

Fasting insulin is a stronger cardiovascular risk factor in women than in men

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Abstract

Background

Diabetes is a stronger risk factor for cardiovascular disease (CVD) in women than in men. It is not known whether there is also a sex difference in the association between hyperinsulinaemia, reflecting insulin resistance, and CVD.

Research design and methods

Fasting insulin was assessed with a specific assay in 6916 fasting, non-diabetic subjects of the PREVEND study without a prior history of CVD. Major Adverse Cardiovascular Events (MACE) (defined as CVD morbidity and CVD mortality) were prospectively recorded after the baseline survey. Cox regression models were used to investigate the association of fasting insulin with subsequent development of MACE.

Results

Fasting insulin was 54 [38-77] pmol/l in women (age 48 ± 12 yrs) and 57 [40-88] pmol/l in men (age 49 ± 13 yrs). During follow-up for 7.5 [6.9-7.8] yr, 98 cardiovascular events were recorded in 3626 women and 242 events in 3290 men. There was a significant ($P < 0.001$) interaction between sex and fasting insulin for MACE, with the strongest association in women. In women, there was a logarithmic association for insulin with MACE, independent of age, alcohol consumption, and smoking (HR = 1.50 [95%CI 1.17-1.91] per doubling of insulin, $P = 0.001$). In men, for a similar multivariate model, there was a logarithmic association (HR = 1.13 [95%CI 0.97-1.32] per doubling of insulin, $P = 0.1$). Further adjustment for components of the insulin resistance syndrome weakened the association more in men than in women. With HOMA instead of insulin, results were essentially similar.

Conclusions

In parallel with diabetes, fasting hyperinsulinaemia reflecting insulin resistance in non-diabetic subjects is associated with an increased risk for cardiovascular disease, which is more pronounced in women than in men.

Introduction

Cardiovascular disease (CVD) is a leading cause of death in both women and men. In general, women have a lower risk for CVD than men. It is, however, well known that diabetes is a stronger CVD risk factor in women than in men, thereby negating the female advantage with respect to CVD.¹⁻³ Whether insulin resistance is an underlying factor in this sex difference is unknown.

Type 2 diabetes is often preceded by insulin resistance and development of cardiovascular disease starts long before diabetes is diagnosed.⁴ Fasting insulin concentrations are considered to mainly reflect compensatory hyperinsulinaemia and the degree of insulin resistance in non-diabetic subjects.⁵ As such, fasting insulin has been shown to be a predictor of CV events, independent of age, sex and body mass index, in three meta-analyses.⁶⁻⁸ Of the studies on fasting insulin as a predictor of CVD risk factor only few included women,⁶⁻⁸ so it is not known whether there is a sex difference for fasting insulin as a predictor of CV events.

The aim of this study was to examine whether there is a sex difference in the association of fasting insulin concentrations with major adverse cardiovascular events (MACE) in a non-diabetic population.

Methods

Study population and design

For this prospective study, we used data of the PREVENDE study (Prevention of Renal and Vascular End-stage Disease). The PREVENDE study is an ongoing longitudinal cohort study based on the general population of the city of Groningen, the Netherlands, between the ages of 28 and 75 years. In brief, 8592 individuals completed the baseline survey (1997-1998). For the present study we first excluded subjects who had not explicitly stated to have been fasting for at least eight hours prior to baseline blood sampling (n=857 subjects). We then excluded subjects with diabetes according to ADA guidelines (defined as a fasting plasma glucose ≥ 7.0 mmol/l or the use of anti-diabetic medication, n=298), subjects with a prior history of CVD (n=363), and finally we excluded those remaining subjects with missing values of insulin at baseline (n=168), eventually leaving 6916 subjects for present analysis. The PREVENDE study was approved by the institutional review board of our institution and is conducted in accordance with the guidelines of the declaration of Helsinki. All participants signed informed consent.

