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Parental history of type 2 diabetes, TCF7L2 variant and lower insulin secretion are associated with incident hypertension. Data from the D.E.S.I.R. and RISC cohorts

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Abstract

Aims/hypothesis. The relation between insulin secretion and the incidence of hypertension has not been well characterized. We hypothesised that both a parental history of diabetes and the *TCF7L2* polymorphism, which increases susceptibility todiabetesbecause of impaired beta cell function, are associated with incident hypertension. We assessed in a separate cohort whether low insulin secretion is related to incident hypertension.

Methods Nine-year incident hypertensionwas studied in 2391 normotensive participants from the D.E.S.I.R. cohort.The relation between insulin secretion and 3-year incident hypertension was investigated in 1047 non-diabetic, normotensive individuals from the RISC cohort. Insulin secretion during an OGTT was expressed in relation to the degree of insulin resistance, as assessed by a hyperinsulinemic-euglycemic clamp.

Results In the D.E.S.I.R. cohort, a parental history of diabetes and the *TCF7L2* at-risk variant were both associated with hypertension incidence at year-9, independently of waist, blood pressure, fasting glucose, insulin levels and the HOMA-IR at inclusion (p=0.02 for parental history, p=0.006 for *TCF7L2*). In the RISC cohort, a lower insulin secretion rate during the OGTT at baseline was associated with both higher blood pressure and a greater risk of hypertension at year-3. This inverse correlationbetween the insulin secretion rate and incident hypertension persisted after controlling for baseline insulin resistance, glycaemia and blood pressureat baseline (p=0.007).

*Conclusions/interpretation*Parental history of diabetes, the *TCF7L2*polymorphism and a reduced insulin secretion rate were consistently associated with incident hypertension. A low insulin secretion rate may be a new risk factor for incident hypertension, beyond insulin resistance.

Keywords: hypertension, insulin secretion,parental history of diabetes, *TCF7L2*, type 2 diabetes

Abbreviations:
D.E.S.I.R.: Data from an Epidemiological Study on the Insulin Resistance syndrome
HOMA2-IR: HOMA of insulin resistance
HOMA2%B: HOMA of beta cell function
OGTT: Oral glucose tolerance test
RISC: Relationship between Insulin Sensitivity and Cardiovascular disease

Introduction

Hypertension and type 2 diabetes are strongly interrelated and may share common environmental risk factors such as sedentary behaviours, visceral adiposity and insulin resistance[1]. Elevated blood pressure confers an increased risk for incident type 2 diabetes in the general population [2] and hypertension treatments may also modulate the risk of diabetes [3]. Fasting blood glucose, hyperinsulinemia and HbA1c have been associated with the development of hypertension in non-diabetic individuals [4-8]. However, the pathophysiology underlying the association between elevated glycemia and incident hypertension has not been well characterized. In particular, the impact on incident hypertension of a parental history of diabetes or genetic predisposition to type 2 diabetes, which is mainly associated with beta cell dysfunction [9], is not known. Furthermore, although the epidemiological link between insulin resistance and hypertension is recognized, the relation between insulin secretion and the incidence of hypertension and elevated blood pressure has not been investigated in the general population.

The aim of our study was to assess in the prospective D.E.S.I.R. cohort, the impact of a genetic predisposition to type 2 diabetes, on the development of hypertension. We analyzedthe impact of the *TCF7L2* polymorphismon incident hypertension, because this polymorphism has the largest effect on susceptibility to diabetes among the predisposing genes discovered to date [10]. As we observed an association with the *TCF7L2* at-risk allele, we tested whether defects in insulin secretion predispose to 3-year incident hypertension, in 1047 non-hypertensive, non-diabetic participants from the RISC cohort, all of whom had an accurate evaluation at baseline of both insulin sensitivity by the hyperinsulinaemic-euglycaemic clamp and insulin secretion from an extended OGTT [11].

Research Design and Methods

The D.E.S.I.R. cohort

We studied men and women aged 30–65 years, who participated in the 9-year follow-up study, Data from an Epidemiological Study on the Insulin Resistance Syndrome (D.E.S.I.R.). Participants were recruited from volunteers offered periodic health examinations free of charge by the French Social Security, in 10 health examinations centres in western France[12, 13]. All signed an informed consent and the protocol was approved by an ethics committee.Cases of hypertension were defined by treatment for hypertension or resting blood pressure ≥ 140 (systolic) or ≥ 90 (diastolic) mmHg at one of the four three-yearly examinations. After exclusion of individuals with hypertension at baseline, we studied 2391 participants with genotype data for *TCF7L2* (745 had incident hypertension during the follow-up).

