Upsala Journal of Medical Sciences

ISSN: 0300-9734 (Print) 2000-1967 (Online) Journal homepage: informahealthcare.com/journals/iups20

# Increased Platelet Volume in Manifest Diabetic Rats

Ulf Eriksson, Uwe Ewald & Torsten Tuvemo

**To cite this article:** Ulf Eriksson, Uwe Ewald & Torsten Tuvemo (1983) Increased Platelet Volume in Manifest Diabetic Rats, Upsala Journal of Medical Sciences, 88:1, 17-23, DOI: 10.3109/03009738309178434

**To link to this article:** https://doi.org/10.3109/03009738309178434

Published online: 18 Jan 2010.

Upsala J Med Sci 88: 17–23, 1983

# Increased Platelet Volume in Manifest Diabetic Rats

Ulf Eriksson[1], Uwe Ewald[2] and Torsten Tuvemo[2]

*Departments of Medical Cell Biology[1], and Pediatrics[2], University of Uppsala, Uppsala, Sweden*

## 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 decribed 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 pregnacy (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 $\pm$ 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 $\pm$ 4 g.

A third group of 21 female (247 $\pm$ 4 g) and 20 male rats (399 $\pm$ 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 ± S.E.M. Probabilities (p) of chance differences between the different means were calculated according to Student's two-tailed t-test (21).

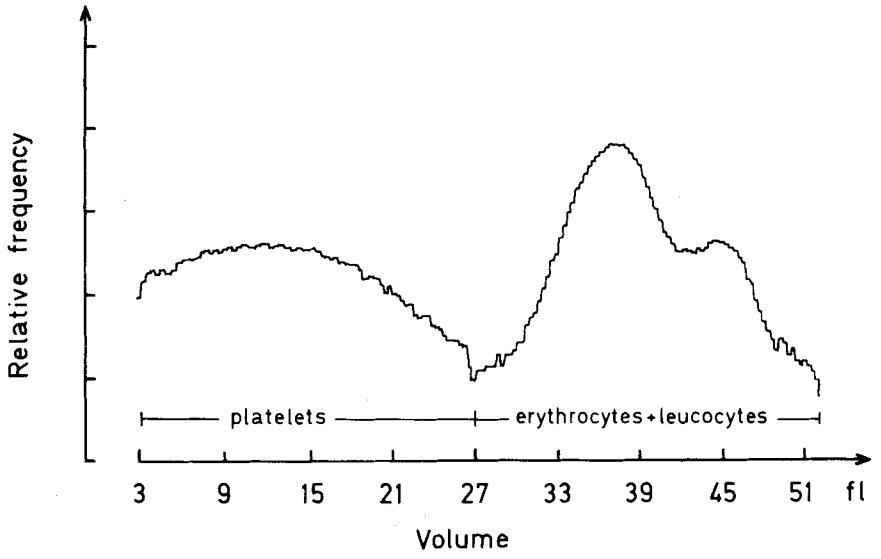


Figure 1. A typical distribution curve of corpuscular volumes in a blood sample from a N male rat. The relative frequency of impedance pulses is shown (arbitrary scale) on the ordinate.

