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Insulin resistance and β-cell function in smokers – results from the EGIR-RISC European multicenter study

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Novelty statements:

- Smoking is not associated with insulin sensitivity or β-cell function in healthy participants, in this large study with insulin sensitivity measured using hyperinsulinaemic, euglycaemic clamps
- Smoking is associated with increased glycaemic and C-peptide responses during an oral glucose tolerance test.

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Abstract

Aims Tobacco smoking is known to increase the long-term risk of developing type 2 diabetes mellitus but the mechanisms involved are poorly understood. This observational, cross-sectional study aims to compare measures of insulin sensitivity and β-cell function in current, ex- and never-smokers.

Methods The study population included 1246 people without diabetes (mean age 44 years, 55% women) from the EGIR-RISC population, a large European multicenter cohort. Insulin sensitivity was measured by a hyperinsulinaemic, euglycaemic clamp and the HOMA-IR index. Two β-cell function parameters were derived from measures during an oral glucose tolerance test: the Early Insulin Response Index and β-cell Glucose Sensitivity. Additionally, the areas under the curve during the oral glucose tolerance test were calculated for glucose, insulin and C-peptide.

Results According to smoking habits, there were differences in insulin sensitivity that was lower in women who smoked and in β-cell Glucose Sensitivity that was lower in men who smoked, but these associations lost significance after adjustment. However, after adjustment, the areas under the glucose and the C-peptide curves during the oral glucose tolerance test were significantly higher in men who smoked.

Conclusions Smoking habits were not independently associated with insulin sensitivity or β-cell function in a healthy middle-aged European population. Health-selection bias, methodological shortcomings, or a true lack of causal links between smoking and impaired insulin sensitivity/secretion/ are possible explanations. Mechanisms behind the observed increased glucose and C-peptide areas under the curve during the oral glucose tolerance test in smoking men need to be further evaluated.

Key words: diabetes, epidemiology, glucose, insulin, smoking

Introduction

Tobacco smoking is known to increase the long-term risk of developing type 2 diabetes mellitus [1, 2]. A recent study showed an association between smoking and incident type 2 diabetes after full adjustment for educational level, physical activity, diet and alcohol consumption, suggesting a causal relationship [3]. Meta analyses have also demonstrated type 2 diabetes to be associated with passive tobacco smoking [2, 4].

If smoking does indeed increase the risk of type 2 diabetes, the underlying mechanisms are not clear but could include changes in insulin sensitivity, insulin secretion or a combination of both. Although smokers are known to have a lower body mass index (BMI) than non-smokers, they tend to exhibit a different body composition with an abdominal fat accumulation [5]. Previous studies have shown an increased insulin resistance among smokers as compared to non-smokers [6] and that smoking cessation improves insulin sensitivity [7], while other studies have shown no such associations [8-12]. Smoking could also cause diabetes by damaging pancreatic β-cells, but conflicting results have been published regarding impaired insulin secretion among smokers compared to non-smokers [8, 9, 12].

The aim of this cross-sectional study was to investigate insulin resistance and β-cell function, as well as glucose, insulin and C-peptide levels during the oral glucose tolerance test, among current smokers, ex-smokers and never smokers in a healthy, European population without diabetes.

Study population and methods

The study population is part of the EGIR RISC database of men and women, aged 29-63 years, from 19 centers in 14 European countries, who were investigated from 2002 to 2004 [13]. The EGIR RISC study aimed to investigate the relation between insulin sensitivity

and atherosclerosis. All participants gave written informed consent prior to study inclusion and ethical approvals were obtained from the respective local ethics committee. The participants were all health-selected and individuals with treatment for diabetes, hypertension, dyslipidemia or cardiovascular disease were excluded. Moreover, individuals with any of these conditions at the study examination were also excluded. A more detailed list of exclusion criteria has previously been published [13]. Data on smoking habits, alcohol consumption, family history of diabetes and physical activity were retrieved from a questionnaire. Smoking status was self-reported and defined as current, ex or never smoker. Alcohol consumption was dichotomized into high and low consumption. A high alcohol intake was defined as a weekly consumption of >168 gram of pure alcohol for men and >108 gram for women, cut-off values for harmful effects of alcohol used by several national health institutes [14, 15]. Physical activity was assessed with the International Physical Activity Questionnaire (IPAQ), a validated instrument for measuring physical activity in adults [16].