Clinical assessment

Two measures of blood pressure, using a mercury sphygmomanometer, were taken in a supine position after 5 minutes rest; mean values were used. Weight and height were measured in lightly clad participants, and body mass index (BMI) calculated. The examining physician noted the parental history of diabetes in a clinical questionnaire; treatment for diabetes and hypertension were recorded at each of the three yearly examinations.Smoking habits and alcohol intake were recorded in an auto-questionnaire.

Biochemical measurements

All biochemical measurements were from one of four health center laboratories located in France at Blois, Chartres, La Riche, and Orléans. The interlaboratory variability for normal and pathological values was assessed monthly. Fasting plasma glucose, measured by the glucose-oxidase method, was applied to fluorooxalated plasma using a Technicon RA100 analyzer (Bayer Diagnostics,

Puteaux, France) or a Specific or a Delta device (Konelab, Evry, France). $HbA1_c$ was determined by high-performance liquid chromatography (L9100 ion-exchange analyzer; Hitachi/Merck-VWR, Fontenay-sous-Bois, France) or an immunoassay (DCA 2000; Bayer Diagnostics). Insulin was quantified by microparticle enzyme immunoassay with an automated analyzer (IMX; Abbott, Rungis, France). Glucose, $HbA1_c$ and insulin have been standardized over laboratories and over the years of the study. Indexes of insulin resistance (HOMA2-IR) [14] and beta cell secretion (HOMA2%B) were computed using software downloaded at http://www.dtu.ox.ac.uk[15].

Genotyping

TCF7L2 single nucleotide polymorphism (SNP) *rs7903146* genotyping was performed with the SNPlex™ Technology (Applied Biosystems, Foster City, CA) based on the Oligonucleotide Ligation Assay (OLA) combined with multiplex PCR target amplification (http://www.appliedbiosystems.com) [12].

The RISC cohort

RISC is a prospective observational cohort study whose rationale and methodology have been published, as well as the characteristics of the individuals recruited[11, 16]. Clinically healthy men and women, aged 30-60 years, were recruited 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 blood pressure ≥140/90 mmHg, fasting plasma glucose ≥7.0 mmol/l, 2-hour 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. We studied 1,047 healthy individuals (579 women and 468 men) who had an evaluation of both insulin sensitivity and insulin secretion at baseline and who

had complete data at the three years follow up. Ethics Committee approval was obtained by each recruiting centre. Volunteers were given detailed written information on the study as well as an oral explanation, and they all signed a consent form.

Height and body weight were measured and BMI calculated.Alcohol and tobacco consumption was assessed using a standardized semi-quantitative questionnaire [17].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 [18].

Blood Pressure

Blood pressure was measured in triplicateafter five minutes of rest, by trained study nurses using an OMRON 705CP (Omron Healthcare GmbH, Hamburg, Germany) with participants sitting, according to a standard protocol; the median of these readings was used in this analysis for both the baseline and the follow-up examinations. Hypertension was defined as median systolic blood pressure ≥ 140 mmHg and/or median diastolic blood pressure ≥ 90 mmHg or treatment for hypertension in routine care at follow-up.

OGTT

Blood samples were taken at fasting and 30, 60, 90 and 120 min into the OGTT, for central analysis of routine blood chemistry. Blood collected during the studies was separated into plasma and serum, aliquoted and stored at -20 ºC for central assays of glucose and insulin.

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 Oy, 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%.

Insulin sensitivity

On a separate day within one month of the OGTT, participants had a hyperinsulinemic-euglycaemic clamp. Exogenous insulin was administered as a primed-continuous infusion at a rate of 240 $pmol.min^{-1}.m^{-2}$ with a variable 20% dextrose infusion adjusted every 5-10 min to maintain plasma glucose level within 0.8 mmol/l (±15%) of the target glucose level (4.5-5.5 mmol/l). The clamp procedure was standardised across centres[11]; 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 is 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 $\mu mol.min^{-1}.kg\text{-}fat\text{-}free\text{-}mass^{-1}(nmol.l^{-1})^{-1}$.

