Induced Hyperglycemia Alters Antithrombin III Activity But Not Its Plasma Concentration in Healthy Normal Subjects

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SUMMARY

The effects of induced hyperglycemia on both antithrombin III (ATIII) biologic activity and plasma concentration in normal subjects are reported. A decrease in ATIII activity parallel to hyperglycemia was observed, while ATIII concentration was unchanged. When the glycemia returned to basal values ATIII activity concomitantly showed values in the basal range. Heparin infusion was able to significantly preserve ATIII activity from glycemia-induced alterations. These data demonstrate that hyperglycemia by itself may alter ATIII biologic activity. Moreover, the effect of heparin administration suggests that both glucose and heparin compete in vivo for the same functional site. Our study, showing the possible role of hyperglycemia in altering the biologic function of some proteins, stresses the role of increased blood glucose in the development of some complications in diabetes. *Diabetes* 36:320–23, 1987

Several studies of the fluid phase of coagulation suggest that diabetes is associated with a hypercoagulable state. However, it is not yet clear whether the reported coagulation abnormalities are causally related or are secondary to increased glycemia (1). Antithrombin III (ATIII) is the principal physiological inhibitor of the blood coagulation system (2), and its alteration has been involved in the pathogenesis of vascular complications in diabetes (3). Despite its normal plasma concentration, decreased ATIII biologic activity has been reported in insulin-dependent diabetics (4), but the direct role of hyperglycemia in determining this effect in vivo is still unclear (5). This study describes the effects of induced hyperglycemia in healthy subjects on both ATIII activity and plasma concentration.

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MATERIALS AND METHODS

Five normal male subjects were recruited from the medical student body of the University of Naples. Ages ranged between 20 and 25 yr, and body weights were within 15% of their ideal body weight (Metropolitan Life Insurance Tables, 1959). They had no family history of diabetes and showed a normal oral glucose tolerance test, according to National Diabetes Data Group criteria (6). The subjects consumed weight-maintaining diets with at least 250 g carbohydrate/day for 3 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.

Study protocol. Two studies were performed in each subject in randomized order and on different days. In the morning, in the postabsorptive state and after a 12- to 14-h fast, an intravenous cannula was introduced into an antecubital vein of each subject 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 heparin plus glucose.

Hyperglycemic studies. After the subjects had rested for 90 min, three basal samples were obtained (−90, −60, and 0 min) before the start of the study. Hyperglycemia was induced according to Cerasi and Luft (7). Each patient received a pulse of 500 mg/kg glucose injected in <2 min; glucose (20 mg · kg^{-1} · min^{-1}) was then infused via a peristaltic pump for 1 h. Blood samples for glycemia, ATIII activity, and ATIII concentration were collected every 10 min during the infusion and every 20 min for 2 h at the end of the infusion.

Heparin studies. After a 30-min baseline period, all subjects were given an initial intravenous bolus injection of 5000 U, followed by a steady intravenous drip at a rate of 750 U/h

