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RELATIONSHIPS BETWEEN METABOLIC AND HEMOSTATIC VARIABLES IN UNCOMPLICATED DIABETES

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Several studies (see the recent review by Colwell and Haluska [14]) have demonstrated a number of abnormalities in the hemostasis tests in diabetes. These findings led to the suggestion that an increased thrombotic tendency might play a primary pathogenetic role in the onset of large- and small-vessel disease. On the other hand, the abnormalities found in diabetics might be a reflection of poor metabolic control and/or a secondary development of vascular disease. Although large prospective studies are needed to solve this dilemma, the combined evaluation of hemostatic parameters and measurements that express the degree of control of glucose and lipid metabolism should help to reveal relationships.

High levels of hemoglobin A_1 (HbA_1), which result from non-enzymatic post-synthetic glycosylation of hemoglobin, are thought to reflect the integrated glucose concentration during the previous 2-3 months and the degree of metabolic control [22]. High density lipoprotein-cholesterol (HDL-C) was found to be inversely correlated to coronary heart disease, and this is considered to be an independent risk factor [11, 30, 38]. Beta-thromboglobulin (B-TG), a platelet-specific protein extruded into plasma during the release reaction, is a sensitive index of platelet activation [3]. Finally, factor-VIII related antigen (VIIIR:Ag), a component of the factor-VIII molecular complex [2], has been proposed as a marker for vascular endothelial damage [4, 15, 18], because the endothelial cells appear to be the principal source of this protein [24]. In order to evaluate whether changes in hemostatic variables are correlated

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to the degree of metabolic control and of lipid abnormalities, we have chosen to study these recently proposed tests and to relate their results to other standard measurements [blood glucose; serum triglycerides; serum cholesterol; factor-VIII procoagulant activity (VIII:C)] in a selected group of 35 patients with insulin-dependent diabetes under poor metabolic control but without apparent micro- and macrovascular involvement.

MATERIALS AND METHODS

Thirty-five subjects with insulin-dependent diabetes mellitus (15 men and 20 women), on insulin and diet therapy only, were examined.

They were aged 15-38 years (mean: 29). The duration of diabetes ranged from 6 to 13 years (mean: 8) and the daily insulin doses ranged from 10 to 80 U, either in a single dose or in two or three spaced doses.

Insulin was discontinued on the morning of the test. Body weight, expressed as a percentage of ideal body weight according to the Metropolitan Life Insurance Company Tables, was 110 ± 18 (mean ± SD). Twelve patients were smokers (10 cigarettes daily). All patients were asked to give up alcohol during the month before testing; none had blood pressure above normal. Patients were selected on the basis of the absence of vascular complications according to the following criteria: retinopathy was excluded on the basis of the absence of microaneurysms, hemorrhages, exudates or neovascularization in both eyes after pupillary dilatation. Neuropathy was excluded if there was no paresthesia and/or ataxia during clinical examination. Nephropathy was excluded in the absence of persistent proteinuria ('Albustix'-negative 24-h urine specimens) or elevated serum creatinine (<150 μmol/l). Ischemic heart and cerebrovascular disease and arterial insufficiency of the limbs were ruled out by negative clinical examination and electrocardiographic and oscillographic findings.

The age-matched healthy control subjects, 20 men and 20 women, were aged 18 to 40 years (mean: 30). They were not taking any drugs and had no history of diabetes or of any other disease that might alter platelet function.

All blood samples were collected after overnight fasting.

Metabolic variables - Total cholesterol and triglycerides were measured by enzymatic methods and glucose by a glucose-oxidase method (Boehringer, Mannheim, FRG). HbA_1 was measured using the cation exchange resin 'Bio-Rex 70', by the method of TRIVELLI et al. [37]. The intra-assay coefficient of variation was 4.1%. HDL-C was determined by the heparin-manganese chloride precipitation method [8]. Completeness of precipitation was assessed by immunoelectrophoresis carried out in 1% agarose gel with barbital buffer (pH 8.6, ionic strength 0.05).