Follow-up and outcomes events

The primary endpoint of this analysis was Major Adverse Cardiovascular Events (MACE). MACE was defined as the combined endpoint of incident cardiovascular morbidity and mortality that occurred during the follow-up of the study. Information (on hospitalisation) for cardiovascular morbidity was obtained from PRISMANT, the Dutch national registry of hospital discharge diagnoses. Data on mortality were received from the municipal register, and cause of death was obtained by linking the number of the death certificate to the primary cause of death as coded by the Dutch Central Bureau of Statistics. Data were coded according to the International Classification of Diseases, 9th revision and the classification of interventions. MACE was defined as the following; acute myocardial infarction (ICD-code 410), acute and subacute ischemic heart disease (411), occlusion or stenosis of the precerebral (433) or cerebral arteries (434) and the following procedures: coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, and other vascular interventions namely percutaneous transluminal angioplasty or bypass grafting of aorta and peripheral vessels. Survival time was defined as the period from the baseline survey to the date of a first major cardiovascular event. Subjects who did not develop MACE were censored on December 31st 2005. In case a person had moved to an unknown destination, the date on which the person was removed from the municipal registry was used as censor date.

Measurements and definitions

Participants underwent two visits to the outpatient research unit for the baseline survey. All participants completed a questionnaire on demographics, cardiovascular disease history, smoking habits, alcohol consumption and medication use prior to their first visit. Height and weight were measured on the first visit and a fasting blood sample was drawn. Subjects were instructed to be fasting for at least 8 hours. During the first and second visit blood pressure was measured, in supine position, every minute for 10 with an automatic device (Dinamap XL Model 9300, Johnson-Johnson Medical, Tampa, FL). Blood pressure values are given as the mean of the last two recordings of both visits. Furthermore, information on medication use was substantiated with use of pharmacy-dispensing data from all community pharmacies in the city of Groningen. This database has complete information on drug-use of approximately 80% of subjects in the PREVENT study.

Diabetes was defined according to the guidelines of the American Diabetes Association as a fasting plasma glucose ≥ 7.0 mmol/L or the use of

antidiabetic medication. Impaired fasting glucose was defined as a fasting glucose >5.6 mmol/l and <7.0 mmol/l. Homeostasis Model Assessment (HOMA) was calculated as: $[\text{glucose (mmol/l)} \times \text{insulin } (\mu\text{U/ml})] / 22.5$. Prior history of CVD at inclusion of the study was defined as self report of cerebrovascular accidents, coronary heart disease, or peripheral vascular disease requiring surgery.

Laboratory measurements

Insulin was measured with an AxSym[®] auto-analyzer (Abbott Diagnostics, Amstelveen, the Netherlands) with a threshold of 7 pmol/l and intra-assay and inter-assay coefficients of variation of 2.6 and 4.3% respectively. This assay has virtually no cross-reactivity with pro-insulin (0.016% at 106 pg/ml). The correction coefficient applied for calculation of HOMA from insulin concentrations is $1 \mu\text{U/ml} = 6.00 \text{ pmol/l}$.⁹ HDL cholesterol, triglycerides, and glucose were measured by standard methods.

Statistical analysis

Analyses were performed using SPSS version 14.0 (SPSS Inc. Chicago IL, USA) and GraphPad Prism version 4.03 (GraphPad Software Inc. San Diego, CA, USA). Normally distributed values are presented as the mean (standard deviation), and a statistical difference was tested with a students t-test. Variables with a skewed distribution are presented as median [inter-quartile range], and a statistical difference was tested with a Mann-Witney U test. A difference between categorical variables was tested using χ -square test. Hazard ratio's (HR) were reported with 95% confidence interval [95%CI]. A two-sided P-value <0.05 indicated statistical significance. Interactions were considered significant at a P-value <0.1 .

First, presence of a potential interaction between sex and fasting insulin for MACE was investigated with Cox regression analyses. All variables did not violate the Cox-regression proportional hazard assumption according to Schoenfeld residuals. Kaplan-Meier curves were made according to quartiles of fasting insulin and tested with Log-Rank test.

