

Increased Platelet Volume in Manifest Diabetic Rats

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ABSTRACT

Platelet function, evaluated as in vitro aggregability, has been reported to be disturbed in diabetes, both in humans and animals. Platelet number and mean volume greatly influence these aggregation tests. The present study was designed to evaluate the impact of two different degrees of experimentally induced glucose intolerance on platelet number and mean volume. For this purpose, we used manifest diabetic and chemically diabetic rats. In the control group, the female rats showed a significantly lower number of platelets compared to the males. The chemically diabetic rats exhibited a tendency towards increased mean platelet volume, whereas the platelet volume of the manifest diabetic females was significantly greater than all other groups. This increase was found to be mainly due to a general shift towards larger volumes of the individual platelets of the manifest diabetic females.

It is suggested, that the enlargement of the mean platelet volume induced by increased severity of the diabetic state may reflect decreased mean age of the circulating platelets. This implies shorter survival time and an increased turnover of the platelet population in diabetes mellitus.

INTRODUCTION

Diabetes mellitus in man is associated with increased risk for atherosclerosis, microvascular complications (28), and arterial thrombosis (18). Changes in the haemostatic system with altered platelet function have been demonstrated by different in vitro tests in diabetic patients (3) and might be involved in the pathogenesis of this disease (19). Determination of survival and turnover is believed to accurately reflect the in vivo function of the platelets (15). Indeed, reduced platelet survival has been noticed in diabetic patients (7, 22).

Estimation of number and mean volume should reflect the turnover rate of the platelets, since small and light platelets generally represent an older population than large and dense platelets (16).

Disturbances, similar to those found in human diabetes mellitus, of the in vitro platelet function have been demonstrated in experimentally diabetic rats (4, 9, 13, 20). Changes

analogous to the proposed angiopathic alterations in human diabetes mellitus, such as changed vascular structure (25) and vascular biochemistry (1, 30, 31) have also been observed in diabetic rats. Furthermore, altered arachidonic acid metabolism in the vessel walls has been described in experimental diabetes in the rat (9-11, 29).

Against this background, the aim of the present study was to evaluate the impact of two different degrees of chronic glucose intolerance on platelet function by estimating the number and mean volume of the platelets. The two different types of experimental diabetes, used in this study, have been previously characterized. The milder chemically diabetic (CD) type was described by Portha and collaborators (23) and the manifest diabetic (MD) type has previously been used in studies of diabetes in pregnancy (5, 6).

MATERIAL AND METHODS

Animals

A first group comprising 19 female Sprague-Dawley rats (Anticimex AB, Sollentuna, Sweden) weighing about 250 g were made manifest diabetic (MD) at 3 months of age by a single i.v. injection of streptozotocin (Lot no. 1613E U-9889, kindly donated by Dr. W.E. Dulin, The Upjohn Co., Kalamazoo, MI, USA) at a dose of 45 mg/kg body weight. At the time of the experiment the animals had been diabetic for 1 - 2 months and exhibited hyperglycemia and glucosuria, as well as a mean weight of 206 ± 3 g.

A second group of 17 female rats of the same strain were given two i.p. injections of about 100 mg/kg body weight of streptozotocin on the second and third days of life (23). After a transient hyperglycemia lasting about one week, these animals (subsequently denoted as CD rats) achieved only slightly increased non-fasting serum glucose levels. The CD animals were 4 months old at the time of the experiment and weighed 232 ± 4 g.

A third group of 21 female (247 ± 4 g) and 20 male rats (399 ± 6 g), of the same strain, 4 months old, served as controls (N) and were not given any injections.

All animals were allowed free access to water and laboratory chow (EWOS AB, Södertälje, Sweden).

Determination of platelet number and mean volume

Blood samples, representing mixed arterial-venous blood, were taken from the cut tip of the tails of non-fasting animals. 10 microliters of free flowing blood was obtained and directly diluted (UNOPETTE, Clay Adams, Laboratory Instrumentation, Parsippany, NJ, USA). Within 20 minutes of blood sampling, the platelet number was determined in an ULTRA-FLO 100 Whole Blood Platelet Counter (Clay Adams) by automatized particle impedance counting. A Partial Sizing Amplifier (Nuclear Data Instruments AB, Uppsala, Sweden) simultaneously sorted the generated impedance signals according to the individual pulse amplitudes in 100 channels, resulting in a distribution curve of the platelet volumes of each sample in the range of 3 - 27 femtolitres (fl., 10^{-15} liter). Cf. Fig. 1. A mean platelet volume could then be calculated for each sample, using the relative frequencies of 8 sub-classes with equal class width of 3 fl. Cf. Fig. 2.