Insulin secretion

Beta-cell function was assessed from the OGTT using a model describing the relationship between insulin secretion (calculated from C-peptide with the method of van Cauter et al [19]and glucose concentration, previously described in detail[20, 21]. 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 subjects with normal glucose tolerance) was estimated. This parameter represents insulin secretion in basal conditions, if basal glucose were 5 mmol/l in each participant. Total insulin secretion was also determined using the model(integral during the OGTT, in $nmol.m^{-2}$)[21]. We considered, for all statistical analyses, that the product of either total insulin secretion or insulin secretion at 5 mmol/l glucose with the M/I value from the

clamp, to express the rate of insulin secretion, in relation with the concomitant degree of insulin resistance.

Statistical analysis

The data are expressed as mean ± SDor 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. Baseline characteristics, means and percentages, were compared using Student's t and χ^2 tests respectively, according to incident hypertension.

For the D.E.S.I.R. cohort, as there was no significant interaction between sex and either parental history of diabetes or the *TCF7L2* genotype on the risk of hypertension, we analysed men and women together. The relations between both parental diabetes and the *TCF7L2* genotype with incident hypertension were assessed by logistic regression analysis, with adjustment for gender, age and waist circumference. Further adjustments for fasting insulinemia, fasting glycemia and mean blood pressure were made. We also tested for an interaction between $HbA1_c$> 5.7% and *TCF7L2* variants on incident hypertension.

In the RISC cohort, blood pressure levels at year 3 were compared according to the quartiles of insulin secretion rate at baseline by ANOVA; we excluded individuals treated by antihypertensive medications at year 3 for this analysis.

A logistic regression analysis was used to test the association between the insulin secretion rate at baseline, assessed both as a continuous variable and stratified into quartiles, and incident hypertension at year-3 with adjustment for age, gender, recruitment centre, physical activity, waist, fasting glycaemia, systolic and diastolic blood pressure levels at baseline.

Statistical analyses used StatView (version 5.0, SAS Institute Inc.,NC) and SAS version 9.2 (SAS Institute, Cary, NC).

Results

The D.E.S.I.R. cohort

In the D.E.S.I.R. cohort,those with incident hypertension had higher fasting glucose, $HbA1_c$, waist circumference, and insulin concentrations at baseline as compared to those who remained normotensive (Table 1).

Fasting glycaemia but not HbA1c levels at baseline remained associated with incident hypertension in a logistic regression analysis, after adjustment for gender and baseline age, waist circumference, and mean blood pressure. Neither HOMA2-IR nor the HOMA2%B indices were significantly related to the risk of incident hypertension in this multivariable model.

Parental history of diabetes

A parental history of diabetes was associated with a higher incidence of hypertension at 9 years (36% vs 30%, p=0.02). This association persisted after controlling for potential confounders such as waist circumference and BMI (Table 2).Further adjustment for baseline fasting glycaemia, fasting insulinaemia, HOMA2-IR andHOMA2%B did not alter the significant relationship (Table 2).

TCF7L2 genotype

The *TCF7L2* variant conformed to Hardy-Weinberg equilibrium. Blood pressure levels at baseline did not differ according to the *TCF7L2* genotype. However, *TCF7L2* at-risk variants (CT + TT) were significantly associated with both higher systolic and diastolic blood pressure at year 9 in univariate analysis [CT+TT vs CC genotype: 137 ± 19 vs 135 ± 19 mmHg, p=0.02 for systolic; 81 ± 10 vs 80 ± 10 mmHg, p=0.01 for diastolic blood pressure].

The presence of the T allele was associated with an increased risk of incident hypertension at year-9, after controlling for gender and age, and the association persisted after further adjustment

for waist circumference, BMI, mean blood pressure, fasting glycaemia, insulinaemia, HOMA2-IR andHOMA2%B at baseline (Table 3).

We further assessed whether baseline BMI or glycaemic status modified the effect of *TCF7L2* at-risk allele on the risk of incident hypertension. There was no significant interaction between BMI (≥ or <27 kg/m^2) and *TCF7L2* on incident hypertension ($p_{interaction}$=0.25). However, there was a significant interaction between baseline HbA1$_c$ above the median value (≥5.7%) and the effect of *TCF7L2* polymorphism on incident hypertension ($p_{interaction}$=0.03). The risk of hypertension after controlling for gender, age and waist circumference was greater for individuals who carried the T allele with HbA1$_c$ ≥5.7% at baseline [OR: 1.82;95% CI (1.21, 2.74), *p*=0.004] than for those with the T allele but normal HbA1$_c$< 5.7% [1.11(0.90, 1.37), *p*=0.3].