## 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 ± 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.

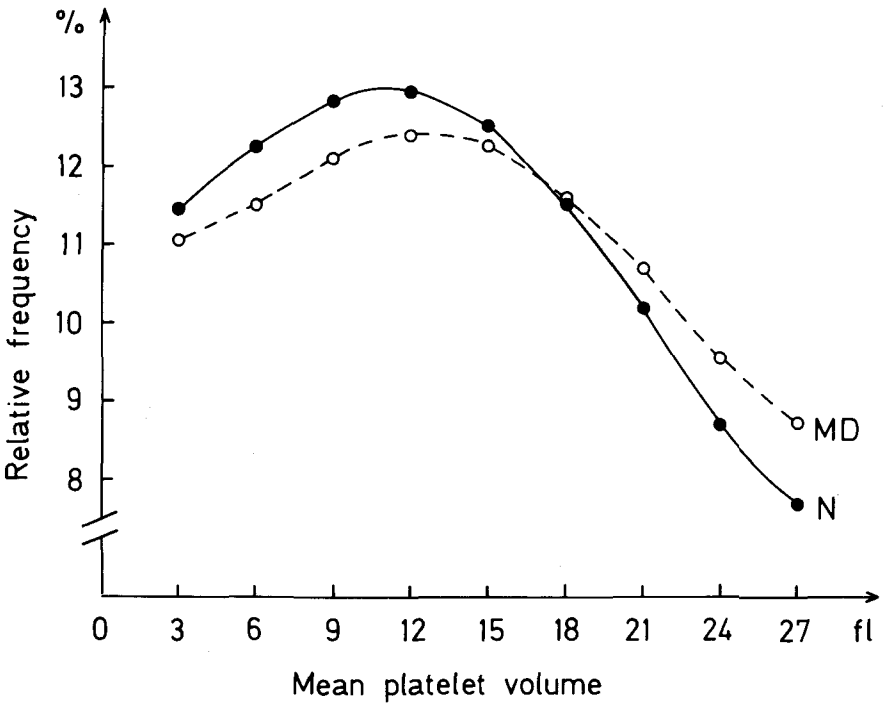


Figure 2. Accumulated mean volume distribution of (female) N and MD rats. The relative frequency of impedance pulses are shown on the ordinate. Note the general shift of the MD population towards larger volumes.

## 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.

## ACKNOWLEDGEMENTS

The authors are indebted to Barbro Westerberg, Astrid Nordin and Eva Törnelius for excellent technical help. Financial assistance has been received from Novo Industri AB, Malmö, Sweden (to Dr. T. Tuvemo) and The Swedish Medical Research Council (Grant no. 12X-109, and Post-Doctoral Fellowship no. K82-12P-6346-01 to Dr. U. Eriksson).

