MEMBRANE FLUIDITY AND CYTOSOLIC Ca^{2+} CONCENTRATION OF THE CIRCULATING POLYMORPHONUCLEAR LEUKOCYTES IN DIABETES MELLITUS

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In the two last decades our haemorheological interest has regarded also the functional aspects played by the polymorphonuclear leukocytes (PMNs) in diabetes mellitus (DM).

Generally, the particular attention to the PMNs is related to the fact that these cells, with their geometric and biological characteristics, influence the microvascular flow and this effect is dependent in particular on their adhesion to the endothelium, on their entrapment or on their spontaneous activation. It must be also underlined the metabolic alteration that characterizes diabetic PMNs that is related to the decreased activity of phosphofructokinase and so of the glycolitic pathway, the increase in glucose metabolism via the hexose monophosphate shunt and the activation of the polyol pathway and so the increased sorbitol concentration[1].

Regarding PMNs, our attention has been directed in particular towards the determination of the membrane fluidity and the cytosolic calcium concentration, both under basal condition and after in vitro activation with chemotactic agents, such as 4-phorbol 12-myristate 13-acetate (PMA), non receptor-mediated, and N-formyl-methionyl-leucyl-phenylalanine (fMLP), receptor-mediated.

PMN membrane fluidity depends in particular on membrane lipid and proteic composition and it is a component of the PMN deformability, influenced also by cytosolic Ca2+ concentration. Both these two PMN parameters alter some functional expressions of these circulating cells, such as phagocytosis, and the increase in cytosolic Ca2+ content is considered a PMN activation marker[2]. As it is known, the PMN calcium concentration depends on the activity of the membrane pumps and this activity is also regulated by the membrane fluidity[3].

Now, we evaluated PMN membrane fluidity and PMN cytosolic Ca2+ concentration in 53 type 1 diabetic subjects (34 men and 19 women; age range 14-58 yrs; fasting blood glucose level 189.6±82.9 mg/dl, total cholesterol 187.7±34.00 mg/dl, serum triglycerides 105.5±61.2 mg/dl), in 68 type 2 diabetic subjects without macrovascular complica-

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tions (33 men and 35 women; age range 20-77 yrs; fasting blood glucose level 181.9±56.8 mg/dl, total cholesterol 210.9±34.8 mg/dl, serum triglycerides 133.4±61.8 mg/dl), and in 60 type 2 diabetic subjects with macrovascular complications (45 men and 15 women; age range 41-77 yrs; fasting blood glucose level 163.8±53.7 mg/dl, total cholesterol 227.7±45.1 mg/dl, serum triglycerides 188.4±89.9 mg/dl). All type 1 diabetic subjects followed a controlled carbohydrate diet and received three daily injections of insulin. All type 2 diabetic subjects followed a controlled carbohydrate diet and received oral hypoglycaemic agents. In type 2 diabetic subjects the presence or absence of macrovascular complications was determined with both physical and instrumental (Doppler, Echo-Doppler, ECG, etc) examination. An unfractioned leukocyte sospension was prepared from fasting venous blood according to the method described by Mikita et al[4]. Leukocytes were separated into mononuclear cells and polymorphonuclear cells using a density gradient[5]. PMN membrane fluidity was obtained by marking PMNs with the fluorescent probe 1-(4-[trimethylamino]phenil)-6-phenyl 1,3,5-hexatriene (TMA--DPH). PMN cytosolic Ca2+ concentration was obtained marking polymorphonuclear cells with the fluorescent probe Fura 2-AM and considering the ratio between the Fura 2-Ca2+ complex fluorescence intensity and the unchelated Fura-2 fluorescence intensity.

The same PMN parameters were examined in 48 normal controls (32 men and 16 women; age range 22-52 yrs; fasting blood glucose level 91.5±8.8 mg/dl, total cholesterol 196.7±35.8 mg/dl, serum triglycerides 102.8±55.5 mg/dl).

The values were expressed as mean s± S.D.. The difference between normal controls and diabetic subjects was evaluated according to the one-way ANOVA model integrated with the Bonferroni multiple post-test. The relationships between PMN membrane fluidity and PMN cytosolic Ca2+ concentration was evaluated using linear regression. Linear regression was also employed for the correlations between PMN membrane fluidity and age and between PMN calcium concentration and age. In normal controls, in type 1 diabetic subjects (DM1) and in type 2 diabetic subjects (DM2) without and with macrovascular complications (MVC) no statistical correlation between age and PMN cytosolic Ca2+ concentration was observed. Between PMN fluidity and age we observed only a significant negative relation DM1 subjects.

