bjh short report

Glucose levels and diabetes are not associated with the risk of venous thrombosis: results from the MEGA case-control study

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Summary

It is unclear whether hyperglycaemia or diabetes mellitus are risk factors for a first venous thrombosis (VT). Self-reported diabetes status and fasting glucose (FG) measures were collected from the Multiple Environmental and Genetic Assessment (MEGA) study to confirm these associations. FG levels were categorized based on the World Health Organization criteria [<6·1 (reference), 6·1–7·0 (2nd), ≥7·0 (3rd) mmol/l]. Logistic regression was performed to quantify the associations. Neither increased FG levels [Odds ratio (95% confidence interval): 0·98 (0·69–1·37) 2nd vs. reference, 0·97 (0·58–1·63) 3rd vs. reference] nor self-reported diabetes [1·12 (0·80–1·58)] were associated with an increased risk of a first VT.

Keywords: hyperglycaemia, venous thrombosis, type 2 diabetes mellitus, case-control studies, odds ratio.

In recent years, several studies indicated that venous thrombosis (VT) and atherosclerotic cardiovascular disease (CVD) might share common risk factors (Lijfering *et al*, 2011). Diabetes mellitus, a chronic metabolic disease that is associated with CVD, is diagnosed by elevated fasting glucose levels and has been increasingly investigated as a possible risk factor for VT (Petrauskiene *et al*, 2005; Heit *et al*, 2009; Stein *et al*, 2009). In several case-control studies, glucose levels were measured at the time of the VT diagnosis; these levels may have been affected by the thrombotic event (i.e., acute phase effect), rather than representing levels before the event (Hermanides *et al*, 2009; Tichelaar *et al*, 2011; Cohn *et al*, 2012).

In the current study, we explored whether fasting glucose levels (after VT events were diagnosed, as a surrogate for glucose levels before the event), are associated with an increased risk of a first VT in a non-diabetic population without a recent cancer diagnosis. Furthermore, we studied the association between self-reported diabetes and the risk of a first VT. Analyses were performed in a large, population-based case-control study, the Multiple Environmental and Genetic Assessment (MEGA) of Risk Factors for VT study.

Materials and methods

Study design

The MEGA study is a population-based case-control study investigating the aetiology of VT. The study design was approved by the Ethics Committee of the Leiden University Medical Centre, the Netherlands, and written informed consent was obtained from all participants. From 1999 to 2004, 4956 consecutive VT patients with an objectively confirmed first event of VT or pulmonary embolism (PE) were included in the study. The control subjects were recruited from two sources, i.e., partners of VT patients without a history of VT (n = 3297); and from the general population, by random-digit dialling (RDD), further matched for age and sex with the VT cases (n = 3000). A detailed description of study design, study population selection and VT risk factor assessment can be found in the Appendix S1.

Laboratory tests

Patients and controls visited one of the anticoagulation clinics for an interview and blood sampling at least 3 months

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after discontinuation of anticoagulation, or during anticoagulant therapy in patients who continued this therapy for more than 1 year. Glucose levels were measured on stored (−80°C) and previously unthawed fasting serum samples by hexokinase method on a Modular P800 Clinical Chemistry analyser (Roche Diagnostics, Mannheim, Germany).

Fasting glucose concentrations were firstly categorized into three categories according to the World Health Organization/International Diabetes Federation (WHO/IDF) criteria for diagnosing diabetes mellitus and intermediate hyperglycaemia (≤6·0, ≥6·1 and <7·0, ≥7·0 mmol/l, respectively) (WHO/IDF 2006). Additionally, they were categorized into quintiles based on the empirical fasting glucose distribution from the entire MEGA control population [<4·5 (<25th), 4·5–4·8 [25th–50th), 4·8–5·2 [50th–75th), 5·2–6·6 [75th–97·5th), ≥6·6 mmol/l (≥97·5th)].

Statistical analyses

Logistic regression models were used to estimate the odds ratios (OR) with 95% confidence intervals (95% CIs) for the associations of continuous and categorized fasting glucose levels with a first event of VT. In the basic model, age- and sex-adjusted ORs with 95% CIs were estimated. In addition, oestrogen use at both index date and blood draw, body mass index, statin use at blood draw and C reactive protein levels were added as confounders in a fully adjusted model. To adjust for lifestyle as a confounder in a model, we performed an additional 1:1 matched analysis by conditional logistic regression, taking only the VT patient partner controls into account. The risk of VT associated with fasting glucose levels was estimated for provoked and unprovoked VT events separately. Unprovoked VT was defined previously (van Hylckama Vlieg *et al*, 2014). Several sensitivity analyses were performed in addition to the main analyses to assess the robustness of the risk estimates (Appendix S1). To determine whether diabetes is a risk factor for VT, two models with different confounders (basic model and fully adjusted model) were taken into account in both the logistic as well as conditional logistic regression, similarly to the analyses described above. In addition, analyses were performed both for provoked and unprovoked VT.

