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Blood glucose may condition factor VII levels in diabetic and normal subjects

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Summary. Increased factor VII levels have been reported in Type 1 (insulin-dependent) diabetic subjects. A direct correlation between fasting plasma glucose and factor VII level was found to exist in both diabetic and normal subjects. Induced-hyperglycaemia was able to increase factor VII levels in both diabetic patients and normal control subjects while, when euglycaemia was achieved in diabetic patients, factor VII values returned to normal range. This study shows that the level of factor VII may be directly conditioned by circulating blood glucose and, therefore, stresses the role of hyperglycaemia in conditioning coagulation abnormalities in diabetes mellitus.

Key words: Factor VII, hyperglycaemia, euglycaemia, diabetes mellitus, coagulation abnormalities.

High levels of factor VII have been associated with an increased risk of both ischaemic heart disease [1] and atherosclerosis [2]. It has long been known that diabetes mellitus is associated with an increased incidence of vascular disease, and several studies of the fluid phase of coagulation suggest that diabetes is associated with a hypercoagulable state [3, 4].

However, it is not yet clear whether the reported coagulation abnormalities are causally related or are secondary to increased glycaemia [3, 4]. Since increased factor VII levels have been reported in diabetes [5, 6], the aim of this study is to evaluate the influence of hyperglycaemia on factor VII in both diabetic and normal subjects.

Materials and methods

Thirty Type 1 (insulin-dependent) diabetic patients, selected according to National Diabetes Data Group (NDDG) criteria [7], 17 males and 13 females (age 32 ± 1.33 years, mean $\pm$ SEM) body mass index (BMI) 23.4 ± 1.48; duration of diabetes 7.35 ± 1.21 years; insulin regimen 20-80 U/day, mean 40.8 ± 2.25 U/day), gave informed consent to this study. Thirty healthy normal subjects, without personal or family history of diabetes, showing a normal oral glucose tolerance test according to NDDG criteria [7], matched for sex (16 males and 14 females), age (31.1 ± 1.42 years), and BMI (22.3 ± 1.27), served as control subjects.

The subjects consumed weight maintaining diets with at least 250 g carbohydrate/day, for three days before the study.

This experiment was approved by the review board for human experiments of our institute, and all subjects gave informed consent to participate in the study after a detailed explanation of its experimental nature.

Basal study

Plasma glucose, glycated HbA_1 and factor VII levels were assayed in fasting conditions in both diabetic patients and normal subjects.

Dynamic studies conditions

In the morning, in the postabsorptive state and after a 12 to 14 h fast, an intravenous cannula was introduced into a antecubital vein of each subjects and kept patent with a slow infusion of 0.9% NaCl. Venous samples for laboratory analysis were obtained from one cannula; another cannula was used for administration of glucose or insulin.

Hyperglycaemic studies conditions

In ten diabetic patients (basal glycaemia 6.7-10 mmol/l) and in ten control subjects, after the subjects had rested for 90 min, two basal samples were obtained (−30 and 0 min). In the diabetic patients, hyperglycaemia was induced by administering a pulse of 0.33 g glucose/kg, and then glucose was maintained between 19.5 and 22.5 mmol/l for 1 h.

In the normal subjects, hyperglycaemia was induced according to Cerasi and Luft [8]. Each subject received a pulse of 0.5 g glucose/kg in less than 2 min; glucose ($20 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was then infused via a peristaltic pump for 1 h. Blood samples were collected every 10 min in the first hour, and at 90, 120 and 180 min.

Normoglycaemic studies design

In ten diabetic patients (fasting glycaemia 11.8-14 mmol/l), after a 30 min baseline period, normoglycaemia was achieved administering 1 mU·kg^{-1}·min^{-1} insulin for 2 h. Samples were removed at −30, 0, 30, 60, 70, 80, 90, 100, 110, 120 and 180 min, and glycaemia and factor VII levels measured.