Statistics

All data are expressed as means \pm S.E.M. Probabilities (p) of chance differences between the different means were calculated according to Student's two-tailed t-test (21).

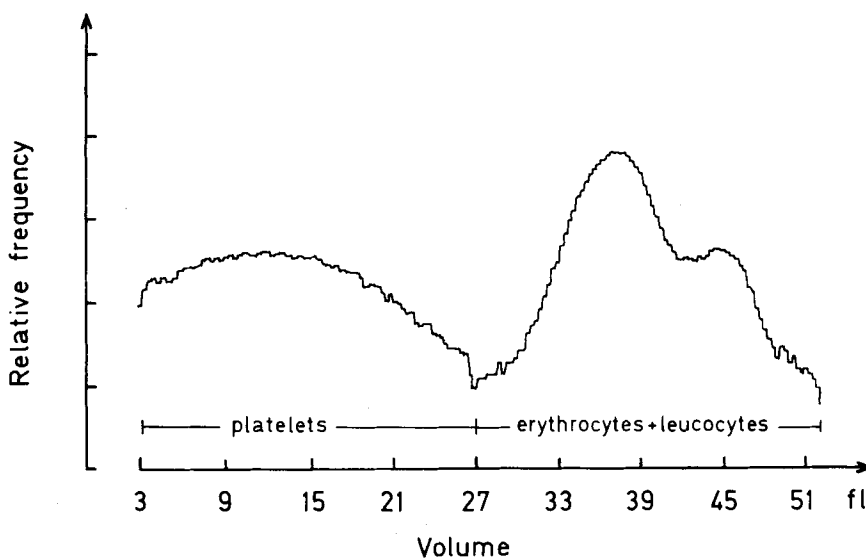


Figure 1. A typical distribution curve of corpuscular volumes in a blood sample from a N male rat. The relative frequency of impedance pulses is shown (arbitrary scale) on the ordinate.

RESULTS

The different levels of serum glucose are shown in Table 1. There was no sex difference in the N group in this respect ($p > 0.05$). On the other hand, both the CD ($p < 0.01$) and MD rats ($p < 0.001$) exhibited increased serum glucose values compared to the N females. In particular, the MD females were markedly abnormal with glucose levels about five times higher than the N females.

Table 1. Serum Glucose Concentrations, Numbers and Mean Volumes of Platelets in the Different Experimental Groups. N, CD and MD denote normal, chemically diabetic and manifest diabetic rats, respectively. Means \pm S.E.M.

Significances: **a** = $p < 0.001$ versus N Female, **b** = $p < 0.01$ versus N Female.

	No. of rats	Serum Glucose (mmol/l)	Platelet Number ($10^9/l$)	Platelet Volume (fl)
N Male	20	5.7 ± 0.1	948 ± 24^a	14.07 ± 0.11
N Female	21	5.8 ± 0.2	745 ± 27	14.08 ± 0.06
CD Female	17	6.7 ± 0.2^b	887 ± 42	14.26 ± 0.11
MD Female	19	28.5 ± 0.7^a	682 ± 54	14.44 ± 0.07^a

The number of platelets in the different experimental groups is shown in Table 1. In the N group, the females had a significantly lower number of platelets compared to the males ($p < 0.001$). Considering the females, the MD rats showed significantly fewer platelets compared to the CD rats ($p < 0.01$) but not in comparison with the N animals ($p > 0.05$).

Table 1 also shows the mean platelet volumes of the different groups of rats. In the N group, no differences could be found between the sexes ($p > 0.05$). A tendency towards increased CD female platelet volume compared to the N females was noted ($0.05 < p < 0.10$). This trend was also observed in the MD rats, who showed a mean platelet volume greater than all other groups, in particular compared to the N females ($p < 0.001$). Inspection of the accumulated mean volume distribution curves of the N and MD female groups (Fig. 2) showed that the increased mean platelet volume of the MD rats was the result of a general shift of the distribution curve towards larger volumes.