Insulin sensitivity was measured by the hyperinsulinaemic, euglycaemic clamp in 1319 individuals [13]. The target plasma glucose concentration was between 4.5 and 5.5 mmol/l. Insulin infusion rate was 240 pmol·min^{-1}·m^{-2}. Plasma or blood glucose was measured at the bedside every 5 to 10 minutes to ensure it remained within 0.8 mmol/l (±15%) of the target glucose concentration. The steady-state period (for calculation of insulin sensitivity) was between 80 and 120 minutes. Additionally, an oral glucose tolerance test (OGTT) was performed with a standard 75 g glucose monohydrate solution.

The study population was reduced from the original 1319 individuals to a population of 1199 individuals due to missing values of key variables and because some individuals met the exclusion criteria (Figure 1). Characteristics of the final study population of 1199 individuals are presented in Table 1. Furthermore for each variable studied, data from a few additional individuals were missing. The study population (n=1199) compared to the excluded

population (n=120) had significantly lower BMI (25.4 vs 26.4 kg/m², *P*=0.03) and a lower percentage of high alcohol consumers (8 % vs 16 %, *P*=0.01). For the other variables listed in Table 1 (age, sex, waist circumference, fasting glucose and physical activity) there were no statistically significant differences.

Insulin sensitivity was calculated as M/I (μmol x min^{-1}x kg_{ffm}^{-1} x nM^{-1}) from clamp data where M is the glucose infusion rate during the last 40 minutes of the 120 minutes clamp, normalized by the fat-free-mass and I is the mean plasma insulin concentration during the same time interval [17, 18]. Additionally, the Homeostatic model assessment - insulin resistance (HOMA-IR) was calculated as described by Matthews *et al* [19], by the formula: (fasting insulin x fasting glucose)/22.5 where insulin is expressed as mIU/liter and glucose as mmol/liter. The rationale for using HOMA-IR in addition to clamp data was to assess insulin sensitivity in a physiological setting more similar to the glucose load following a meal as well as to facilitate the comparison with previous studies not using a clamp measure of insulin sensitivity.

Two separate measurements of β-cell function were calculated from the OGTT. The Early Insulin Response Index (EIRI), the ratio of the increment in insulin concentration during the first 30 minutes of an OGTT to the glucose concentration at 30 minutes (ΔInsulin 30 min / Glucose 30 min) [20]. EIRI is a measurement of the early phases of insulin secretion and it has been described as a physiological estimation. β-cell Glucose Sensitivity (GS) is described by Mari *et al*, [21, 22]. GS is the slope of the dose response curve and reflects the sensitivity of the β-cell to changes in plasma glucose levels after the initial rise in glucose following an OGTT.

The areas under the curves (AUC) during the OGTT were calculated for glucose, insulin and C-peptide, to compare levels during all of the OGTT, rather than just at specific time points.

Descriptive data are presented as mean ± SD or %, stratified according to sex and smoking status.

The differences in insulin sensitivity, β-cell function and AUC measurements between smoking groups were analyzed with ANOVA including Levene's test of equality of variances and in case of significant differences the *P*-value from the Welch test was used. Analyses were then adjusted for age and study center with ANCOVA. Further adjustments were made for BMI, family history of diabetes, physical activity and alcohol consumption. Indices of β-cell function were also adjusted for insulin sensitivity (M/I). All analyses were stratified by gender. M/I, EIRI, GS and insulin AUC were transformed by logarithms to achieve more symmetrical distributions.

Statistical differences in glucose and C-peptide levels at each time point during the OGTT were compared with the Mann-Whitney U test, between current smokers and the combination of ex-smokers and never smokers.

A *P*-value of less than 0.05 was considered significant.

Results

In all, 26.5 % were current smokers, 27.4 % ex-smokers and 46.1 % never smokers. The study population was aged between 29 and 61 years, with a mean age of 44 years, and 55 % were women (Table 1).

In women, M/I differed significantly between smoking groups (*P*=0.03) and was higher in smokers in an unadjusted model (Table 2). In men, there were significant differences (*P*=0.04) in GS between smoking categories in unadjusted models. In fully adjusted models no significant differences remained for M/I, HOMA-IR, EIRI or GS for either sex.