Analytical methods

Citrated venous blood (1 ml of anticoagulant to 9 ml of blood) was obtained with plastic syringes; a solution of 0.1 mol/l sodium citrate was used as anticoagulant. Blood was collected in polystyrene tubes and centrifuged at 1500 g for 20 min at 4° C. Glycaemia was measured with a Beckman Analyzer (Beckman Instruments Inc, Galway, Ireland).

HbA_1 was evaluated by aminophenylboronic acid affinity chromatography [9]. Factor VII coagulant activity was assayed in triplicate with a one stage method, using an artificial factor VII deficient plasma as reagent [10], supplied by Boehringer Biochemia Robin (Mannheim, FRG).

In this method, factor VII level is evaluated measuring the prothrombin time when the sample is added to the factor VII depleted plasma. This value is compared to a reference curve obtained with a large pool of healthy blood donors, and expressed as U/dl. The prothrombin time obtained with the plasma pool diluited 1/10 is considered as the reference value of 1 U/dl.

The intra and inter-assay coefficients of variation were 4.8% and 6.1%, respectively.

To exclude the possibility that glucose itself does not interfere with the factor VII assay, three sets of control experiment were performed. First, simple solution of glucose, at concentrations of 5, 10 and 20 mmol/l respectively, were run in the factor VII assay system; second, glucose (5, 10 and 20 mmol/l) was added to five samples with normal basal glycaemia (4.48-5.04 mmol/l), and factor VII assayed before and after addition; third, five blood samples with high basal glycaemia (11.2-16.8 mmol/l) were dissolved in Owren buffer, pH 7.35, and dialised against the same buffer to remove free glucose, and factor VII measured before and after dialysis.

Statistical analysis

Statistical evaluation was performed by Student's t-test for paired and unpaired data, and by single linear regression analysis.

Results

Factor VII levels were increased in diabetic patients (122.6 ± 5.87 U/dl, mean ± SEM, vs 77.1 ± 3.86 U/dl; $p > 0.001$).

A good linear correlation between factor VII levels and fasting blood glucose, in both diabetic and normal subjects was found (Fig. 1). Induced-hyperglycaemia was able to increase factor VII in both diabetic and normal subjects ($p > 0.01$ vs basal values at 10-120 min, Fig. 2), while when euglycaemia was achieved in diabetic patients, factor VII showed values in the normal range (Fig. 3).

Glucose did not influence factor VII assay. In the first protocol, coagulation was not activated in factor VII depleted plasma, in the second study, factor VII levels before (75.2 ± 2.99 U/dl) and after (76.4 ±

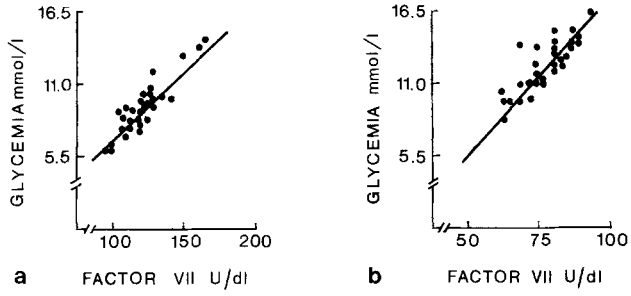

Fig. 1a and b. Factor VII and blood glucose correlation in diabetic (●, $r = 0.89$, $p > 0.001$) and normal (●, $r = 0.83$, $p > 0.001$) subjects, **a** = diabetic patients; **b** = normal subjects