Fasting insulin was entered as a continuous variable in Cox-regression analyses. First, the crude model was adjusted for age and sex (model 1), and subsequently for smoking and alcohol consumption as these are considered confounders between the association of insulin and cardiovascular disease (model 2).^{10, 11} Finally, we further adjusted model 2 for waist circumference, HDL cholesterol, fasting triglycerides, systolic blood pressure, and fasting glucose (as components of what is considered the insulin resistance syndrome). Model 2 was first adjusted for each separate

component and then for all components combined (model 3). In a sensitivity analysis those with impaired glucose tolerance were excluded. In a second sensitivity analysis HOMA was used instead of fasting insulin as measure of insulin resistance and all analyses were repeated

Results

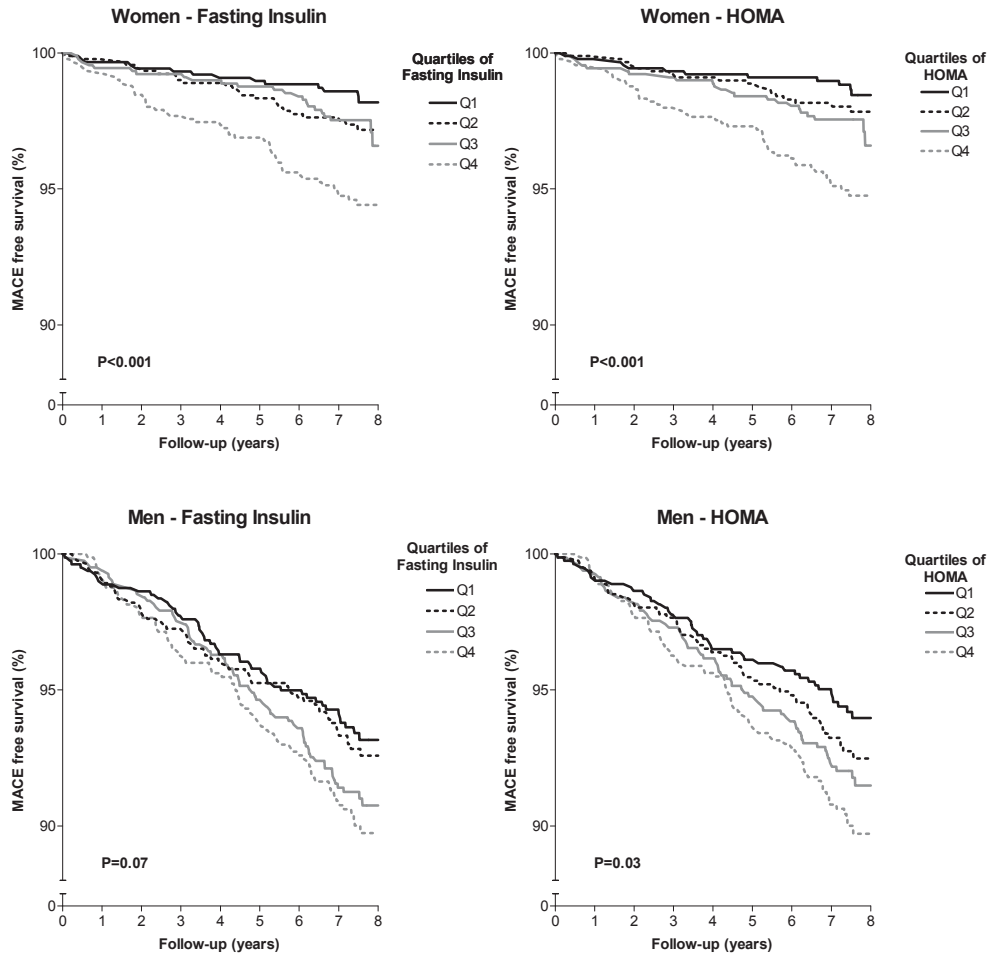
Fasting insulin concentrations were lower in women than in men (54 [38-77] pmol/l versus 57 [40-88] pmol/l, $P < 0.001$) and also less women had impaired fasting glucose than men (5.8% vs 10.8%, $P < 0.001$) (table 1). Both women and men were predominantly Caucasian (>95%). Women consumed less alcohol, had a lower BMI (25.8 ± 4.6 kg/m² vs 26.2 ± 3.6 kg/m²), a lower blood pressure (systolic 124 ± 21 mmHg vs 133 ± 18 mmHg), and lower triglycerides (1.0 [0.8-1.5] mmol/l vs 1.3 [0.9-1.8] mmol/l) with higher HDL cholesterol concentrations (1.5 ± 0.4 mmol/l vs 1.2 ± 0.3 mmol/l) (all $P < 0.001$).