In men, after full adjustment in Model 2, there were differences between smoking groups for glucose AUC (*P*=0.02) and for C-peptide AUC (*P*=0.005), with smokers showing

higher levels (Table 2). In women, the AUC of C-peptide was highest among ex-smokers, but similar to current smokers, with a significant (*P*=0.03) difference between the three groups after full adjustment.

The time curves of plasma glucose and C-peptide levels during the OGTT are presented for men and women, in Appendix 1. At 30 minutes (*P*=0.04) and 60 minutes (*P*=0.02) into the OGTT the corresponding glucose levels were significantly higher in smoking men compared to never and ex-smoking men, in unadjusted analyses. For women there were no such differences. There were no differences in C-peptide or insulin levels at any time point during OGTT.

Conclusion

Smokers are at higher risk of developing type 2 diabetes [1, 3]. As this process takes a long time, a cross-sectional analysis should be able to show either a contributing increased insulin resistance or decreased β-cell function in smokers without diabetes. In this health-selected all-European population, smokers did not show a decreased insulin sensitivity or β-cell function compared to ex-smokers and never-smokers after adjustment.

Some previous studies have compared insulin sensitivity between smokers and non-smokers using clamp data in a similar way. In a small study including 40 men, it was shown that insulin sensitivity improved after 8 weeks in the 17 men who quit smoking compared to the remaining 23 participants [7]. However, several larger studies have investigated the relationship between smoking and insulin sensitivity using HOMA-IR [8, 9], insulin at three time points during an OGTT [10] or an intravenous glucose tolerance test [11], without showing any evidence of an increased insulin resistance in smokers.

Despite the absence of decreased insulin sensitivity or secretion in smokers, we found that smoking men showed an increased glucose and C-peptide AUC during the OGTT. The

increased AUCs in smokers are due to a more rapid increase in glucose and C-peptide 30 to 90 minutes into the OGTT. Similar patterns have been previously reported in a study of 1454 men without diabetes, where blood glucose levels were higher in smokers 40 and 60 minutes into the OGTT, while no differences were observed at 0 or 120 minutes [23]. It has previously been shown in a small study including 20 smokers and 20 non-smokers, that the acute effect of smoking was a delayed insulin response during the OGTT [24]. The results are also consistent with results from a meta-analysis of 35,425 people without diabetes showing a higher HbA1c in smokers, while also reporting no differences in fasting glucose but a lower glucose 2 hours after an OGTT [25].

We speculate that the increased plasma glucose and C-peptide during the OGTT that we found in this study could be explained by small, early disturbances in glucose metabolism associated with smoking, that are not detectable by the insulin sensitivity or secretion indices used in this study. Alternatively the increased plasma glucose in smokers observed at 30 and 60 minutes into the OGTT could be due to faster gastric emptying as this has previously been described in abstaining smokers [26]. This would cause the glucose solution to reach the duodenum faster, resulting in a quicker glucose uptake.

A meta-analysis has shown that the increased risk of type 2 diabetes associated with smoking is greater in men than in women [3]. Thus, the mechanisms behind the association between smoking and diabetes seem more evident in men, a fact that could explain the sex differences during the OGTT found in our study.

This is, to our knowledge, the largest study so far using the gold standard clamp methodology to investigate insulin sensitivity in relation to smoking. This is a major strength. Smoking is well-known to be associated with other life-style related factors that might influence insulin secretion or β-cell function. We chose to adjust for age and research center to account for national and cultural differences. Otherwise, the lack of differences between

smoking categories in our study could be interpreted as over-adjustment. It is unlikely that lack of adjustment caused the absence of significant results in this study.

The crude division of the population into three smoking categories, with no information on pack-years of smoking exposure, limits the ability to measure associations between exposure and outcome.

In conclusion, in a health-selected European population without diabetes, smokers did not show evidence of decreased insulin sensitivity or β-cell function compared to ex-smokers and never-smokers. However increased glycaemic and C-peptide responses were evident in smoking men following an OGTT, a finding that needs to be further evaluated.

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Further information on the RISC Study and participating centres can be found on www.egir.org.