## REFERENCES

1. Arnqvist, H.J. & Dahlkvist, H.H.: Effect of experimental diabetes on the

incorporation of amino acids into protein in rat aorta. Horm. Metab. Res. **11**: 384-388, 1979.
2. Bonne, C., Romquin, N. & Regnault, F.: Modified sensitivity of diabetic platelets to prostacyclin. In: 10th Eur. Conf. Microcirc., Calgliari 1978, Biblthca anat. pp. 98-103. Karger, Basel, 1979.
3. Colwell, J.A. & Halushka, P.V.: Platelet function in diabetes mellitus. Br. J. Haematol. **44**: 521-526, 1980.
4. Eldor, A., Merin, S. & Bar-On, H.: The effect of streptozotocin diabetes on platelet function in rats. Thromb. Res. **13**: 703-714, 1978.
5. Eriksson, U., Andersson, A., Efendić, S., Elde, R. & Hellerström, C.: Diabetes in pregnancy: effects on the foetal and newborn rat with particular regard to body weight, serum insulin concentration and pancreatic contents of insulin, glucagon and somatostatin. Acta Endocrinol. (Copenh.) **94**: 354-364, 1980.
6. Eriksson, U.: Diabetes in pregnancy: a rat model for the study of fetal complications. Uppsala J. Med. Sci. **86**: 207-212, 1981.
7. Ferguson, J.E., Mackay, N., Phillip, J.A.D. & Summer, D.J.: Determination of platelet and fibrinogen half life with ($^{75}$Se)selenomethionine: studies in normal and in diabetic subjects. Clin. Sci. Mol. Med. **49**: 115-120, 1975.
8. Garg, S.K., Lackner, H. & Karapatkin, S.: The increased percentage of megathrombocytes in various clinical disorders. Ann. Int. Med. **77**: 361-369, 1972.
9. Gerrard, J.M., Stuart, M.J., Rao, G.H.R., Steffes, M.W., Mauer, S.M., Brown, D.M. & White, J.G.: Alteration in the balance of prostaglandin and thromboxane synthesis in diabetic rats. J. Lab. Clin. Med. **95**: 950-958, 1980.
10. Harrison, H.E., Reece, A.H. & Johnson, M.: Decreased vascular prostacyclin in experimental diabetes. Life Sci. **23**: 351-356, 1978.
11. Harrison, H.E., Reece, A.H. & Johnson, M.: Effect of insulin treatment on prostacyclin in experimental diabetes. Diabetologia **18**: 65-68, 1980.
12. Holme, S. & Murphy, S.: Influence of platelet count and size on aggregation studies. J. Lab. Clin. Med. **97**: 623-630, 1980.
13. Ishibashi, T., Tanaka, K. & Taniguchi Y.: Platelet aggregation and coagulation in the pathogenesis of diabetic retinopathy in rats. Diabetes **30**: 601-606, 1981.
14. Johnson, M., Ramey, E. & Ramwell, P.W.: Sex and age differences in human platelet aggregation. Nature **253**: 355-357, 1975.
15. Jones, R. L., Paradise, C. & Peterson, C.M.: Platelet survival in patients with diabetes mellitus. Diabetes **30**: 486-489, 1981.
16. Karpatkin, S.: Heterogeneity of human platelets. VI. Correlation of platelet function with platelet volume. Blood **51**: 307-315, 1978.
17. Kemona, H., Prokopowicz, J. & Wotosowicz, N.: The count of blood platelets and sex in humans. Experentia **34**: 257, 1978.
18. Kwaan, H.C., Colwell, J.A. & Suwanwela, N.: Disseminated intravascular coagulation in diabetes mellitus, with reference to the role of increased platelet aggregation. Diabetes **21**: 108-113, 1972.
19. Kwaan, H.C., Colwell, J.A., Cruz, S., Suwanwela, N. & Dobbie, J.G.: Increased platelet aggregation in diabetes mellitus. J. Lab. Clin. Med. **80**: 236-246, 1972.
20. Muhlrad, A., Eldor. A., Bar-On, H. & Kahane, I.: The distribution of platelet contractile proteins in normal and streptozotocin diabetic rats. Thromb. Res. **14**, 621-629, 1979.
21. Ostle, B.: Statistics in research. 2nd ed. pp. 1-585. The Iowa State University Press, Ames, Iowa, 1963.
22. Paton, R.C.: Platelet survival in diabetes mellitus using an aspirin-labelling technique. Thromb. Res. **15**: 793-802, 1979.
23. Portha, B., Picon, L. & Rosselin, G.: Chemical diabetes in the adult rat as the spontaneous evolution of neonatal diabetes. Diabetologia **17**: 371-377, 1979.
24. Reading, H.W. & Rosie, R.: Age and sex differences related to platelet aggregation. Biochem. Soc. Trans. **8**: 180-181, 1980.
25. Reinilä, A.: Long-term effects of untreatred diabetes on the arterial wall in rat. An ultrastructural study. Diabetologia **20**: 205-212, 1981.
26. Sagel, J., Colwell, J.A. & Crook, L.: Increased platelet aggregation in early diabetes mellitus. Ann. Int. Med. **82**: 733-738, 1975.
27. Stevens, R.F. & Alexander, M.K.: A sex difference in the platelet count. Br. J. Haematol. **37**: 295-300, 1978.

28. Stout, R.W., Bierman, E.L. & Brunzell, J.D.: Atherosclerosis and disorders of lipid metabolism in diabetes. In: Diabetes: Its physiological and biochemical basis (ed. Vallance-Owen, J.), pp. 125-169. M T M Press, Lancaster, 1975.
29. Turlapaty, P.D.M.V., Lum, G. & Altura, B.M.: Vascular responsiveness and serum biochemical parameters in alloxan diabetes mellitus. Am. J. Physiol. **239**: E412-E421, 1980.
30. Wolinsky, H., Capron, L., Goldfischer, S., Capron, F., Coltoff-Schiller, B. & Kasak, L. Hydrolase activities in the rat aorta. II. Effects of hypertension alone and in combination with diabetes mellitus. Circ. Rec. **42**: 831-839, 1978.
31. Wolinsky, H., Goldfischer, S., Capron, L., Capron, F., Coltoff-Schiller, B. & Kasak, L. Hydrolase activities in the rat aorta. I. Effects of diabetes mellitus and insulin treatment. Circ. Res. **42**: 821-831, 1978.

Adress for reprints
Ulf Eriksson M.D.
Department of medical cell biology
University of Uppsala
S-751 23 Uppsala
Sweden