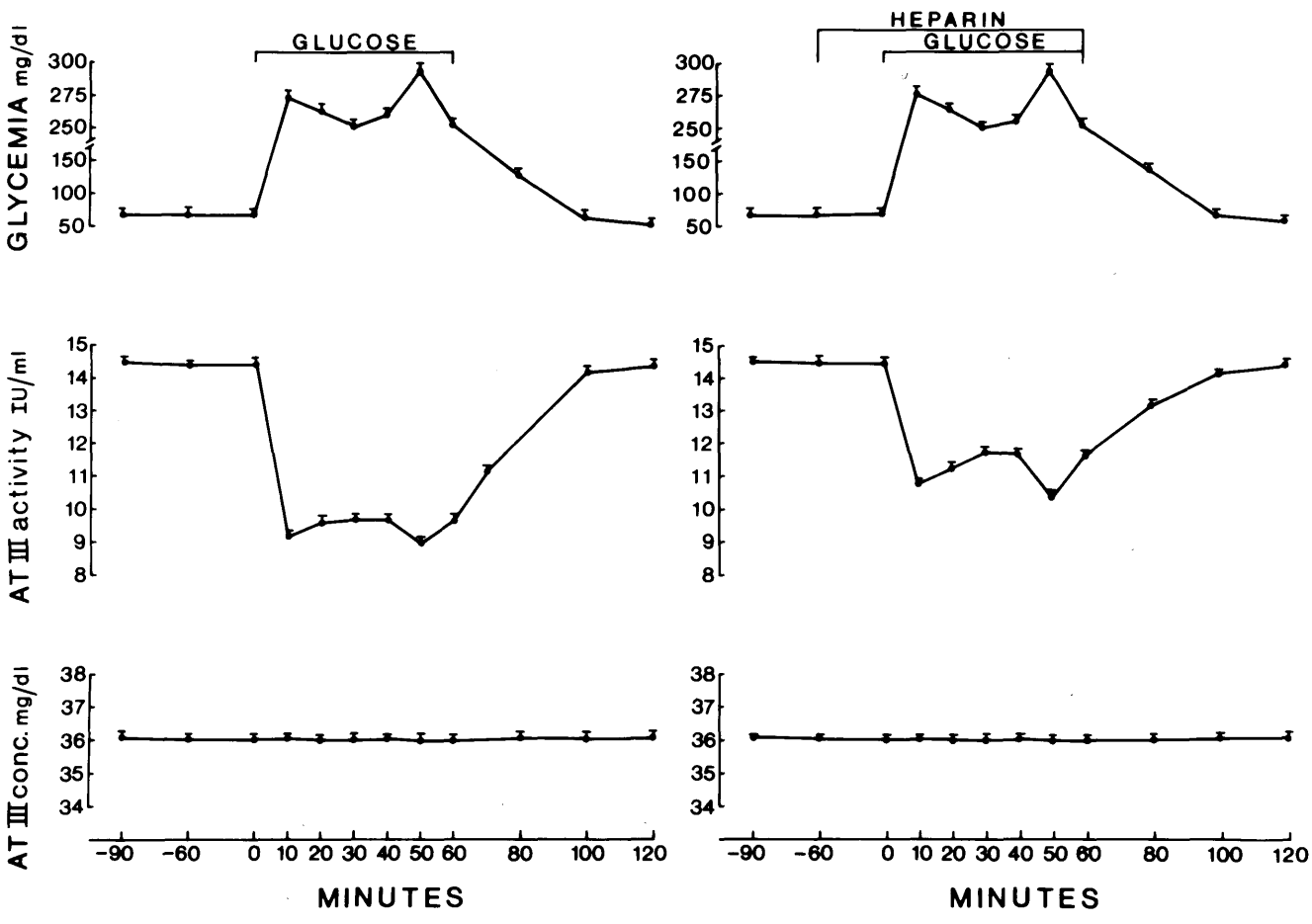

FIG. 1. Antithrombin III activity and glycemia-induced variations in healthy normal subjects.

for 2 h (8). After 1 h of heparin administration, glucose infusion was started. Blood samples were obtained as in the hyperglycemic protocol.

Analytical and statistical methods. Citrated venous blood (1 ml of anticoagulant to 9 ml of blood) was obtained with plastic syringes; a solution of 0.1 M sodium citrate was used as anticoagulant. Blood was collected in polystyrene tubes and centrifuged at 1500 × *g* for 20 min at 4°C.

Glycemia and ATIII activity were measured immediately, and the remaining plasma was frozen at −40°C until assayed for ATIII concentration. Plasma glucose was measured with a Beckman analyzer.

ATIII activity was determined in triplicate as heparin cofactor according to Abildgaard et al. (9). ATIII activity is measured as the percentage of a large pool of healthy blood donors and expressed as IU/ml. The intra- and interassay coefficients of variation for this method were 4.5 and 7%, respectively. ATIII plasma concentration was determined in triplicate by radial immunodiffusion according to Mancini (10), on M-Partigen agarose plates (Behring, Scoffito, Italy). The intra- and interassay coefficients of variation were 3 and 5%, respectively.