During a follow-up of 7.5 [7.2-7.8] years, 98 cases of MACE were recorded in women (incidence: 38 events per 10,000 person years). During a follow-up of 7.5 [6.9-7.8] years, 242 cases of MACE were recorded in men (incidence: 106 events per 10,000 person years). In the exploratory Cox-regression analysis, there was a significant interaction between fasting insulin concentrations and sex for MACE, as indicated by a significant product-term of insulin and sex ($\beta = 1.006$ [1.002-1.009], $P = 0.001$), in a model with $\beta = 1.002$ [1.001-1.004], $P = 0.01$ for insulin (per pmol/l) and $\beta = 0.23$ [0.16-0.34], $P < 0.001$ for sex. We thus proceeded with the analyses separately in women and men.

Kaplan Meier curves for quartiles of fasting insulin concentrations in women and men are shown in *figure 1*. Log Rank tests indicated that quartiles of fasting insulin concentrations were significantly associated with MACE in women ($P < 0.001$), while in men there was only a trend ($P = 0.1$). In the 1st quartile of fasting insulin 12 (1.4%) women developed MACE, but comparably in the 4th quartile 43 (4.7%) women developed MACE (table 2). The age adjusted HR of the 4th versus the 1st quartile was 2.30 [1.20-4.39], $P = 0.01$. In men, the incidence of MACE also increased with higher fasting insulin, but not as strongly as compared to women. In the 1st quartile, 47 (5.8%) men had MACE, while in the 4th quartile 71 (8.7%) had MACE, but the 4th quartile did not have significantly more MACE when adjusted for age (HR: 1.11 [0.77-1.60], $P = 0.6$). Because analyzing the data in quartiles is arbitrary and no established cut off points exist for

Figure 1

Kaplan-Meier curves for MACE in quartiles of fasting insulin and HOMA in women and men.



Kaplan-Meier for MACE in quartiles of fasting insulin and HOMA in women and men. Significance was tested with Log-rank test.

In women fasting insulin concentration for the 1st quartile was <38 pmol/l (n=887), 2nd quartile 38-53 pmol/l (n=916), 3rd quartile 53-77 pmol/l (n=910), and 4th quartile >77 pmol/l (n=913). In men fasting insulin concentration for the 1st quartile was <40 pmol/l (n=807), 2nd quartile 40-58 pmol/l (n=844), 3rd quartile 58-87 pmol/l (n=821), and 4th quartile >87 pmol/l (n=818).

In women HOMA in the 1st quartile was <1.11 (n=906), 2nd quartile 1.1-1.5 (n=907), 3rd quartile 1.5-2.3 (n=908), and 4th quartile >2.3 (n=905). In men HOMA in the 1st quartile was <1.2 (n=823), 2nd quartile 1.2-1.7 (n=821), 3rd quartile 1.7-2.7 (n=824), and 4th quartile >2.7 (n=822).

Table 1. Baseline characteristics by sex

	Women	Men	P-value
n=	3626	3290	
Age, yr	48±12	49±13	<0.001
Race, n(%)			
Caucasion	3472 (96)	3146 (96)	0.9
Other	154 (4)	144 (4)	
Alcohol consumption, n (%)			
None	1184 (33)	535 (16)	
1-4 units/month	714 (20)	404 (12)	<0.001
2-7 units /week	1129 (31)	1232 (38)	
>1 units /day	599 (16)	1119 (34)	
Smoking, n (%)			
No	2294 (63)	2056 (63)	0.5
Current	1332 (37)	1234 (38)	
BMI, kg/m²	25.8±4.6	26.2±3.6	<0.001
Waist circumference, cm	83±12	93±11	<0.001
Blood pressure, mmHg			
Systolic	124±20	133±18	<0.001
Diastolic	71±9	77±9	<0.001
Lipids, mmol/l			
HDL cholesterol	1.50.4	1.20.3	<0.001
Triglycerides	1.0[0.8-1.5]	1.2 [0.9-1.8]	<0.001
Glucose, mmol/l	4.6±0.6	4.9±0.6	<0.001
Impaired fasting glucose, n (%)	208 (5.8)	347 (10.6)	<0.001
Insulin, pmol/l	54 [38-77]	57 [40-87]	<0.001
HOMA	1.88±1.39	2.17±1.68	<0.001

Abbreviations: BMI: Body Mass Index, HDL: High Density Lipid, HOMA: Homeostasis Model Assessment

Normally distributed variables are given as mean (standard deviation). Variables with a skewed distribution are presented as median [inter-quartile range]. Categorical variables are presented as number (percentage). Differences between men and women were tested by t-test for variables with a normal distribution, by Mann-Whitney U-test for variables with a skewed distribution and by Chi-square test for categorical variables

Table 3. Cox-regression analyses of fasting insulin concentrations and HOMA in women and men.

