmol/l) sensitivity <1 pmol/l, within-assay CV <5% at 20 pmol/l, between-assay CV <12% [11].

Insulin sensitivity On a separate day within 1 month of the OGTT, a hyperinsulinaemic–euglycaemic clamp was performed. Exogenous insulin was administered as a primed continuous infusion at a rate of 240 pmol $min^{-1}m^{-2}$ simultaneously with a variable 20% dextrose infusion adjusted every 5–10 min to maintain the plasma glucose concentration within 0.8 mmol/l (±15%) of the target glucose level (4.5–5.5 mmol/l). The clamp procedure was standardised across centres; the data from each clamp study were transferred to the coordinating centre where they underwent quality-control scrutiny according to pre-set criteria.

Insulin sensitivity was expressed as the ratio of the *M* value during the final 40 min of the 2 h clamp, to the mean plasma insulin concentration measured during the same interval (*M*/*I*), normalised to fat-free mass and expressed in units of $\mu mol\,min^{-1}$ (kg fat free mass)$^{-1}$ (nmol/l)$^{-1}$

Insulin secretion Beta cell function was assessed from the OGTT using a model describing the relationship between insulin secretion (calculated from C-peptide using the method of van Cauter et al [12]) and glucose concentration, previously described in detail [13–15]. From the model-estimated beta cell dose–response, relating insulin secretion (in pmol $min^{-1}m^{-2}$) to glucose concentration, insulin secretion at 5 mmol/l glucose (the average basal glucose in the participants with normal glucose tolerance) was determined. This variable represents insulin secretion in basal conditions if basal glucose was 5 mmol/l in each individual. Basal and total (integral during the OGTT, in $nmol/m^2$) insulin secretion was also determined using the model.

Insulin clearance Peripheral insulin clearance during the clamp (in l $min^{-1}m^{-2}$) was calculated as the ratio between insulin infusion rate and steady-state insulin concentration in the last 40 min of the clamp.

The endogenous 'pre-hepatic' clearance in basal conditions was defined as: (basal insulin secretion)/(basal insulin concentration) where basal insulin secretion is calculated from the beta cell modelling analysis. This clearance value is directly dependent on hepatic insulin extraction. The endogeneous 'pre-hepatic' insulin clearance during the OGTT (in l $min^{-1}m^{-2}$) was defined as the ratio of mean insulin secretion and mean insulin concentration.

Statistical analysis The data are expressed as mean±SD or as median (interquartile range) for variables with a skewed distribution, and categorical data as percentages. Variables that were not symmetrically distributed were log transformed before analyses. Data were analysed for men and women separately.

The mean values of the participant characteristics were compared across categories of alcohol intake by ANOVA. Spearman correlation coefficients were first assessed between alcohol consumption, as expressed in g/week as a continuous variable, and insulin sensitivity.

The relationships between alcohol intake categories, and insulin sensitivity, insulin secretion, insulin clearance and glucagon were assessed by linear regression analysis with adjustment for age, recruitment centre, physical activity, current smoking and waist circumference. Trend tests were determined across the categories of alcohol intake. Next, HDL-cholesterol, triacylglycerol and adiponectin levels were added into the regression model. We also tested for an interaction between BMI ≤25 and >25 kg/m^2 with alcohol consumption categories on insulin sensitivity. Logistic regression analysis was used to assess the relationship between alcohol consumption and the risk of impaired glucose tolerance or type 2 diabetes at year3, after adjustment for age and waist circumference. Statistical analyses used StatView for Windows (version 5.0; SAS Institute, Cary, NC, USA) and SAS version 9.2.

Results

The percentage of abstainers was higher in women (n=172, 24.4%) than in men (n=72, 12.6%). When excluding the abstainers, the median alcohol intake was 84 g/week (range 7.5–350 g/week) in men and 42 g/week in women (range 7.5–270 g/week).

In women only, moderate alcohol consumption was associated with a lower BMI and waist circumference and with a higher prevalence of current smoking (Tables 1, 2). Fasting and 2 h glycaemia did not differ according to alcohol consumption categories in either men or women.

In both men and women, there was a graded increase in plasma HDL-cholesterol and gamma glutamyl transferase (GGT) across categories of alcohol intake (Tables 1, 2). Adiponectin concentrations did not significantly differ according to alcohol consumption categories in either men or women, but there was a trend for a positive relation in women (p=0.08).