The RISC cohort

Baseline glycaemia and hypertension at year 3

In univariate analysis, both fasting and 2hr glycaemia at baseline were significantly associated with incident hypertension at year-3 (Table 4). However, in a multivariable analysis, the association between both fasting glycaemia [OR: 1.34; 95% CI (0.96, 1.86); *p*=0.09] and 2hr glycaemia [1.03; (0.92, 1.16); *p*=0.63] with the risk of hypertension at year-3 was no longer significant after controlling for gender, age, recruitment center and waist circumference respectively.

Baseline insulin secretion and hypertension at year 3

Individuals who developed hypertension at year-3 had at baseline, a reduced total insulin secretion during the OGTT and also a reduced basal beta-cell function at 5 mmol/l glucose, as compared to those who remained normotensive (Table 4). This suggests that individuals with subtle alterations in insulin secretion, after accounting for the concomitant degree of insulin resistance, were at higher risk of developing hypertension at follow-up.

A lower insulin secretion rate at baseline (1st vs 4th quartile of total insulin secretion during OGTT x M/I) was associated with higher blood pressure at year 3 [SBP: 123 ± 14 vs 118 ± 15 mmHg, p=0.0001; DBP: 78 ± 9 vs 74 ± 9 mmHg, p<0.0001]. A similar inverse association was observed for basal insulin secretion at a fixed normal glucose level of 5 mmol/l from the beta-cell dose-response [1st vs 4th quartile, SBP: 125 ± 13 vs 116 ± 15 mmHg, p<0.0001; DBP: 79 ± 8 vs 73 ± 9 mmHg, p<0.0001].

The incidence of hypertension at year-3 decreased progressively across the quartiles of both total insulin secretion and insulin secretion at 5 mmol/l at baseline (Figure 1). Individuals with the lowest total insulin secretion rate during OGTT (quartile 1) had a higher incidence of hypertension as compared to those with the highest insulin secretion (quartile 4), after adjusting for other baseline risk factors (centre, age, gender, physical activity, waist circumference, smoking, alcohol intake, fasting glucose, systolic and diastolic blood pressures) [OR: 2.02; 95% CI (1.06, 3.86), p=0.03].

Similarly, the inverse association between total insulin secretion rate during the OGTT, expressed as a continuous value, and the risk of incident hypertension at 3 years remained significant in a multivariable model after controlling for centre, age, gender, physical activity, waist, smoking, alcohol intake,systolic and diastolic blood pressure levels at inclusion [OR per 1 SD log total insulin secretion: 0.75; 95% CI (0.61, 0.93), p=0.007]. The addition of fasting glucose and 2h glucose levels into the model did not alter the significant relationship, [OR: 0.75; 95% CI (0.61, 0.92), p=0.007] and [OR: 0.75; 95% CI (0.61, 0.93), p=0.007] respectively.

Discussion

The main finding of this study is that a genetic predisposition to type 2 diabetes and in particular the *TCF7L2* at-risk allele, was significantly associated with the incidence of hypertension after a 9-year follow-up in the general population; *TCF7L2* conferred an increased risk of type 2 diabetes, through alterations in insulin secretion capacity. Furthermore, we provide support for the first time, that a

lower insulin secretion rate during an OGTT is a risk factor for the development of elevated blood pressure in a non-diabetic cohort, even after taking into account insulin resistance, as assessed by the gold standard method of the clamp.

Previous evidence has shown that offspring of patients with type 2 diabetes have an increased risk of developing type 2 diabetes, with defects in early beta-cell secretion[22-25]. It has also been suggested that these offspring have early autonomic dysfunction, even in the absence of glucose intolerance[26], and more often display metabolic abnormalitieswith high insulin levels and arterial hypertension[27, 28].In a previous community-based cohort, parental diabetes appeared as an independent predictor of longitudinal changes in both systolic and diastolic blood pressures in the offspring, regardless of race and gender[29]. Taken together, these observations and our results suggest that heritable factors related with type 2 diabetes, increase the risk of various disorders, including hypertension.