	Women				Men			
Subjects, n	3626				3290			
MACE, n (%)	98 (2.7)				242 (7.4)			
MACE, incidence ^a	38				106			
	Fasting insulin		HOMA		Fasting insulin		HOMA	
	(per log base 2)		(per log base 2)		(per log base 2)		(per log base 2)	
	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value	HR [95% CI]	P-value
Crude Model	1.83 [1.45-2.32]	<0.001	1.83 [1.49-2.26]	<0.001	1.20 [1.03-1.39]	0.02	1.22 [1.06-1.40]	0.004
Model 1	1.47 [1.16-1.88]	0.002	1.45 [1.17-1.80]	0.001	1.06 [0.91-1.23]	0.5	1.05 [0.92-1.21]	0.5
Model 2	1.50 [1.17-1.91]	0.001	1.48 [1.19-1.84]	<0.001	1.13 [0.97-1.32]	0.1	1.12 [0.97-1.29]	0.1
Model 2A	1.28 [0.96-1.69]	0.09	1.29 [1.00-1.67]	0.05	1.04 [0.87-1.24]	0.7	1.04 [0.89-1.23]	0.6
Model 2B	1.26 [0.95-1.66]	0.1	1.27 [0.98-1.64]	0.07	1.05 [0.88-1.25]	0.6	1.05 [0.89-1.24]	0.5
Model 2C	1.40 [1.08-1.82]	0.01	1.40 [1.11-1.78]	0.005	1.03 [0.88-1.21]	0.7	1.03 [0.89-1.19]	0.7
Model 2D	1.41 [1.08-1.84]	0.01	1.40 [1.11-1.78]	0.005	1.08 [0.93-1.26]	0.3	1.07 [0.93-1.24]	0.3
Model 2E	1.37 [1.07-1.75]	0.01	1.36 [1.08-1.70]	0.008	1.06 [0.91-1.24]	0.5	1.05 [0.91-1.22]	0.5
Model 2F	1.45 [1.12-1.89]	0.006	-	-	1.10 [0.93-1.30]	0.3	-	-
Model 3	1.13 [0.84-1.52]	0.4	1.14 [0.88-1.48]	0.3	0.96 [0.80-1.15]	0.7	0.96 [0.80-1.16]	0.7

a) Incidence per 10.000 person years, BMI=Body Mass Index, SBP=Systolic Blood Pressure

Model 2 A: Model 2 + BMI, kg/m2
 Model 2 B: Model 2 + Waist circumference, cm
 Model 2 C: Model 2 + HDL-cholesterol, mmol/l
 Model 2 D: Model 2 + Triglyceride, mmol/l
 Model 2 E: Model 2 + SBP, mmHg
 Model 2 F: Model 2 + Glucose, mmol/l

Crude Model: Model with fasting insulin
 Model 1: adjustment for age
 Model 2: further adjustment for alcohol consumption and smoking
 Model 3: further adjustment for waist circumference, HDL cholesterol, glucose, triglyceride, and systolic blood pressure

fasting insulin, we further analyzed our data with insulin as a continuous variable.

In women, every doubling of fasting insulin increased the risk of MACE by 83% ($P < 0.001$), which was reduced to 50% after adjustment for age, alcohol consumption, and smoking ($P = 0.002$) (model 2, table 3). In women, with separate adjustment for components of the insulin resistance syndrome other than BMI or waist circumference, insulin remained significantly associated with MACE. After adjustment for BMI or waist circumference the association weakened to borderline significance ($P = 0.09$ and $P = 0.1$ respectively).

In men, every doubling of fasting insulin increased the risk of MACE by 20% ($P = 0.02$), which became non-significant after adjustment for age ($P = 0.5$, Crude model and model 1, table 3). The association was confounded by alcohol consumption and smoking, as the HR slightly increased with adjustment in model 2. Fasting insulin was not associated with further adjustment of each separate component (models 2) of the insulin resistance syndrome or all components together (model 3, table 3).