No significant difference (Figure 1) in PMN membrane fluidity was observed among normal controls, DM1 subjects, DM2 subjects without and with MVC (N= 0.341±0.024; DM1= 0.343±0.015; DM2 without MVC= 0.345±0.020; DM2 with MVC= 0.345±0.021).

Regarding PMN cytosolic Ca2+ concentration, among normal controls (0.805±0.057), DM1 subjects (0.837±0.058), DM2 subjects without MVC (0.826±0.066), and DM2 subjects with MVC (0.865±0.034) we found a significant difference (Figure 2) even if, employing the Bonferroni multiple post-test, a significant difference was evident only between normal controls and DM1

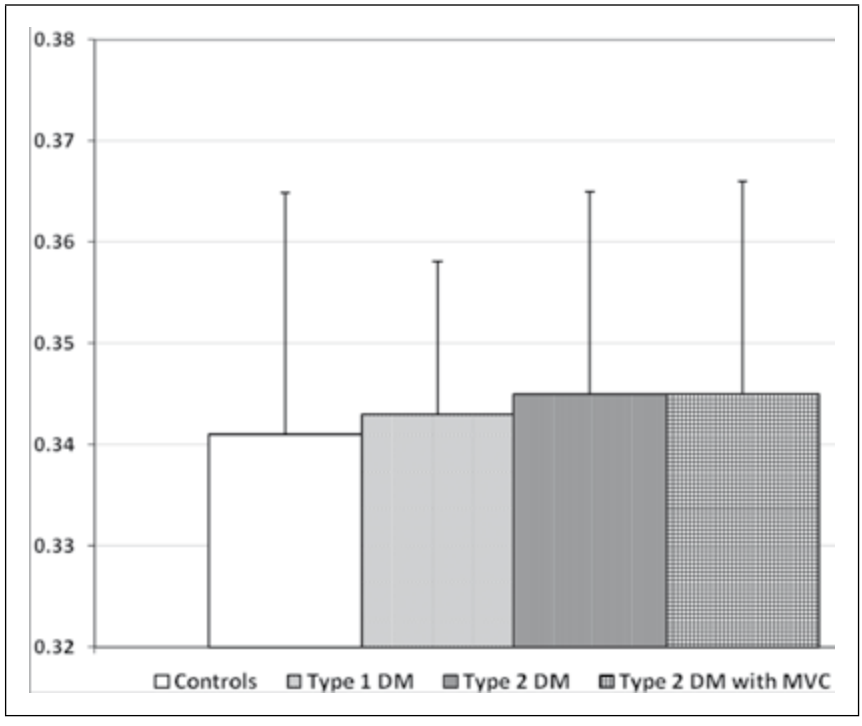
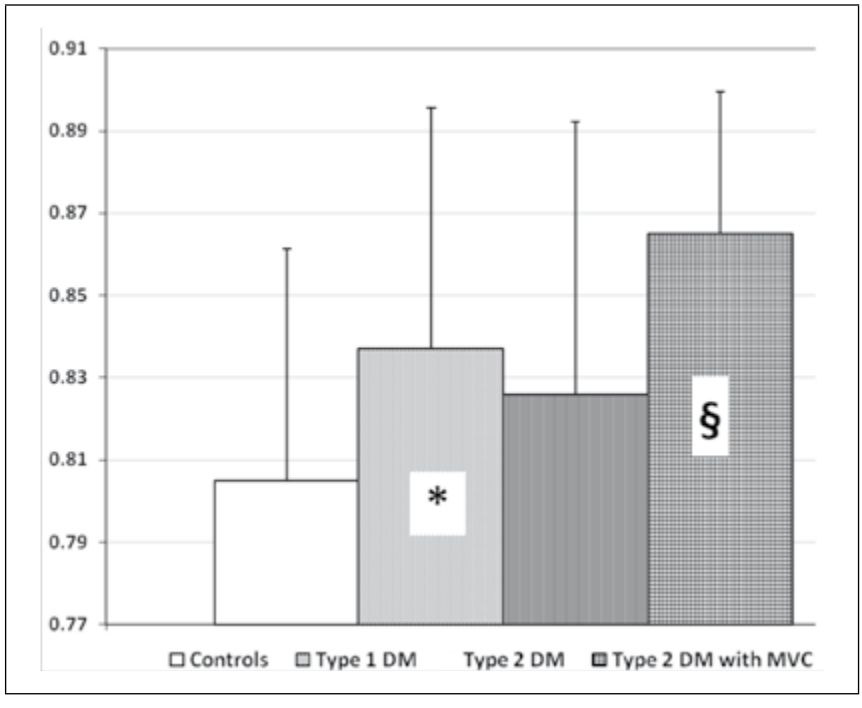