Genetic susceptibility totype 2 diabetesseems to be more related to early beta-cell dysfunction rather than to insulin resistance [30, 31]. We selected the *TCF7L2* polymorphism as this variant that has the largest effect on type 2 diabetessusceptibility among the predisposing genes discovered to date[10, 32]. To our knowledge, this is the first report to study the longitudinal relation of the*TCF7L2* genotypeon incident hypertension in the general population. It has been demonstrated that the at-risk *TCF7L2* genotype is associated with impaired insulin secretion[33] and a reduced sensitivity of the beta-cell to incretins[34].The fact that the effect of the *TCF7L2* polymorphism on blood pressure was independent of waist circumference, glycaemia or the insulin-resistance HOMA2-IR index, underscores the potential role of insulin secretion defects on the evolution of blood pressure over time. This is highlighted by our data on the relationship between both total and basal insulin secretion and 3-year incident hypertension in the RISC cohort. However, we suggest that the association of the *TCF7L2* genotype with incident hypertension might be related, at least in part, to reduced GLP-1 secretion in these individuals, as recent evidence

suggested that incretins may modulate blood pressure [35]. The *TCF7L2* variant may then affect primarily the incretin levels, and as a consequence, have independent effects on blood pressure and on insulin secretion.

Our longitudinal results are in favor of a positive interaction between subtle elevations in glycaemia and genetic predisposition leading to defects in insulin secretion in the development of hypertension. Our results are consistent with recent genetic data showing an association between the TCF7L2 variant and increased systolic blood pressure in an endogamous ethnic group from India [36].However, it should be noted that a recent large GWAS study did not report a significant association between the TCF7L2 variant *rs7903146* and blood pressure, although a trend was observed for systolic blood pressure (p=0.06) and pulse pressure (p=0.04) [37].A regional plot around *TCF7L2*locus from the data publicly available of the ICBP GWAS study suggest that a signal near *rs7903146*may modulate BP, although this variant doesn't appear to be a glycaemic signal (data not shown). The differences between our present study and the GWAS report may be related to the longitudinal follow-up used in our study as opposed to the case-control design used in the GWAS study. Indeed, we did not observe a significant association between the TCF7L2 variant and the presence of hypertension in a cross-sectional analysis of the entire D.E.S.I.R. cohort at baseline (data not shown). Furthermore, in our study, we found that a large part of the association of the *TCF7L2* variant with hypertension was observed in people who already had $HbA1_c$ at baseline above the median value (≥5.7%), which may explain why TCF7L2 locus did not appear as being associated with hypertension in large GWAS cross-sectional studies that were not stratified on glucose. Our results need to be confirmed in other large prospective cohorts with a long follow-up.

The causal role of insulin in the development of hypertension has been much debated. It has been proposed that insulin resistance and/or hyperinsulinaemia promote the development of elevated blood pressure over time [5, 6, 38, 39] with a positive association between insulin levels and incident hypertension [39, 40]. In these studies, the insulin secretion rate was not specifically

assessed. Our findings confirm an association between fasting insulinaemia and blood pressure but however suggest a greater role for reduced insulin secretion in the pathogenesis of hypertension. Fasting insulin is a marker of insulin resistance which could be confounded by the presence of subtle defects in insulin secretion.

To our knowledge, the effects of insulin secretion on blood pressure evolution have not been investigated previously, in contrast to the role of insulin resistance.In the present study, we found that a lower total insulin secretion rate was significantly associated with the incidence of hypertension in non-diabetic people, and that this effect was independent of insulin sensitivity. This observation 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. Interestingly, in the present study, the inverse association between insulin secretion and incident hypertension was not related to weight gain over the follow-up (data not shown) and was also independent of insulin resistance, as assessed by the gold-standard method of the clamp, suggesting alternative mechanisms.

Potential mechanisms that may explain the increase in hypertension risk with a reduced insulin secretion rate, may be the protective action exerted by insulin on the blood vessel. Hemodynamic actions of insulin have been suggested [41] and a small blood-pressure lowering effect of insulin has been described in non-diabetic individuals [42]. Furthermore, a partial or complete deficiency in the insulin receptor and/or the insulin-receptor substrate-1 in the endothelial cells, is associated in mice, with endothelial dysfunction and increased blood pressure [43, 44]. Together these findings suggest that a reduced activation of the insulin pathway through diminished insulin secretion capacity, particularly in the presence of insulin resistance, may lead to endothelial dysfunction that favors the development of hypertension. Hyperinsulinaemia has to be interpreted as a compensation for enhanced insulin resistance. If this compensation is insufficient, the net effect at the cellularlevel, including probably the endothelium, is a down-tuned insulin signal.

Furthermore, recent evidence suggests that improving insulin secretion via the modulation of incretin concentrations may lower blood pressure, even in non-diabetic hypertensive individuals [45, 46].