In a sensitivity analysis we excluded those with impaired fasting glucose, which left 3418 women with 80 cases of MACE and 2943 men with 206 cases of MACE for analysis. The interaction between fasting insulin and sex remained significant ($P = 0.008$ for the product term of insulin and sex). Results changed little as there was a logarithmic association between fasting insulin and MACE in women the hazard ratio was 1.46 [1.11-1.92], per doubling of fasting insulin ($P = 0.007$), but not significantly in men with a hazard ratio of 1.13 [0.96-1.33], per doubling of fasting insulin ($P = 0.1$) after adjustment of age, alcohol consumption and smoking. Both in men and in women fasting insulin was not associated with MACE independent of other factors of the insulin resistance syndrome in women, HR was 1.17 [0.84-1.63] per doubling of fasting insulin, $P = 0.4$ and in men HR was 0.94 [0.77-1.15] per doubling of fasting insulin, $P = 0.6$). All results presented were also analyzed with HOMA instead of fasting insulin, which revealed similar results as the primary analyses (see table 2, table 3, and *figure 1*).

Discussion

In this study, we found that higher fasting insulin concentrations had a stronger and steeper association with more CVD events in women than in men, although this was not independent of components of what is considered the insulin resistance syndrome. The greater susceptibility of insulin

resistant women than men for CVD events could perhaps be an underlying reason why diabetes negates the female advantage for less CVD.

In our study, women in the 4th quartile versus the 1st quartile of fasting insulin, had an age adjusted HR of 2.30 ($P=0.01$) for MACE. In men this HR was 1.11 ($P=0.6$). In women the association was also logarithmic, indicating a steep increase in the risk with higher insulin levels. In women, it seems that obesity underlies the association between insulin and MACE because BMI and waist circumference made the association between insulin and MACE non-significant ($P=0.08$). Dyslipidaemia and hypertension most likely do not underlie the association because the association remained after adjustment for HDL-cholesterol, triglycerides, and systolic blood pressure. Similarly in both women and men, all components when entered together made the association non-significant. Especially in women, non-significance most likely indicates a statistical over-correction, and not that increased insulin concentrations (reflecting of insulin resistance) do not increase CVD risk. Furthermore, these results are not necessarily contradictory to earlier studies, because not all studies reported independent affects of fasting insulin for CVD events.⁶⁻⁸

To date approximately twenty-five prospective studies have been published whether fasting insulin concentrations predict CVD in men, but few studies have included women.⁶⁻⁸ Results from the ARIC study reported a greater risk for coronary heart disease (CHD) associated with fasting insulin in women compared to men. In 13,466 non-diabetic people, free of baseline CHD, 209 men and 96 women developed CHD with an adjusted relative risk of 2.8 ($P=0.02$) in women, but 0.9 ($P=0.6$) in men.¹² However, this study was performed with a non-specific insulin assay, making it possible that effects are the consequence of pro-insulin or pro-insulin like molecules rather than insulin itself, as proinsulin has been shown to be associated with CVD independent of fasting insulin concentrations.^{13, 14} A recent meta-analysis suggested that women had a greater risk for development of coronary heart disease in association with fasting insulin concentrations, but this could not be formally tested because of a lack of power.⁸ It is indicative of the little data currently available in women that the results of this recent meta-analysis were based on 2427 cases in men and only 155 cases in women, which is a comparable number of cases as in this present study.⁸ In the San Antonio Heart Study, HOMA and fasting insulin a gender interaction was not found for (self reported) CVD events, possibly because it concerned less subjects ($n=2569$) and less events ($n=187$) compared to the present study.¹⁵ In conclusion, data in woman are sparse, and not conclusive, though a recent meta-analysis suggested

that insulin is a greater CVD risk factor in women than in men.⁸

It is debated whether high insulin levels by themselves lead to cardiovascular disease.^{10, 11} The consensus is that a causal relationship between insulin and CVD is not likely, but rather between insulin resistance and CVD.¹⁶ Insulin resistance leads to compensatory hyperinsulinaemia and increased fasting insulin concentrations, which are associated with increased CVD risk.^{5, 6} Two studies have measured insulin resistance per se in smaller cohorts, and both indicate that insulin resistance is associated with CVD risk.^{17, 18} Unfortunately, a specific sex difference could not,¹⁷ or was not investigated.¹⁸ It has also been suggested that the associations found with fasting insulin, as measure of insulin resistance, were due to an association between insulin and smoking related CVD, or greater mortality due to underweight and greater alcohol consumption as an indication of overall poor health or malignancies.^{10, 11} In our regression models we show that smoking, alcohol consumption and by design we show that CVD history (as persons with known CVD were excluded) do not underlie the association between insulin and MACE in women.