Hemostatic variables - VIIIR:Ag was assessed by quantitative immunoelectrophoresis [26], using monospecific rabbit antiserum to human factor VIII (Istituto Behring, Scoppito-L'Aquila, Italy). VIII:C was measured by a two-stage assay [16]. Values are expressed as percentages of those of pooled plasma from 20 normal subjects, snap-frozen and stored at –70 °C. B-TG was measured by radioimmunoassay (Thromboglobulin RIA kit, Radiochemical Center, Amersham) and values were expressed in ng/ml of platelet-poor plasma, prepared as described by LUDLAM and CASH [27].

Statistical analysis - The significance of differences between the control and patient groups was assessed by Student's standard *t*-test. The method of least squares showed a nonlinear relationship between the variables examined. Therefore, linear regression analysis was carried out by applying to the functions the conventional method of logarithmic transformation. The statistical significance of the correlation coefficient (r) was determined by Student's *t*-test for small-sized samples. Fisher's Z-transformation [20] established that the observed correlation coefficients in all instances were representative of the correlation coefficients of the entire groups.

RESULTS

Table 1 summarizes the results of laboratory tests in healthy subjects and diabetics. The correlation coefficients between the different variables in diabetics are reported in tab. 2. The mean values for all metabolic and hemostatic variables were significantly different in the group of diabetics from those of healthy subjects. Blood glucose and total cholesterol were not correlated significantly to other parameters (data not shown). HbA_1 was positively correlated to triglycerides, whereas the relationship between HDL-C and HbA_1 appeared to be negative, but was not statistically significant. Triglycerides were negatively correlated to HDL-C. B-TG

groups	glucose (mg/dl)	HbA_1 (%)	cholesterol (mg/dl)	triglycerides (mg/dl)	HDL-C (mg/dl)	B-TG (ng/ml)	VIIIR:Ag (%)	VIII:C (%)	$\frac{\text{VIIIR:Ag}}{\text{VIII:C}}$
normals	84±10	7.2±0.5	179±28	94±31	46±18	26±14	94±37	92±35	0.98±0.23
diabetics	199±89	9.2±2.9	199±41	152±120	39±16	62±39	196±103	146±46	1.42±0.57
p	<0.001	<0.001	<0.01	<0.01	<0.05	<0.001	<0.01	<0.001	<0.001

Tab. 1 - Concentrations of metabolic and hemostatic parameters in 40 healthy subjects and in 35 diabetics. Data are expressed as means ± SEM.

	HDL-C	HbA_1	VIIIR:Ag	VIII:C	$\frac{\text{VIIIR:Ag}}{\text{VIII:C}}$	B-TG
triglycerides	–0.41*	0.39*	0.55**	n.s.	n.s.	n.s.
HDL-C		n.s.	–0.45**	n.s.	–0.41*	n.s.
HbA_1			0.55***	n.s.	0.39*	n.s.
VIIIR:Ag				n.s.	0.53***	n.s.
VIII:C					n.s.	n.s.
$\frac{\text{VIIIR:Ag}}{\text{VIII:C}}$						

* $p<0.05$; ** $p<0.01$; *** $p<0.001$; n.s. – not significant

Tab. 2 - Simple correlation coefficients between metabolic and hemostatic parameters in 35 diabetics.