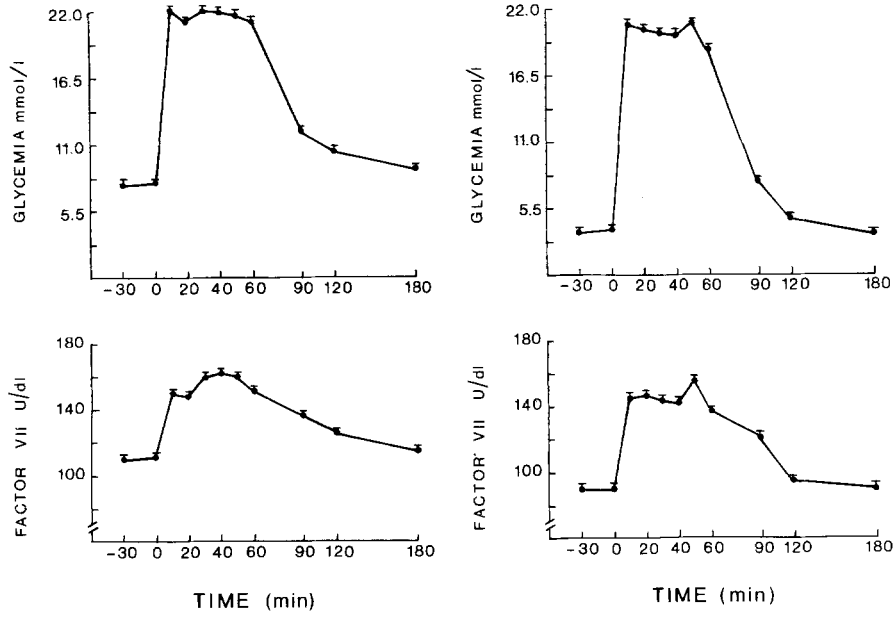

Fig. 2. Effect of induced-hyperglycaemia on factor VII level in diabetic patients (left) and control subjects (right). Data are reported as mean ± SEM

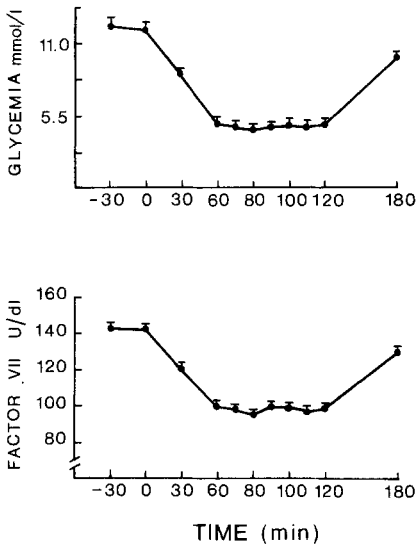

Fig. 3. Effect of normoglycaemia on factor VII levels in diabetic patients. Data are reported as mean ± SEM

2.54 U/dl, 5 mmol/l glucose; 76.8 ± 3.25, 10 mmol/l glucose, 75.7 ± 2.88 U/dl, 20 mmol/l glucose) glucose addition unchanged, as well as when glucose was removed in the third protocol (128 ± 3.89 U/dl vs 126 ± 4.75 U/dl).

Discussion

This study confirms increased factor VII levels in Type 1 diabetes, and, furthermore, stresses the role of hyperglycaemia in conditioning this phenomenon. Interestingly, a good correlation between factor VII levels and fasting blood glucose is present in both diabetic and normal subjects. More recently, Ballesein et al. reported an association between glucose levels and factor VII in a large normal population [2].

Our study confirms these data, and, moreover, showing the effect of induced hyperglycaemia in healthy subjects, suggests a possible role of circulating blood glucose in conditioning factor VII levels in man. In light of this, factor VII increase would be the direct results of increased glycaemia.

Of interest, moreover, is to consider that an increased risk of ischaemic heart disease, associated with high levels of factor VII [1], is frequently associated with impaired glucose tolerance [11]. This hypothesis supports the possibility to obtain normal factor VII levels, when euglycaemia is achieved in diabetic patients. The mechanism of this phenomenon remained unexplained.

Glucose is a toxic agent for endothelial cells of arterial walls [12]. The consequent release of tissue thromboplastin from vascular lesions might activate factor VII. In that case, the increase of factor VII might be due to a feedback phenomenon following the consumption of factor VII. This study shows, for the first time, that factor VII levels may be conditioned by circulating blood glucose in both diabetic and normal subjects. Moreover, this report confirms the existence of coagulation abnormalities in diabetes mellitus; and especially demonstrates that hyperglycaemia is responsible, by itself, in conditioning this phenomenon.

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