In parallel, subtle defects in beta cell function may facilitate development of hypertension through possible repeated post-prandial elevations in glucose levels over time. Glycemic variability may contribute to increase blood pressure through chronic induction of inflammation and enhanced oxidative stress [47, 48]. Oxidative stress has been shown to correlate with both glycemic fluctuations and blood pressure variability, suggesting possible alternative mechanisms to explain the increased incidence of hypertension in relation to subtle defects in beta cell function [49]. In addition, the STOP-NIDDMtrial has shown that acarbose treatment, which reduces postprandial hyperglycemia, was associated with a significant reduction in the development of incident hypertensionin those with impaired glucose tolerance, suggesting an impact of postprandial excursions on blood pressure [50].This point needs to be specifically investigated in another prospective study with ambulatory, continuous assessment of glucose values.

Limitations of the present study include the absence of accurate measures of both insulin resistanceand beta cell function over time in the D.E.S.I.R. cohort, which precludes us from demonstrating that the association between parental history, the *TCF7L2*genotype and incident hypertension is directly related to defects in beta cell function. It is possible that the association observed between parental history and hypertension may be related, at least in part, to enhanced insulin resistance in the parents.The number of individuals with the at-risk *TCF7L2*genotype was limited, in comparison with large genetic consortium studies and our results, albeit being significant, need to be replicated in other populations. Furthermore, the absence of postprandial glucose levels did not allow us to investigate the exact role of this genotype on the development of elevated blood pressure. The strengths of the present study are the use of two complimentary

cohorts specifically dedicated to the study of glucose metabolism, with a large number of normotensive participants at inclusion, aligned laboratory assays, continuous quality control of data. The RISC cohort is the largest cohort available with both a systematic evaluation of insulin secretion and gold standard measures of insulin sensitivity by the glucose clamp technique, which provided the opportunity to clarify the role of alterations of insulin secretion in hypertension. For this important question to be addressed, a large number of individuals with high quality measures of glucose metabolism is required.

In conclusion, our study shows that a parental history of type 2 diabetes and at-risk variants in the *TCF7L2*genotype predispose to incident hypertension. The novel finding of the association between reduced insulin secretion and the development of elevated blood pressure deserves further investigations with the study of the impact of treatments targeting insulin secretion and postprandial hyperglycemia on blood pressure. Our results suggest that defects in insulin secretion may be a new independent risk factor for the development of hypertension.

The list of the D.E.S.I.R. Study Group and RISC investigators is presented in the ESM.

Author contributions

FB was responsible for the conception of the study, analysed data, and wrote the manuscript; RR, BB, EF and AN reviewed the manuscript andcontributed to the discussion, CL, SC and LY analysed the data, ML, JP, PF, OL and MM reviewed the manuscript. All authors approved the final version.

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Duality of interest The authors declare that there is no duality ofinterest associated with this manuscript.

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Table 1. Baseline characteristics of participants in the D.E.S.I.R. cohort according to incident hypertension (HTA) over the 9-year follow-up.

	Without incident HTA n=1646	**With incident HTA** n=745	*P*
Age (years)	44 ± 9	49 (9)	0.0001
Men (%)	41%	52%	0.0001
BMI (kg/m^2)	23.4 ± 3.0	24.8 ± 3.3	0.0001
Waist circumference (cm)	78 ± 10	84 ± 10	0.0001
Smoking	21%	20%	0.4
Systolic blood pressure (mmHg)	121 ± 9	127 ± 7	0.0001
Diastolic blood pressure (mmHg)	75 ± 7	78 ± 6	0.0001
Fasting glucose (mmol/l)	5.17 ± 0.55	5.41 ± 0.88	0.0001
$HbA1_c$ (%)	5.35 ± 0.40	5.50 ± 0.55	0.0001
HbA1c (mmol/mol)	33.2 ± 2.48	34.1 ± 3.41	0.0001
Fasting insulin (pmol/l) *	39.9 (20.8)	45.3 (23.3)	0.0001
HOMA2-IR*	1.55 (0.91)	1.29 (0.73)	0.0001
HOMA2%B*	83.6 (25.7)	82.5 (25.2)	0.3

Data shown are as mean (standard deviation), median (interquartile range) or %

* log transformation for statistical analysis

Table 2. Association between parental history of diabetes and incident hypertension in the D.E.S.I.R. cohort