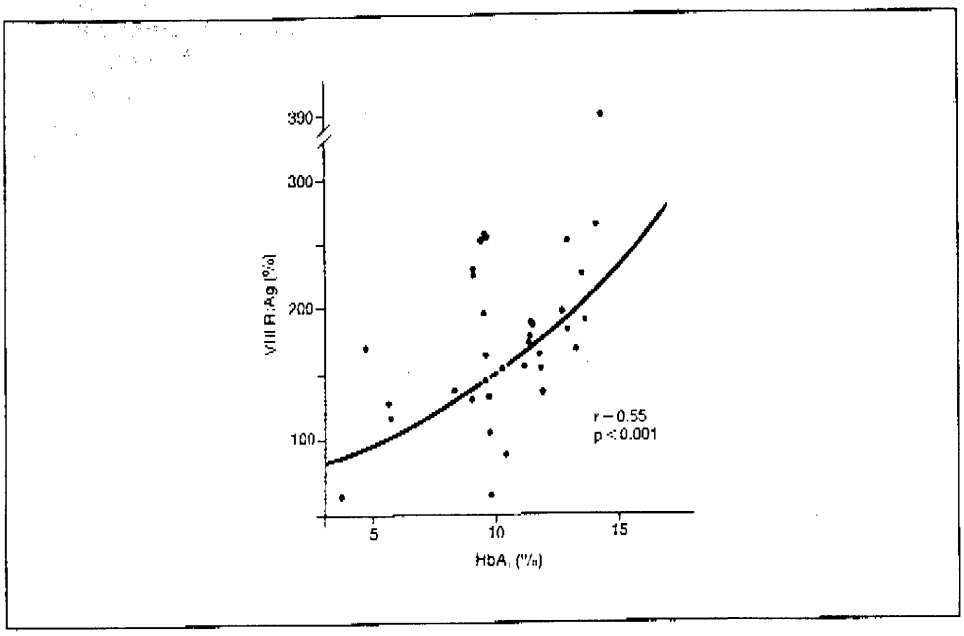

Fig. 1 - Relationship between VIIIR:Ag and HbA_1 in 35 diabetics. The line represents the best fitting curve obtained by non-linear regression analysis.

and VIII:C were not correlated to either the metabolic or the hemostatic variables. VIIIR:Ag had positive exponential correlations with triglycerides and HbA_1 (fig. 1) and a negative hyperbolic relationship with HDL-C (fig. 2). The latter two factors showed similar correlations with VIIIR:Ag/VIII:C but were not correlated to triglycerides. None of the tests showed any significant correlations in the control group of healthy subjects, except for VIII:C to VIIIR:Ag ($r = 0.78$; $p<0.001$). There were also no significant correlations of these metabolic and hemostatic variables to patient's weight or age or to the dose of insulin administered.

DISCUSSION

Contrasting results have previously been obtained when the relationship between HbA_1 and HDL-C was examined [6, 10, 19]. In the present study, no significant inverse correlation between these parameters was found, whereas HDL-C was hyperbolically related to triglycerides, which is in agreement with MYERS et al. [31] and CHAN et al. [12]. An inverse relationship between these variables appears to be consistently observed [36], and it is apparently based on a precursor product relationship between very low density lipoproteins (VLDL) (which transport triglycerides) and HDL in the lipoprotein-lipase reaction [32].

Our study also demonstrates the presence of increased plasma levels of B-TG, confirming recent reports [5, 9, 23, 34]. However, high B-TG was not correlated with HbA_1 or parameters of lipid metabolism, making it unlikely that the abnormality is a reflection of the metabolic disturbance, as suggested by PRESTON et al. [34]. Similarly, there was no significant correlation between B-TG and factor-VIII components, supporting the view that B-TG changes are independent of other hemostatic

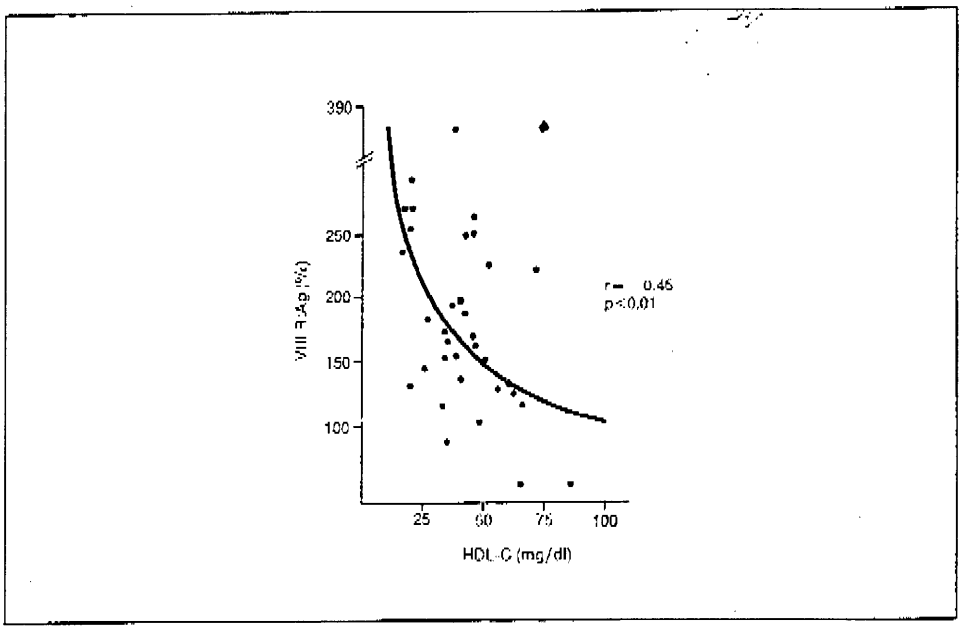

Fig. 2 - Relationship between VIIIR:Ag and HDL-C in 35 diabetics. The line represents the best fitting curve obtained by non-linear regression analysis.