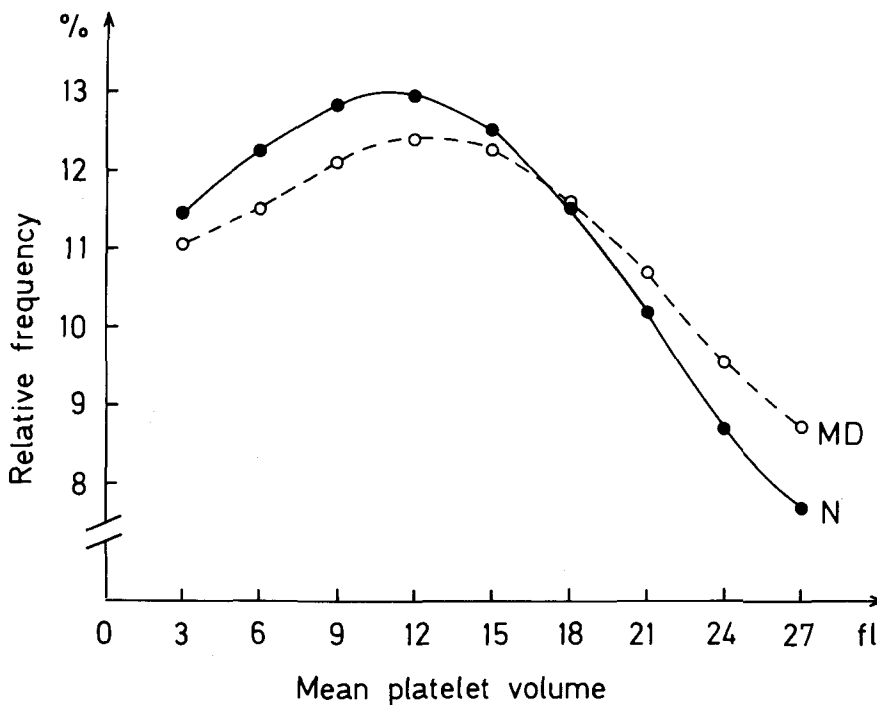


Figure 2. Accumulated mean volume distribution of (female) N and MD rats. The relative frequency of impedance pulses are shown on the ordinate. Note the general shift of the MD population towards larger volumes.

DISCUSSION

The decreased number of platelets in the females compared to the males in the control group has not been previously demonstrated in the rat. Platelet number and volume greatly influence the results of *in vitro* platelet aggregation studies (12), therefore the

demonstrated sex difference may be of importance for the evaluation of platelet aggregability. In humans, differences between the sexes in platelet number (17, 27), and platelet aggregability (14, 24) have been reported. The results of the present study may illustrate the necessity to consider the sex as well as both the platelet number and mean platelet volume in the future evaluations of platelet function.

The major finding in this study, the marked increase in MD platelet volume, would indicate a generally decreased mean age in the platelet population of the manifest diabetic rats (16). This notion is further substantiated by the finding of a right-hand side shift in the distribution curve of the platelets. A decreased mean age would be the result of increased turnover and shortened survival time of platelets (7, 16, 22). Furthermore, the number of MD platelets tended to be decreased, an observation also in agreement with an increased rate of platelet turnover. Since younger and larger platelets are known to be more metabolically active (16), this finding would be consistent with both the view of a general hyperaggregability of the platelets in diabetes (3, 8), and increased platelet turnover in vivo (2, 7, 22).

A similar tendency towards increased mean volume of the platelets was also observed in the CD rats. Changes in platelet function have been noticed in the very early phases of human diabetes (26). The accumulated distribution curve of platelet volumes in the CD group did not show a general shift towards larger volumes similar to that seen in the MD rats. There was instead rather a localized increase in the proportion of large platelets.

The present findings of increased platelet volumes correlating with an increased severity of the diabetic state suggest, that a disturbed carbohydrate metabolism may directly, or indirectly, cause an increased turnover of the circulating platelets. This suggestion would support the notion of increased platelet aggregation in diabetes mellitus, since shorter survival time, decreased mean age and general enlargement of the mean platelet volume implicates increased platelet activity, and thereby increased aggregability.

The precise reason for the dysfunction of the platelets in diabetes mellitus is at present not completely understood. Animal models with different degrees of glucose intolerance may therefore play an important role in future research on altered platelet function in diabetes mellitus.

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