Insulin sensitivity In univariate analysis, alcohol consumption, expressed as a continuous variable, was positively correlated with insulin sensitivity in women (r_{Spearman}= 0.16, p=0.0001) but not in men (r_{Spearman}=0.05, p=0.20). When alcohol was used as a categorical variable the results were very similar, with a significant association in women (p=0.005) but not in men (p=0.07), after controlling for age, recruitment centre, waist, current smoking and physical activity (Fig. 1). This association persisted after excluding women who were alcohol abstainers from the analysis. The significance of the relation between alcohol consumption categories and insulin sensitivity was only borderline after controlling for HDL-cholesterol concentration in women (p=0.06), and there was no relation in men (p=0.24). Further adjustment for adiponectin, triacylglycerol or GGT levels did not alter the significant association between alcohol consumption and the M/I value in women. In men, the relationship between alcohol intake and insulin sensitivity was stronger after controlling for GGT levels (p=0.02). There was no significant interaction between BMI ≤/ >25 kg/m^2 and alcohol consumption on insulin sensitivity in either sex.

Insulin secretion Basal insulin secretion decreased across alcohol consumption categories in women but not in men, after adjusting for age, recruitment centre, smoking, physical activity and waist circumference (Fig. 2). The significant inverse association between increased alcohol consumption and basal insulin secretion in women persisted after controlling for insulin sensitivity (log M/I) (p_{trend}=0.017) or for HDL-cholesterol, albeit being attenuated after taking this variable into account (p_{trend}=0.03).

We also quantified basal insulin secretion, at a fixed normal glucose level of 5 mmol/l glucose, from the beta cell dose–response. There was an inverse association between alcohol intake and insulin secretion at 5 mmol/l glucose in women only, with a progressive decrease in insulin secretion rate across alcohol consumption categories (see electronic supplementary material [ESM] Fig. 1). This association persisted after controlling for the M/I value, suggesting an effect independent of changes in insulin sensitivity. Total insulin secretion during the OGTT did not differ according to the alcohol intake categories, in either sex (ESM Fig. 2).

NEFA concentration In both men and women, there was a significant increase in NEFA levels across alcohol intake categories (Tables 1 and 2). This association persisted after controlling for age, recruitment centre, waist, current smoking and physical activity in men (p_{trend}=0.0003) but not in women (p_{trend}=0.32).

Insulin clearance Alcohol consumption, expressed in categories, was significantly associated with an increased peripheral clearance of insulin during the clamp and enhanced endogenous ‘pre-hepatic’ clearance of insulin in both basal

Table 1 Baseline characteristics according to categories of alcohol consumption in men. The RISC study

Characteristic	Alcohol consumption (g/week)				p_{ANOVA}
	Abstainers (n=72)	<57 (n=169)	57–130 (n=167)	>130 (n=162)	
Age (years)	41.2±7.9	43.5±8.4	43.5±8.8	43.6±8.6	0.20
BMI (kg/m^2)	26.7±4.2	26.5±3.3	26.1±3.8	26.2±3.1	0.59
Waist circumference (cm)	93.8±11.8	93.4±9.7	93.0±11.0	93.6±9.2	0.93
Current smoking (%)	25.0	25.0	28.5	31.5	0.13
Physically inactive (%)	17.6	25.6	23.6	16.6	0.38
Systolic blood pressure (mmHg)	121.3±10	122.6±10	121.6±11	122.8±11	0.62
Diastolic blood pressure (mmHg)	75.7±7	76.9±7	75.9±8	76.9±7	0.43
Fasting glycaemia (mmol/l)	5.4±1.8	5.1±0.6	5.3±0.5	5.3±0.5	0.17
Fasting insulinaemia (pmol/l)	34.5 (38.0)	27.0 (22.9)	26.2 (21.2)	30.0 (25.0)	0.02
Fasting NEFA (mmol/l)	0.41 (0.26)	0.43 (0.25)	0.44 (0.20)	0.47 (0.28)	0.007
Total cholesterol (mmol/l)	4.64±0.9	4.86±0.9	4.90±0.8	4.98±0.9	0.05
LDL-cholesterol (mmol/l)	2.95±0.8	3.10±0.8	3.08±0.8	3.08±0.8	0.59
HDL-cholesterol (mmol/l)	1.14±0.3	1.24±0.3	1.24±0.3	1.26±0.3	0.05
Triacylglycerol (mmol/l)	1.04 (0.5)	1.08 (0.6)	1.08 (0.8)	1.16 (0.9)	0.13
Adiponectin (mg/l)	5.6 (2.4)	6.0 (3.2)	6.3 (3.7)	5.9 (3.1)	0.19
GGT (U/l)	26.0 (15.0)	25.0 (14.5)	25.0 (12.7)	28.0 (18.0)	0.007

Data are presented as mean±SD, median (interquartile range) or %

conditions and during the OGTT in men and women (Tables 3 and 4). The increased insulin clearance across quartiles of alcohol intake in men was independent of insulin sensitivity (M/I) in the multivariable model (Tables 3 and 4).