	OR (95% CI)	*P*
Unadjusted	1.30 (1.04-1.64)	0.02
Adjusted for gender, age	1.45 (1.14-1.84)	0.003
Adjusted for gender,	1.41 (1.10-1.80)	0.006
Adjusted for gender, age, waist	1.41 (1.11-1.81)	0.006
Adjusted for gender, age, waist, HbA1c	1.40 (1.09-1.79)	0.008
Adjusted for gender, age, waist, fasting glucose, fasting insulin	1.37 (1.07-1.76)	0.01
Adjusted for gender, age, waist, mean BP, smoking, HOMA-IR	1.37 (1.06-1.77)	0.02
Adjusted for gender, age, waist, fasting glucose, mean BP, smoking, alcohol intake	1.39 (1.04-1.86)	0.03
Adjusted for gender, age, waist, mean BP, smoking, alcohol intake, HOMA-IR	1.39 (1.04-1.87)	0.03

Table 3. Association between *TCF7L2*at-risk variants and incident hypertension (CT+TT vs CC as reference) in the D.E.S.I.R. cohort

	OR (95% CI)	*P*
Unadjusted	1.15 (0.96-1.36)	0.12
Adjusted for gender, age	1.23 (1.02-1.47)	0.03
Adjusted for gender, age, BMI	1.22 (1.02-1.47)	0.03
Adjusted for gender, age, waist	1.22 (1.01-1.47)	0.03
Adjusted for gender, age, waist, HbA1c	1.22 (1.02-1.47)	0.03
Adjusted for gender, age, waist, fasting glucose, fasting insulin	1.22 (1.01-1.47)	0.03
Adjusted for gender, age, waist, mean BP, smoking, HOMA-IR	1.31 (1.08-1.59)	0.006
Adjusted for gender, age, waist, fasting glucose, mean BP, smoking, alcohol intake	1.38 (1.11-1.73)	0.004
Adjusted for gender, age, waist, mean BP, smoking, alcohol intake, HOMA-IR	1.40 (1.12-1.75)	0.003

Table 4. Baseline characteristics in the RISC cohort according to incident hypertension (HTA) over the 3-year follow-up

	Without incident HTA (n=881)	**With incident HTA** (n=166)	*P*
Age (years)	43.9 ±8.2	47.7 ±8.1	<0.0001
Waist (cm)	86 ±12	92 ±12	<0.0001
BMI (kg/m²)	25.1 ±3.7	27.2 ±4.3	<0.0001
Smoker (%)	26.3	26.7	0.92
Physically inactive (%)	19.3	22.0	0.43
Systolic blood pressure (mmHg)	116 ±12	128 ±10	<0.0001
Diastolic blood pressure (mmHg)	74 ±8	80 ±7	<0.0001
Fasting glucose (mmol/L)	5.1 ±0.5	5.3 ±0.6	<0.0001
2hr glucose (mmol/l)	6.1 ±1.7	5.7 ±1.5	0.02
Fasting insulin (pmol/l)*	29.5 (21.0)	36.0 (25.7)	<0.0001
Clamp Insulin Sensitivity (M/I) *	134 (88)	114 (76)	<0.0001
Total insulin secretion† x M/I*	50.8 (29.7)	46.2 (25.8)	0.0005
Insulin secretion at 5 mmol† x M/I*	90.0 (72.7)	74.2 (71.5)	0.002

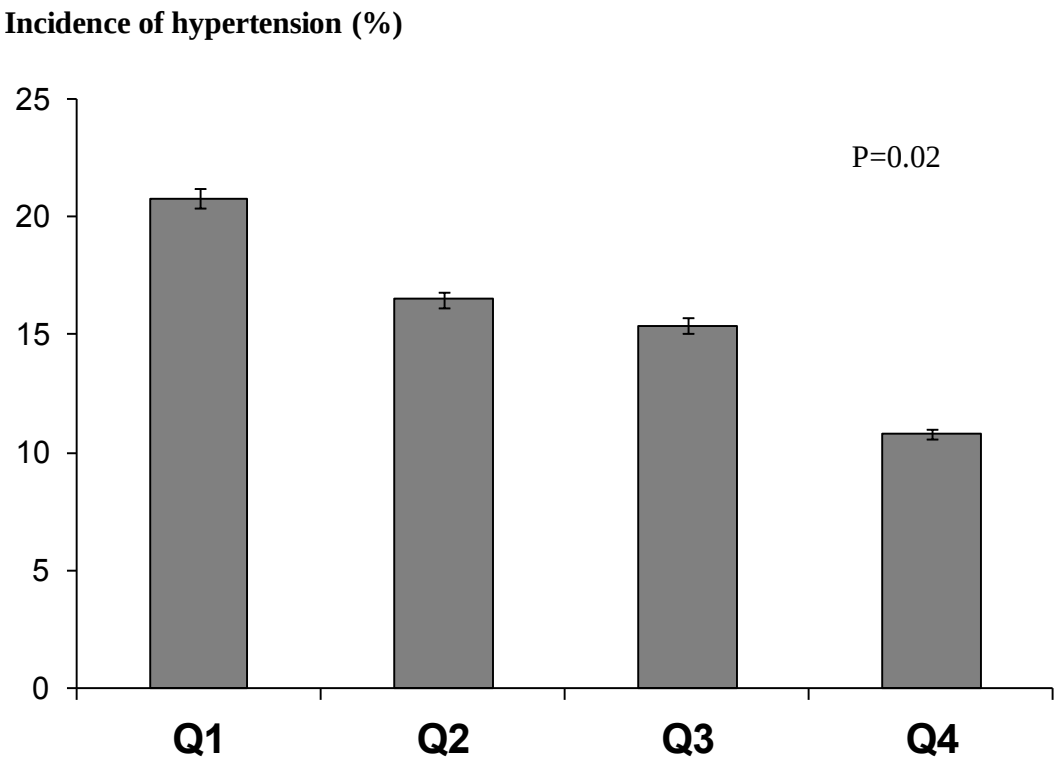
Data shown are as mean ± standard deviation, median (interquartile range) or n