For each time point of the study, the ratio of the mean normoglycemic period hematocrit to the observed hematocrit ranged between 1.04 and 1.06. This factor was used to correct the dilutional error introduced by blood volume expansion. Statistical significance was determined by the paired Student's *t* test. Data are expressed as means ± SE.

RESULTS

All subjects had substantial blood glucose elevations in the hyperglycemic range as a result of glucose infusion. No significant difference between mean glycemic area (from 0 to 180 min) existed in the two experiments (270.95 ± 17.93 vs. 275.24 ± 18.33 mg/dl). Time zero glycemia and both ATIII activity and plasma concentration were similar in hyperglycemic and hyperglycemic plus heparin tests (Fig. 1; Table 1).

A decrease in ATIII biologic activity versus fasting basal values concomitant with hyperglycemia was observed at 10–80 min ($P < .001$–$.01$), whereas ATIII concentration remained unchanged (Fig. 1; Table 1). When the glycemia returned to basal values, ATIII activity concomitantly showed values in basal range (100–120 min).

Heparin infusion did not change basal ATIII activity or ATIII concentration, as shown by time zero values in both Fig. 1 and Table 1. Heparin administration was able to preserve a partial but significant amount of ATIII activity from hyperglycemia-induced alteration at 10–80 min ($P < .05$; Table 1).

TABLE 1
Antithrombin III variations during glucose and heparin plus glucose infusion

Patient	Age (yr)	Body wt (kg)		ATIII (IU/ml) at various times (min)											
				−90	−60	0	10	20	30	40	50	60	80	100	120
1	21	65	Glucose	13.2 ± 0.40	13.0 ± 0.37	13.0 ± 0.40	9.0 ± 0.31	9.5 ± 0.35	9.7 ± 0.41	9.6 ± 0.32	9.1 ± 0.41	9.5 ± 0.42	11.8 ± 0.44	12.8 ± 0.37	13.1 ± 0.41
			Glucose + heparin	13.1 ± 0.29	13.0 ± 0.38	13.1 ± 0.38	9.8 ± 0.33	10.4 ± 0.38	10.8 ± 0.39	10.6 ± 0.41	9.9 ± 0.30	10.4 ± 0.42	12.4 ± 0.41	13.0 ± 0.38	13.1 ± 0.44
2	25	74	Glucose	16.3 ± 0.41	16.3 ± 0.42	16.0 ± 0.25	10.0 ± 0.38	10.5 ± 0.41	10.7 ± 0.43	10.7 ± 0.44	10.0 ± 0.35	10.8 ± 0.44	12.4 ± 0.33	15.7 ± 0.38	16.2 ± 0.38
			Glucose + heparin	16.3 ± 0.42	16.6 ± 0.34	16.3 ± 0.31	11.5 ± 0.40	11.8 ± 0.45	12.2 ± 0.43	12.0 ± 0.41	11.0 ± 0.36	12.0 ± 0.38	15.2 ± 0.48	15.8 ± 0.29	16.4 ± 0.41
3	25	61	Glucose	13.7 ± 0.36	13.5 ± 0.19	13.5 ± 0.24	9.2 ± 0.41	8.9 ± 0.48	9.2 ± 0.44	9.2 ± 0.37	9.0 ± 0.41	9.4 ± 0.36	10.8 ± 0.41	13.7 ± 0.40	13.6 ± 0.44
			Glucose + heparin	13.5 ± 0.36	13.5 ± 0.31	13.5 ± 0.41	10.1 ± 0.42	10.9 ± 0.50	11.9 ± 0.29	11.8 ± 0.29	10.1 ± 0.40	11.5 ± 0.41	11.9 ± 0.38	13.9 ± 0.44	13.4 ± 0.45
4	22	69	Glucose	14.0 ± 0.18	14.1 ± 0.27	14.1 ± 0.39	8.8 ± 0.44	9.2 ± 0.22	9.3 ± 0.45	9.1 ± 0.33	8.9 ± 0.32	9.2 ± 0.27	10.7 ± 0.35	14.3 ± 0.45	14.2 ± 0.38
			Glucose + heparin	14.2 ± 0.37	14.2 ± 0.29	14.2 ± 0.40	11.0 ± 0.43	11.4 ± 0.39	11.9 ± 0.40	11.9 ± 0.35	10.9 ± 0.30	11.7 ± 0.30	13.5 ± 0.48	14.1 ± 0.48	14.0 ± 0.39
5	20	72	Glucose	15.4 ± 0.41	15.3 ± 0.34	15.1 ± 0.47	9.5 ± 0.37	9.8 ± 0.37	9.8 ± 0.41	9.9 ± 0.25	8.7 ± 0.27	9.7 ± 0.45	11.5 ± 0.42	14.8 ± 0.39	15.4 ± 0.40
			Glucose + heparin	15.3 ± 0.38	15.7 ± 0.38	15.2 ± 0.39	11.5 ± 0.29	11.8 ± 0.44	12.3 ± 0.38	12.2 ± 0.41	11.2 ± 0.39	12.5 ± 0.47	12.9 ± 0.40	14.6 ± 0.41	15.2 ± 0.44