Glucagon concentration Fasting glucagon levels progressively decreased across alcohol consumption categories in both sexes, but with a significant trend for women only (p_{trend}=0.01, Fig. 3). In women, this association remained significant after controlling for insulin sensitivity (M/I) in

Table 2 Baseline characteristics according to categories of alcohol consumption in women. The RISC study

Characteristic	Alcohol consumption (g/week)				p_{ANOVA}
	Abstainers (n=172)	<28 (n=168)	28–64 (n=181)	>64 (n=185)	
Age (years)	43.4±8.4	44.1±8.1	44.5±8.1	44.8±8.1	0.41
BMI (kg/m^2)	25.8±5.1	24.9±4.2	24.6±42	24.0±3.5	0.0007
Waist circumference (cm)	83.6±4.3	81.4±11.6	80.1±11.3	79.4±.7	0.006
Current smoking (%)	20.2	22.1	28.2	28.6	0.01
Physically inactive (%)	15.3	22.4	18.4	21.5	0.28
Systolic blood pressure (mmHg)	114.9±13	113.2±12	112.9±12	113.3±13	0.47
Diastolic blood pressure (mmHg)	73.9±8	72.5±8	71.9±8	72.7±8	0.10
Fasting glycaemia (mmol/l)	4.9±0.6	4.9±0.6	5.0±0.5	5.0±0.5	0.17
Fasting insulinaemia (pmol/l)	28.0 (25.7)	26.0 (18.0)	24.0 (21.1)	23.4 (16.0)	0.19
Fasting NEFA (mmol/l)	0.60 (0.28)	0.54 (0.25)	0.53 (0.25)	0.59 (0.26)	0.03
Total cholesterol (mmol/l)	4.71±0.9	4.89±0.9	4.70±0.8	4.81±0.8	0.13
LDL-cholesterol (mmol/l)	2.82±0.8	2.91±0.9	2.70±0.8	2.68±0.8	0.03
HDL-cholesterol (mmol/l)	1.45±0.3	1.54±0.3	1.59±0.4	1.70±0.4	<0.0001
Triacylglycerol (mmol/l)	0.86 (0.55)	0.85 (0.47)	0.85 (0.42)	0.80 (0.48)	0.56
Adiponectin (mg/l)	8.5 (5.1)	9.1 (5.0)	9.6 (5.1)	9.8 (4.8)	0.08
GGT (U/l)	16.0 (9.0)	17.0 (9.0)	17.0 (8.0)	19.0 (12.0)	<0.0001

Data are presented as mean±SD, median (interquartile range) or %

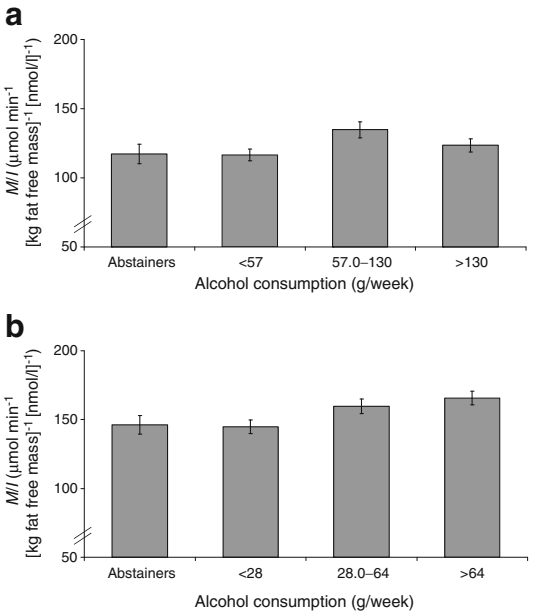

Fig. 1 Insulin sensitivity, expressed as the *M*/*I* value according to categories of alcohol consumption (in g/week) in men (**a**) and women (**b**). Data are means±SEM. p_{trend}=0.07 for men and p_{trend}=0.005 for women, adjusted for age, recruitment centre, physical activity, smoking and waist circumference after a logarithmic transformation for *M*/*I*