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Table 1 Characteristics of the study population (n=1199), mean and ± SD or %, stratified by sex and smoking habits. The EGIR-RISC Study

	Men (n=539)			*Women (n=660)*		
	Current smoker	**Ex-smoker**	**Never smoker**	**Current smoker**	**Ex-smoker**	**Never smoker**
n (%)	152 (28.2)	145 (26.9)	242 (44.9)	166 (25.2)	183 (27.7)	311 (47.1)
Age (years)	41.4 ± 8.1	45.0 ± 8.1	43.0 ± 8.8	43.0 ± 8.0	45.9 ± 8.1	44.1 ± 8.2
BMI (kg/m2)	26.3 ± 3.8	26.5 ± 3.5	26.3 ± 3.3	24.2 ± 4.1	25.2 ± 4.2	24.7 ± 4.3
Waist circumference (cm)	93.3 ± 10.8	93.9 ± 9.9	93.1 ± 9.9	79.7 ± 10.7	82.4 ± 11.1	80.3 ± 11.9
Fasting glucose (mmol/l)	5.2 ± 0.5	5.3 ± 0.5	5.2 ± 0.5	4.8 ± 0.6	5.0 ± 0.5	4.9 ± 0.6
IPAQ Physical activity (met mins/wk)	2.2 ± 0.7	2.1 ± 0.7	2.2 ± 0.7	2.1 ± 0.8	2.2 ± 0.7	2.2 ± 0.7
High alcohol consumption (men: >168 g/wk ; women >108 g/wk)	39 (25.7)	34 (23.4)	40 (16.5)	28 (16.9)	26 (14.2)	30 (9.6)

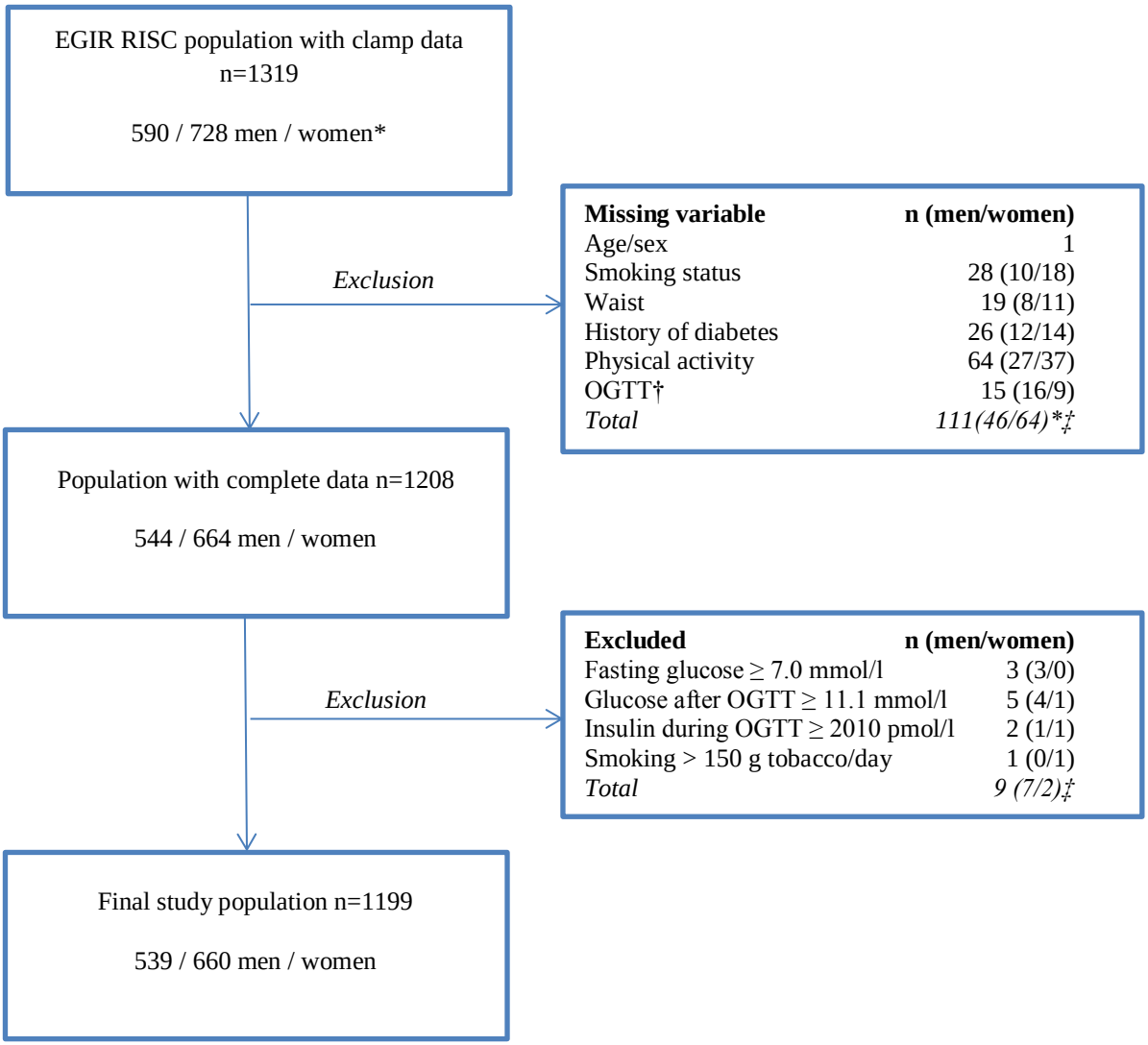
Table 2 Mean ± SD of insulin sensitivity, β-cell function and glucose, insulin and c-peptide area under the curve (AUC) during the oral glucose tolerance test (OGTT) according to sex and smoking categories. *P*-values are for differences between groups. The EGIR-RISC Study