*log-transformed for analysis

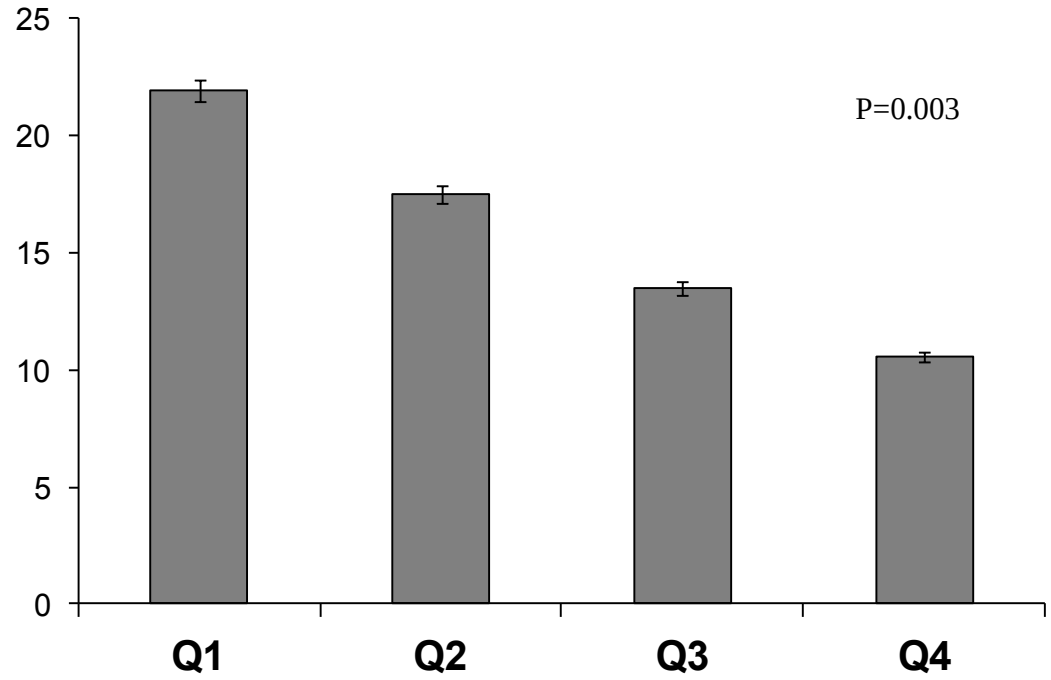
†Total insulin secretion during the OGTT at baseline, and insulin secretion at 5 mmol/l are both expressed in relation to the M/I value (nmol m^{-2}µmol min^{-1}kgffm^{-1} $nmol^{-1}$) and multiplied by 10^{-2} for simplification of presentation

Fig 1

A


Incidence of hypertension (%)
25
20
15
10
5
0
P=0.02
Q1
Q2
Q3
Q4

B


Incidence of hypertension (%)
25
20
15
10
5
0
P=0.003
Q1
Q2
Q3
Q4

Legends for figure

Fig.1 Incidence of hypertension at year-3 according to the quartiles of (A) total insulin secretion rate x M/I during the OGTT (B) basal insulin secretion rate at 5 mmol glucose x M/I at inclusion in the RISC cohort. The *p* value is for trend.