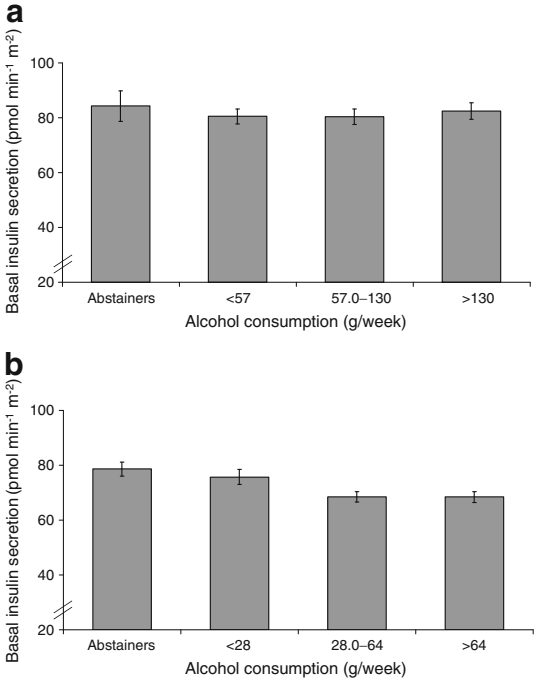

Fig. 2 Basal insulin secretion across alcohol consumption categories in men (**a**) and women (**b**). Data are means±SEM. p_{trend}=0.56 for men and p_{trend}=0.004 for women, adjusted for age, recruitment centre, physical activity, smoking and waist circumference after a logarithmic transformation for insulin secretion

the multivariable model (p_{trend}=0.02) and was also independent of fasting glycaemia and fasting insulinaemia.

Impaired glucose tolerance and type 2 diabetes at 3 years Alcohol consumption categories were not associated with either fasting or 2 h glycaemia at year3 in either men or women, analysed separately in multivariate models. There was a reduced risk of IGT/diabetes at year3 for the women with a baseline alcohol consumption above the median value (>30 g/week) (OR 0.57, 95% CI 0.34, 0.94, p=0.03) after adjusting for age and waist circumference. In men, baseline alcohol consumption above the median value (>71 g/week) was not associated with a reduction in the risk of IGT/diabetes at year3 (OR 1.02, 95% CI 0.57, 1.81, p=0.95).

Discussion

The main finding of this study is that moderate alcohol consumption is associated with an enhanced peripheral insulin sensitivity, a reduced basal insulin secretion and a lower fasting plasma glucagon concentration in healthy women. This is in agreement with a large body of evidence showing that moderate alcohol consumption is associated with a decreased risk of type 2 diabetes in the general population [1, 7, 16–18], including women at low risk [6, 19]. A meta-analysis reported a much more marked relation in women than in men [7]. Similarly, a recent multicentre prospective European study, with 12,403 incident cases of type 2 diabetes and 16,154 controls, showed that moderate alcohol consumption was associated with a reduced risk of type 2 diabetes in women only [20].

Enhanced insulin sensitivity among moderate alcohol drinkers has been reported previously, but most of these studies used proxy estimates of insulin sensitivity, such as the fasting insulin concentration or the HOMA of insulin resistance (HOMA-IR) index, instead of the gold-standard method of the euglycaemic–hyperinsulinaemic clamp, as used in the present study [21–23].

Short-term intervention studies have shown an increase in both insulin sensitivity and adiponectin concentration within 3–8 weeks after alcohol intake [24–27]. Moderate alcohol consumption for 4 weeks has been associated with a 12% increase in plasma adiponectin and a 57% increase in its high-molecular-weight form, without concomitant changes in subcutaneous and abdominal fat content or body weight [28]; adiponectin has been shown to account for 25–30% of the inverse association between alcohol consumption and the risk of type 2 diabetes in women [19]. Interestingly, in the present study, the significant association between moderate alcohol intake and improved insulin sensitivity in women persisted after controlling for adiponectin concentration, suggesting that other mechanisms may be involved. The pathophysiology

Table 3 Insulin clearance according to categories of alcohol consumption in men. The RISC study