Data are mean values of 3 determinations and are expressed as means ± SE.

In the last experiment, ATIII concentration was not affected by glucose plus heparin administration (Fig. 1).

DISCUSSION

Diabetes mellitus is characterized by the development of long-term sequelae such as vascular complications. The prevalent view among clinicians is that improving blood glucose control will prevent or retard diabetic vascular diseases. Despite this belief, a causal relationship between the metabolic abnormalities and the vascular complications of diabetes has not been established.

Recent studies suggest that abnormalities of the hemostatic system may be involved in the initiation or propagation of vascular lesions (1), but the role of abnormal glucose levels in determining the increased activity of the coagulation system is not clear (1).

ATIII is a major protease inhibitor for the coagulation system (2). Recently, decreases in its activity in the presence of normal plasma concentration have been demonstrated (4). However, the direct role of hyperglycemia in determining this phenomenon is unclear (5).

Our study, which showed a decrease of ATIII activity inversely related to induced hyperglycemia in normal subjects at its normal plasma concentration, demonstrates that hyperglycemia itself may alter ATIII biologic function. As previously reported (8), a single intravenous heparin administration did not alter ATIII activity or ATIII concentration but was able to significantly preserve ATIII activity from effects due to hyperglycemia.

These data suggest the competition of both glucose and heparin for the same functional site of the ATIII molecule. Inhibition of ATIII activity in vitro by nonenzymatic glycosylation has been reported (11,12), and this effect is overcome by incubation with excess heparin (11).

A role in vivo for nonenzymatic glycosylation to explain our results might be hypothesized. Our study, demonstrating a direct role of increased blood glucose in conditioning alterations of a coagulation inhibitor protein, seems to agree with the results of Jones and Peterson, who showed abnormal fibrinogen survival (13) and increased fibrinopeptide A levels (14) more directly related to abnormal glucose levels than to long-term control. Interestingly, normalization of both fibrinogen disappearance rates and fibrinopeptide A could be achieved by addition of heparin to the hyperglycemic glucose infusion (13,14). In addition, these studies indicate that an alteration in the fibrinogen molecule is not responsible for the reduced fibrinogen survival seen in hyperglycemic diabetic patients, so that an involvement of ATIII in the hyperglycemia-dependent coagulopathy of diabetes was hypothesized (13). Our data support this hypothesis.

This study shows for the first time the ability of induced hyperglycemia in healthy normal subjects to alter a biologic protein function and stresses the role of increased blood glucose in directly conditioning some alterations in diabetes.

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