The hallmark of diabetes is hyperglycaemia, which results from β -cell dysfunction. In type 1 diabetes, a disease in which primarily β -cell failure occurs and not insulin resistance, women also have a greater CVD risk than men, as shown in several studies.^{19, 20} This could indicate that β -cell dysfunction plays a role in the sex difference of CVD in type 2 diabetes. However, in the studies in type 1 diabetes it is unknown whether hyperglycaemia or β -cell dysfunction account for the sex difference.^{19, 20} Moreover, insulin resistance increases CVD risk in type 1 diabetes, and a sex difference with insulin resistance for coronary artery calcifications has been shown.^{21, 22} In non-diabetic persons, elevated pro-insulin levels can be attributed to β -cell dysfunction, and fasting pro-insulin is a stronger predictor for CVD than fasting insulin.^{13, 14} However, in the Hoorn study, homeostasis model assessment of β -cells (HOMA-B), and the pro-insulin to insulin ratio (a better marker of β -cell dysfunction than pro-insulin) did not predict CVD mortality, indicating that pro-insulin itself could be atherogenic, and that the role of β -cell dysfunction in CVD is uncertain.¹⁴

Whether hyperglycaemia increases CVD risk in non-diabetic subjects is controversial. Meta-analyses show an association between fasting glucose and CVD in non-diabetic subjects,^{23, 24} but not all studies indicate an independent association, thus the topic remains somewhat controversial.^{25, 26} Importantly, meta-analyses indicating an independent effect of glucose did not include measures of insulin resistance, whilst the meta-analyses of fasting insulin did include glucose.⁶⁻⁸ The notion that insulin resistance

is more important than elevated glucose levels in non-diabetic women at least is corroborated by a recent study that showed that fasting insulin was a stronger predictor of coronary heart disease than fasting glucose or glycated haemoglobin.²⁷ In summary, β -cell dysfunction and hyperglycaemia could well play a role in the sex difference of CVD risk in non-diabetic persons, but there is plausible contradictory data against this.

A limitation of the study is that insulin resistance was not specifically measured using a hyperinsulinaemic euglycaemic clamp. However, this is not easily feasible in such a large population study. We did corroborate our results with the HOMA index, which gave essentially similar results as with fasting insulin. Secondly, this study is limited to Caucasian populations, and results can not be directly extrapolated to other populations. An important strength of this study is that we used a highly specific insulin assay, i.e. with very little cross-reactivity with pro-insulin, thereby attributing the current findings solely to insulin and not to pro-insulin or pro-insulin like molecules. Importantly, we excluded those with known CVD, thus the effects of insulin are not diluted or confounded by prevalent CVD. Finally, results remained essentially similar after exclusion of those with impaired fasting glucose, indicating that diabetic persons who were perhaps misclassified as non-diabetic also do not confound our results.

The increased CVD risk in women with hyperinsulinaemia in women should perhaps warrant greater attention for treatment in women. This could be important because treatment of modifiable CVD risk shows a sex disparity in which women often receive less treatment.^{28, 29} Perhaps, women could benefit more from treatment aiming at improvement of insulin sensitivity than men by weight loss or physical exercise. Intriguingly, in healthy young women muscle can utilize more glucose per kilogram than in healthy males.³⁰ It should furthermore be noted that many women in our study may have been around menopause. Although we found no effect-modification by age for the effect of insulin, it can not be excluded that estrogenic state in women plays a role, and that the difference between men and women is mainly determined by lack of oestrogen in postmenopausal women.

In conclusion, this study shows that higher fasting insulin concentrations assessed with a specific assay are more strongly associated with an increased risk for cardiovascular disease in women than in men, although the effect of insulin was not independent of variables related to insulin resistance. Obesity was the most important factor in women negating the association between insulin and cardiovascular disease.

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