Insulin clearance	Alcohol consumption (g/week)				p_{trend}	p_{trend}[a]
	Abstainers (n=70)	<57 (n=167)	57–130 (n=165)	>130 (n=162)		
Peripheral clearance of insulin during the clamp (1 $min^{-1}m^{-2}$)	0.58±0.15	0.59±0.15	0.63±0.2	0.63±0.26	0.003	0.02
Endogenous 'pre-hepatic' clearance of insulin in basal conditions (1 $min^{-1}m^{-2}$)	2.11±0.71	2.45±0.94	2.36±0.78	2.44±0.78	0.04	0.05
Endogenous 'pre-hepatic' clearance of insulin during OGTT (1 $min^{-1}m^{-2}$)	1.47±0.55	1.55±0.57	1.57±0.50	1.68±0.68	0.003	0.01

Data are presented as mean±SD

p_{trend} is adjusted for age, centre, physical activity, current smoking, waist circumference

[a] p_{trend} is additionally adjusted for insulin sensitivity, log M/I

underlying the effects of moderate alcohol intake on insulin sensitivity is complex and not fully understood.

The final product of ethanol oxidation is acetate, which is converted to acetyl-CoA in peripheral tissue [29]. Acetate may modulate fat oxidation and decrease lipolysis. Therefore, an increase in skeletal muscle oxidative capacity after alcohol intake has been suggested even though evidence is limited in humans [28].

HDL-cholesterol concentration increases with alcohol consumption and is also related to insulin sensitivity and adiponectin concentrations, and could be a key mediator in the association between alcohol consumption and improved glucose use [30]. In the present study, the relationship between alcohol intake and HDL-cholesterol levels was stronger in women than in men. Furthermore, the observation that the association between alcohol and insulin sensitivity lost significance after further adjustment for HDL-cholesterol concentration supports a greater role for HDL-cholesterol, rather than for adiponectin, in the modulation of insulin sensitivity by alcohol, in particular among women.

The effects of alcohol consumption on insulin secretion have been investigated less extensively than its effects on insulin sensitivity, in particular with a conjoint assessment of insulin sensitivity. In the present study, we found that light-to-moderate alcohol consumption was associated with a decreased basal insulin secretion rate in non-diabetic women, and this effect was independent of insulin sensitivity. This finding was confirmed when we assessed basal insulin secretion at a fixed normal glucose level of 5 mmol/l glucose from the beta cell dose–response. These results suggest that alcohol has a more pronounced effect on basal insulin secretion rate than on glucose-load-induced insulin response. Data on the effects of alcohol consumption on insulin secretion are conflicting: some studies show the absence of an association in men [31, 32] and others show a decreased insulin secretion with men and women combined [17]. The reasons for the absence of a significant association between alcohol intake and insulin secretion in men in contrast to women remain unclear, and may rely on sex specificity. Adjustment for menopausal status did not substantially modify the association between alcohol consumption and either insulin sensitivity or secretion (data not shown), suggesting that sex-specific effects may be related to effects other than differences in hormone levels.

In agreement with our findings, an inverse association between alcohol intake and insulin secretion has been noted

Table 4 Insulin clearance according to categories of alcohol consumption in women. The RISC study

Insulin clearance	Alcohol consumption (g/week)				p_{trend}	p_{trend}[a]
	Abstainers (n=171)	<28 (n=166)	28–64 (n=181)	>64 (n=184)		
Peripheral clearance of insulin during the clamp (1 $min^{-1}m^{-2}$)	0.62±0.41	0.63±0.19	0.63±0.13	0.64±0.22	0.04	0.49
Endogenous 'pre-hepatic' clearance of insulin in basal conditions (1 $min^{-1}m^{-2}$)	2.28±0.76	2.29±0.72	2.43±0.81	2.53±0.80	0.22	0.53
Endogenous 'pre-hepatic' clearance of insulin during OGTT (1 $min^{-1}m^{-2}$)	1.62±0.60	1.61±0.56	1.71±0.61	1.74±0.54	0.28	0.91

Data are presented as mean±SD

p_{trend} is adjusted for age, centre, physical activity, current smoking, waist circumference