All statistical analyses were performed with SPSS for Windows, release 23 (SPSS Inc, Chicago, IL, USA).

Results

The baseline characteristics of all participants in both analyses are summarised in Table SI. No association was found between fasting glucose levels as a continuous variable and the risk of VT (OR: 0·97, 95% CI 0·89–1·05) in the fully adjusted model (Table I). After categorizing fasting glucose levels according to the WHO criteria for diabetes mellitus diagnosis and adjusting for all potential confounders, there was still no association observed between increased levels of fasting glucose and the risk of VT, with an OR of 0·97 (95% CI 0·58–1·63) for the highest (≥7 mmol/l) *versus* the reference category (<6·1 mmol/l). Similar results were observed for separate analyses for provoked and unprovoked VT. The sensitivity analysis, which added self-reported diabetic individuals to the highest glucose level group, also yielded no association by any of the different models. (Table SII).

Subsequently, fasting glucose concentrations were categorized into quintiles. If anything, there was a weakly decreased risk, with an 0·84-fold (95% CI 0·69–1·03), 0·77-fold (95% CI 0·63–0·94), 0·74-fold (95% CI 0·61–0·92) and 0·73-fold (95% CI 0·48–1·14) decreased risk of VT in the 2nd, 3rd, 4th and 5th category of fasting glucose levels, respectively, compared with the lowest reference category (Table SIII). The sensitivity analysis in which the blood samples tested were restricted to 2- to 4-h room temperature transportation showed no association in different models. (Table SIV).

Self-reported diabetes was not associated with an increased risk of VT (Table II), with an OR of 1·12 (95% CI 0·80–1·58) in the fully adjusted model.

Discussion

Previous case-control studies provided evidence that hyperglycaemia was associated with the risk of VT, but in nearly all these studies blood was drawn at the time of the thrombotic event, where stress-induced hyperglycaemia either by the thrombotic event (Hermanides et al, 2009; Tichelaar et al, 2011) or surgery (Cohn et al, 2012) could have occurred, leading to spurious results. In two large cohort studies, no association was found between HbA1c and the incidence of subsequent VT (Bell *et al*, 2013; Lerstad *et al*, 2014), where reverse causation could not have occurred. While a limited number of cases were identified during follow-up (n = 345 VT cases out of 12 298 participants and n = 333 VT cases out of 16,156 participants, respectively), these findings are consistent with our observations in the current analysis.

Type 2 diabetes is considered a prothrombotic condition in some studies, with the hypothesized mechanism of suppressing fibrinolysis through increasing fibrinolytic inhibitor PAI-1 (Plasminogen activator inhibitor-1) levels (Grant, 2007). Our null findings may for this reason come as surprising. Nevertheless, both a recent meta-analysis as well as an individual patients meta-analysis of cohort studies are in line with our findings, in which confounding was meticulously taken into account (Gariani *et al*, 2016; Mahmoodi *et al*, 2017).

There are some strengths in the current study. Firstly, the large sample size allowed for subgroup analysis. Secondly, detailed information was available regarding many risk factors for VT to adjust for potential confounders. Thirdly, conditional logistic regression in partner controls alone enabled us to fully adjust for further confounding according to socioeconomic factors and lifestyle.

Table I. The association of fasting glucose levels categorized by the diabetes mellitus diagnosis according to World Health Organization criteria (WHO/IDF 2006) and the risk of a first VT event.

FG level (mmol/l)	Patients (n = 1888)	Controls (n = 2531)	Odds ratio* (95% CI)	Adjusted odds ratio† (95% CI)	Matched patients (n = 869)	Matched controls (n = 869)	Odds ratio‡ (95% CI)	Adjusted odds ratio§ (95% CI)
Total population with all patients								
<6·1	1777	2402	Ref	Ref	818	822	Ref	Ref
≥6·1, <7·0	76	92	1·12 [0·82,1·53]	0·98 [0·69,1·37]	32	37	0·88 [0·54, 1·42]	0·68 [0·38, 1·22]
≥7·0	35	37	1·29 [0·80,2·05]	0·97 [0·58,1·63]	19	10	1·85 [0·86, 4·00]	2·19 [0·78, 6·11]
Continuous variable	1888	2531	1·05 [0·98,1·13]	0·97 [0·89, 1·05]	869	869	1·11 [0·97,1·27]	1·08 [0·96, 1·22]
VT patients with provoked VT								
<6·1	1221	2402	Ref	Ref	575	566	Ref	Ref
≥6·1, <7·0	38	92	0·96 [0·65,1·42]	0·85 [0·55,1·32]	14	28	0·53 [0·27, 1·04]	0·44 [0·18, 1·04]
≥7·0	18	37	1·24 [0·70,2·20]	0·95 [0·51,1·80]	12	7	1·58 [0·60, 4·15]	2·42 [0·62, 9·41]
Continuous variable	1277	2531	1·06 [0·98,1·15]	0·99 [0·90, 1·08]	601	601	1·12 [0·96, 1·31]	1·13 [0·98, 1·29]
VT patients with unprovoked VT								
<6·1	534	2402	Ref	Ref	238	251	Ref	Ref
≥6·1, <7·0	38	92	1·34 [0·89,2·01]	1·13 [0·74,1·71]	18	9	0·89 [0·35,2·23]	0·71 [0·26, 1·91]
≥7·0	16	37	1·23 [0·66,2·27]	1·01 [0·54,1·92]	7	3	1·18 [0·24,5·80]	1·95 [0·31, 12·24]
Continuous variable	588	2531	1·02 [0·90,1·16]	0·92 [0·81, 1·06]	263	263	1·00 [0·73, 1·37]	0·98 [0·69, 1·38]