			Men			
	n	**Current smoker**	**Ex-smoker**	**Never smoker**	***P* - no adj**	***P* - Model 1**	***P* - Model 2**
Insulin sensitivity or resistance							
M/I	539	120 ± 62	133 ± 71	124 ± 63	0.3	0.1	0.053
HOMA-IR	517	1.32 ± 0.87	1.30 ± 0.69	1.20 ± 0.63	0.4	0.8	0.8
Beta cell function							
EIRI	508	34.3 ± 27.1	30.4 ± 17.2	29.6 ± 18.6	0.3	0.3	0.2
GS	537	109 ± 67	120 ± 70	118 ± 67	0.04	0.07	0.1
Area under the curve (AUC) during the oral glucose tolerance test							
Glucose	539	29.4 ± 6.1	28.1 ± 4.8	28.5 ± 5.3	0.1	0.01	0.02
insulin	497	1094 ± 750	997 ± 582	973 ± 535	0.5	0.6	0.5
C-peptide	532	9116 ± 3305	8672 ± 2339	8336 ± 2716	0.03	0.007	0.005
			Women				
	n	**Current smoker**	**Ex-smoker**	**Never smoker**	***P* - no adj**	***P* – Model 1**	***P* - Model 2**
Insulin sensitivity or resistance							
M/I	660	169 ± 97	158 ± 71	149 ± 64	0.03	0.2	0.2
HOMA-IR	642	1.06 ± 0.70	1.08 ± 0.62	1.09 ± 0.69	0.6	0.6	0.9
Beta cell function							
EIRI	631	30.1 ± 23.1	30.6 ± 19.4	32.3 ± 21.4	0.1	0.3	0.3
GS	660	150 ± 115	146 ± 108	143 ± 95	0.95	0.95	0.98
Area under the curve (AUC) during the oral glucose tolerance test							
Glucose	660	26.5 ± 6.1	26.6 ± 5.4	26.6 ± 6.1	0.98	0.7	0.4
Insulin	610	926 ± 566	1002 ± 660	949 ± 530	0.5	0.6	0.8
C-peptide	657	8497 ± 2850	8532 ± 2737	8176 ± 2466	0.3	0.06	0.03

Abbreviations: M/I, Insulin sensitivity measured by the hyperinsulinaemic euglycaemic clamp; HOMA-IR, Homeostatic model assessment - insulin resistance; EIRI, Early Insulin Response Index; GS, β-cell Glucose Sensitivity; AUC, Area under curve during OGTT. Model 1: Adjustment for age and study centre. Model 2: Adjustment for age, study centre, BMI, family history of diabetes, physical activity and a high alcohol consumption. β-cell function adjustment also includes adjustment for M/I in both Models 1 and 2.