[a] p_{trend} is additionally adjusted for insulin sensitivity, log M/I

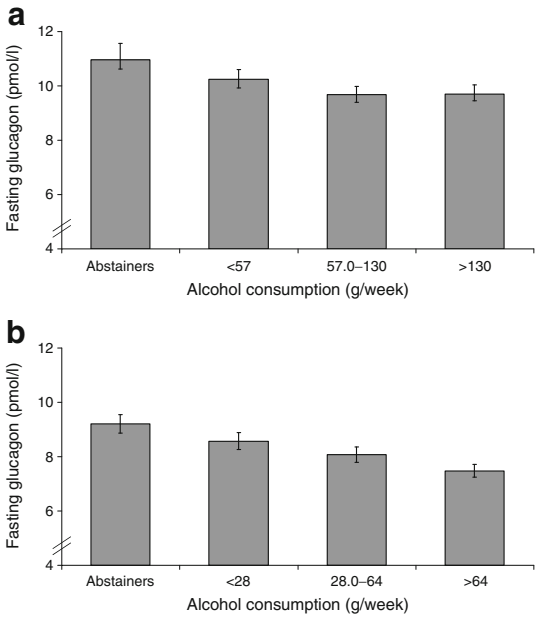

Fig. 3 Fasting glucagon concentration according to categories of alcohol consumption (in g/week) in men (**a**) and women (**b**). Data are means±SEM. p_{trend}=0.37 for men and p_{trend}=0.01 for women, adjusted for age, recruitment centre, physical activity, smoking and waist circumference after a logarithmic transformation for glucagon

previously in non-diabetic women [33]. The decrease in basal insulin secretion rate across alcohol consumption categories in women could be viewed as a direct consequence of enhanced insulin sensitivity. Furthermore, exposure to physiological hyperinsulinaemia stimulates insulin secretion in non-diabetic individuals [14]. However, adjustment for insulin sensitivity did not alter the inverse association, suggesting alternative mechanisms. Furthermore, the effect of alcohol on insulin secretion appears to be independent of the concomitant increase in either HDL-cholesterol or adiponectin concentrations. Therefore, the possibility of a specific effect of alcohol on beta cell function needs to be further explored.

The present study showed that alcohol consumption was significantly associated with an increased endogenous clearance of insulin. This aspect has not previously been recognised. Enhanced rates of insulin clearance across alcohol intake categories may be related to concomitant increased insulin sensitivity and reduced basal insulin secretion rate as seen in the present study. Previous studies have underscored the tight interactions between insulin sensitivity, insulin secretion and insulin clearance [34–36]. In the presence of insulin resistance, glucose homeostasis is maintained by an increase in plasma insulin via increased secretion and a decrease in insulin extraction. Animal studies have shown that induction of insulin resistance induces an initial increase in insulin secretion followed by a decrease in hepatic insulin extraction [37].

The graded increase in NEFA concentration across alcohol consumption categories observed in the present study is consistent with both the increased clearance of insulin and reduced basal insulin secretion rate, observed in parallel with increasing alcohol intake, as insulin inhibits lipolysis and NEFA release in healthy individuals [38].

To our knowledge, the relationship between alcohol and glucagon concentration has not been investigated previously. Our finding of an inverse correlation between alcohol consumption and fasting glucagon levels in women suggests a putative new mechanism to explain why moderate alcohol consumption is protective for the development of type 2 diabetes. A direct effect of alcohol on the regulation of glucagon release warrants specific investigation. This inverse association between glucagon and alcohol consumption appeared to be independent of fasting glycaemia and of insulinaemia, suggesting a specific, yet poorly characterised, direct effect of alcohol on the regulation of glucagon release. This novel finding of an inverse correlation between moderate alcohol consumption and fasting glucagon concentrations should also be tested in type 2 diabetes.

Limitations of the present study include its cross-sectional design, which precludes drawing causal inferences from the observed associations. Furthermore, our results may have been influenced by differences in factors other than alcohol intake. We cannot rule out confounding by unmeasured variables. The difficulty of an accurate evaluation of alcohol consumption with possible under-reporting and misclassification should be acknowledged. However, we intentionally excluded heavy drinkers in our study to focus on abstainers and light-to-moderate drinkers.

The strengths of the present study are the large RISC cohort of healthy participants, the use of the gold-standard methodology for measurement of insulin sensitivity, use of centralised laboratory assays with a quality control of data and the assessment of both insulin secretion and insulin clearance [8]. This provided the opportunity to systematically explore, in both men and women, the relationship between alcohol consumption and insulin sensitivity, insulin secretion, insulin clearance and glucagon concentrations.

In conclusion, our study shows that in healthy women, light-to-moderate alcohol consumption is associated with enhanced insulin sensitivity, decreased basal insulin secretion rate, decreased fasting glucagon levels and an increased insulin clearance. These findings provide potential explanations for the reduced risk of type 2 diabetes associated with moderate alcohol consumption.

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Contribution statement FB was responsible for the conception of the study, analysed data and wrote the manuscript, ED, ML, AM, KH, CHA, PP and BB analysed data and revised the manuscript. All authors approved the final version.

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