BMI, body mass index; CI, confidence interval; CRP, C reactive protein; FG, fasting glucose; VT, venous thrombosis.

*adjusted for sex and age.

†adjusted for age, sex, BMI, statin use, oestrogen use and CRP.

‡adjusted for sex, age and matched by lifestyle.

§adjusted for age, sex, BMI, statin use, oestrogen use and CRP, and matched by lifestyle.

Table II. The association of self-reported diabetes with the risk of a first event of VT.

	Patients (Diabetic patients), *n*	Controls (Diabetic controls), *n*	Odds ratio* (95% CI)	Adjusted odds ratio† (95% CI)	Matched patients (Diabetic patients), *n*	Matched controls (Diabetic controls), *n*	Odds ratio‡ (95% CI)	Adjusted odds ratio§ (95% CI)
Total population	3280 (149)	4930 (184)	0·90 [0·72,1·13]	1·12 [0·80,1·58]	1565 (64)	1565 (63)	1·02 [0·71,1·46]	1·26 [0·69,2·29]
Provoked VT patients	2068 (78)	4930 (184)	0·94 [0·72,1·24]	1·14 [0·75,1·72]	1030 (36)	1030 (35)	1·09 [0·66,1·81]	1·47 [0·64,3·37]
Unprovoked VT patients	1152 (67)	4930 (184)	0·84 [0·62,1·13]	1·06 [0·68,1·66]	520 (27)	520 (27)	1·29 [0·68,2·45]	1·08 [0·40,2·90]

BMI, body mass index; CI, confidence interval; CRP, C reactive protein; VT, venous thrombosis.
*adjusted for sex and age.
†adjusted for age, sex, BMI, statin use, oestrogen use and CRP.
‡adjusted for sex, age and matched by lifestyle.
§adjusted for age, sex, BMI, statin use, oestrogen use and CRP, and matched by lifestyle.

Several limitations should also be considered. Firstly, HbA1c levels, as a more accurate measure than fasting glucose to reflect hyperglycaemia and diabetes status, were not available in the study. Meanwhile, fasting glucose levels were measured in patients after the first VT to surrogate glucose levels before the event. However, it is possible that the VT patients, in particular, adopted healthier lifestyles after the disease, which led to decreased levels of fasting glucose compared with the controls and therefore a weakening of the association with VT. Secondly, we cannot rule out misclassification by self-reported diabetes. However, this misclassification was minimalized by including medication use. Moreover, around 4% of current study population self-reported as diabetes, which is comparable to the Dutch diabetes prevalence of 4% in the period of 2001/2002 (data from Dutch Centraal Bureau voor de Statistiek). Thirdly, the number of diabetic individuals was limited in both the VT patients and controls, which resulted in limited power in some of the subgroup analyses.

In conclusion, our current findings confirm that neither elevated fasting glucose levels in the non-diabetic population nor self-reported diabetes are associated with an increased risk of VT.

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Author contributions

R. Li-Gao analysed and drafted the manuscript; R. Li-Gao, V. M. Morelli, W. M. Lijfering, A. van Hylckama Vlieg interpreted the data; F. R. Rosendaal, S.C. Cannegieter and A. van Hylckama Vlieg designed the study. All the authors reviewed the manuscript.

Conflict of interest

The authors have nothing to disclose.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1.

Fig S1. Flowchart of sample selection from the MEGA study. (A). The selection for the analysis of self-reported diabetes to the risk of a first event of VT, with the number of cases and controls in the brackets. (B). The selection for the analysis of hyperglycaemia in non-diabetic population to the risk of VT.

Fig S2. Group average fasting glucose levels across seven time interval categories, tested by one-way ANOVA and controlled by age, sex, BMI and case/control status. The numbers labelled above the dots in the figure corresponded to the numbers of patients/controls falling into a time interval category.

Table SI. Baseline characteristics

Table SII. Sensitivity analysis of the association of fasting glucose levels categorized by the diabetes mellitus diagnosis according to WHO criteria and the risk of a first event of VT, by including self-reported diabetic individuals

Table SIII. The association of fasting glucose levels categorized by the percentiles of the empirical fasting glucose distribution in the control population and the risk of VT

Table SIV. Sensitivity analysis of taking the blood samples with the time interval between two and four hours, and fasting glucose were categorized by the percentiles of the empirical fasting glucose distribution in the controls

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