Figure 1. TMA-DPH polarization degree in PMNs of control subjects, type 1 and 2 diabetics and type 2 diabetics with macrovascular complications



*** p < 0.**05 vs Controls § p < 0.001 vs Controls and type 2 DM; p < 0.05 vs type 1 DM

Figure 2. PMN Cytosolic Ca2+ content in control subjects, in type 1 and 2 diabetics and in type 2 diabetics with macrovascular complications.

subjects and between normal controls and DM2 subjects with MVC, but not between normal controls and DM2 without MVC. It must be underlined that the values of PMN cytosolic calcium concentration observed in DM2 subjects with MVC are significantly higher in comparison with DM1 subjects and DM2 subjects without MVC. In normals, in DM1 subjects, in DM2 subjects without MVC and in DM2 subjects with MVC no significant correlation between PMN membrane fluidity and PMN cytosolic Ca2+ concentration was observed.

Even if, in our experience, the behaviour of the PMN membrane fluidity in diabetes mellitus results to be controversial[6-10] and seems to depend on the extent of survey and on the glycometabolic profile, in the re-examination of our case-record of diabetic subjects subdivided for type and for macrovascular complications this microrheological parameter has the same trend observed by us in other chronic clinical conditions, such as vascular atherosclerotic disease[10,11], essential arterial hypertension[12,13] and chronic kidney disease[14] and is different from that observed in acute clinical conditions, such as juvenile myocardial infarction[15] and ischemic stroke[16,17].

Interesting is instead the behaviour of PMN cytosolic Ca2+ concentration in all the groups of diabetic subjects now examined. From these data it is evident the different trend of PMN cytosolic Ca2+ in the two types of DM. Regarding the group of DM2 with MVC these data are in agreement with our previous observation[18] concerning a small number of subjects. However other authors found in type 2 diabetic sub-

jects without any vascular complication (19, 20) a significant increase in PMN cytosolic Ca2+ concentration.

The different behaviour of cytosolic calcium in DM1 subjects in comparison with DM2 subjects is similar to that regarding PMN membrane fluidity (lo presti 98) and β_2-integrin pattern[21] during in vitro activation with PMA and fMLP.

Several studies have regarded instead the role of leukocyte count in this metabolic condition. An elevated leukocyte count has been associated with different glucose metabolism abnormality[22], such as insulin resistance[23], impaired glucose tolerance[24], impaired fasting glucose[24], and type 2 DM[25,26,27,28]. Even in normoglicemic subjects with parental type 2 DM an elevated leukocyte count predicted the presence of several components of the metabolic syndrome and therefore the cardiometabolic risk[29]. A higher leukocyte count seems to be related to the development of micro- and macro-vascular complications in type 2 diabetic subjects and, in fact, leukocyte count results correlated with the albumin excretion rate[30] and it increases the risk of peripheral artery disease[31]. The leukocyte count, and in particular the polymorphonuclear count, is associated with the presence and the severity of diabetic retinopathy as well as DM itself[32].

Bearing in mind that a secondary hyperviscosity syndrome is present in diabetes mellitus, it is interesting to underscore the behaviour, at rest, of PMN membrane fluidity and PMN cytosolic Ca2+ concentration. As it is known, in fact, circulating leukocytes contribute to the blood hyperviscosity that characterizes this clinical condition. In our experience, however, major informations regarding these two parameters of PMN cells in diabetes mellitus may be obtained when their evaluation, including PMNs filtration, is effected during "in vitro" activation with chemiotactic agents. Employing this technique it seems also avident the different trend of these two parameters in type 1 diabetics and in type 2 diabetics.

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