Legends to figure

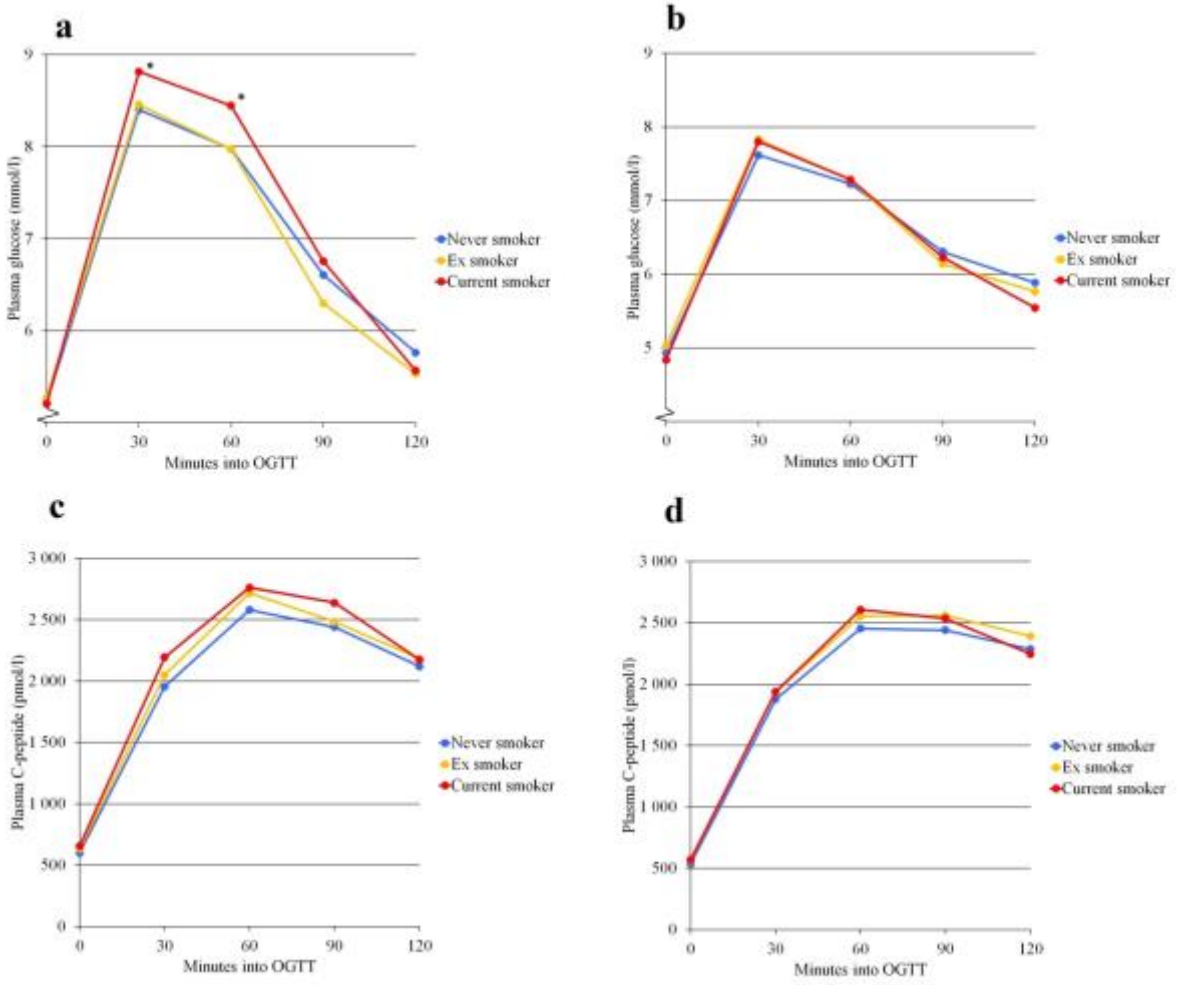
FIGURE 1 Flow chart of missing data and exclusions.



*One individual had no data on sex, therefore numbers do not add up.
†Missing at least one of the five glucose values during the OGTT.
‡Some individuals had more than one missing or excluded variable and the same individual can therefore be listed more than once.
Abbreviations: BMI; Body Mass Index, OGTT; Oral Glucose Tolerance Test

Legend to appendix

APPENDIX 1
a Plasma glucose in men; b plasma glucose in women; c plasma c-peptide in men; d plasma c-peptide in women during the OGTT, stratified for smoking status The EGIR-RISC Study



*Significant difference ($p<0.05$) between current smokers compared to never and ex smokers at this time point.