parameters. Elevated plasma levels of B-TG in diabetes might indicate *in vitro* rather than *in vivo* platelet activation, particularly when the antiplatelet anticoagulant provided with the commercial radioimmunoassay kit is used without added prostaglandin E_1 [5] as was done in this study.

A disproportionately greater rise in VIIIR:Ag than in VIII:C, resulting in an increased VIIIR:Ag/VIII:C ratio, was observed in our patients. High VIIIR:Ag values were also observed in previous studies, particularly in complicated diabetes [3,28,33]. The VIIIR:Ag and/or VIIIR:Ag/VIII:C ratio, but not VIII:C, were significantly positively correlated to HbA_1 and triglycerides, indicating that these hemostatic parameters are linked to the quality of diabetic control as measured by HbA_1 concentration. A study of the changes induced in patients with poor metabolic control by continuous insulin infusion techniques will be useful for further validation of these findings. However, the observed non-linear pattern of the relationship indicates that mechanisms other than the degree of metabolic control contribute to the abnormalities of factor VIII components in diabetics. DENSON [17] postulated that disseminated intravascular coagulation might increase the VIIIR:Ag/VIII:C ratio, whereas ATICHARTAKARN et al. [1] suggested that a more marked elevation of VIIIR:Ag, compared to that of VIII:C, can also be produced by the action of proteolytic enzymes. The absence of clinical and laboratory evidence for disseminated intravascular coagulation and/or increased plasma proteolysis makes these possibilities unlikely in our patients. There is a report of a relationship between plasma growth hormone (GH) and factor VIII components in healthy volunteers, and therefore high factor VIII in uncontrolled diabetics might be accounted for by elevated GH [35]. However, subsequent studies by the same investigators showed no clear-cut relationship between GH and factor VIII in diabetics [23]. Finally, another

possible explanation is that the sustained increase of VIIIR:Ag might be non-specific, simply representing an 'acute phase' reaction to disturbed homeostasis [25]. VIII:C is also an acute phase reactant [7], but its synthesis does not appear to take place in the endothelial cells [2]. Therefore, the demonstration of a greater rise in VIIIR:Ag than in VIII:C supports the view that increased plasma VIIIR:Ag, and particularly a high VIIIR:Ag/VIII:C ratio, might be a consequence of endothelial damage and is indicative of vascular involvement. The negative correlation between VIIIR:Ag and VIIIR:Ag/VIII:C with HDL-C provides further indirect evidence that the VIIIR:Ag increase might also be a reflection of the extent of the endothelial lesions, because low HDL-C concentrations are known to be associated with a high incidence of ischemic heart disease [11] and thus, presumably, with the presence of extensive vascular involvement. The absence of detectable complications in our patients does not invalidate such a view, because the clinical and instrumental criteria adopted cannot rule out with certainty the presence of clinically mute vascular lesions. Provided that other studies confirm the value of VIIIR:Ag as an indirect index of endothelial damage, the measurement might have long-term prognostic significance and this could usefully be ascertained by a prospective study.

SUMMARY

Several metabolic (HbA_1, HDL-C, triglycerides) and hemostatic (VIIIR:Ag, VIII:C, B-TG) variables were investigated in 35 non-obese, insulin-dependent diabetics without clinically evident vascular complications. B-TG was high but did not correlate with other metabolic and hemostatic parameters, suggesting that elevated B-TG in diabetes might be an expression of *in vitro* platelet activation. VIIIR:Ag and the ratio of VIIIR:Ag to VIII:C were markedly increased. There was a significant correlation of the HbA_1 and HDL-C levels with VIIIR:Ag, indicating that VIIIR:Ag is another reflection of metabolic control in diabetes. Additional pathogenic mechanisms, however, appear to be involved in causing the changes in VIIIR:Ag in diabetes.

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