Diabetes Mellitus: Polymorphonuclear Leukocyte (PMN) Filtration Parameters and PMN Membrane Fluidity After Chemotactic Activation

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The goal of this research was to determine leukocyte rheology at baseline and after chemotactic activation in type I and type II diabetics. In 19 normal subjects, 21 type I diabetics, and 16 type II diabetics at baseline and after in vitro chemotactic activation (prolonged for 5 and 15 minutes) with two stimulating agents (4-phorbol 12-myristate 13-acetate [PMA] and *N*-formyl-methionyl-leucyl-phenylalanine [fMLP]), we evaluated polymorphonuclear (PMN) filtration parameters (using a St. George filtrometer [Carri-Med, Dorking, UK] and considering the initial relative flow rate [IRFR] and the concentration of clogging particles [CP]) and PMN membrane fluidity (obtained by marking PMNs with the fluorescent probe 1-(4-[trimethylamino]phenyl)-6-phenyl-1,3,5-hexatriene (TMA-DPH). At baseline, there was a difference between normals and type I and II diabetics for PMN membrane fluidity only. After activation in normals and diabetics of both types, a significant variation was present in PMN filtration parameters (IRFR and CP) at both 5 and 15 minutes. In normals, no variation was present in PMN membrane fluidity after activation with PMA or fMLP. After PMN activation, only in type I diabetics was a significant decrease in PMN membrane fluidity present at both 5 and 15 minutes. After PMN activation with either PMA or fMLP in comparison to basal values, only the mean variation (Δ%) of the IRFR was significantly different between normals, type I diabetics, and type II diabetics at both 5 and 15 minutes. From the data obtained, it is evident that after activation, the PMN filtration pattern shows a specific behavior in diabetics of both types, while PMN membrane fluidity changes only in type I diabetics. The latter finding may be the basis of a metabolic pattern present in PMNs of this type, revealed after in vitro activation.

THERE HAVE BEEN contrasting reports regarding leukocyte rheology expressed as filtration parameters in diabetes mellitus. By filtering unfractionated leukocytes, some investigators demonstrated a reduction of the filtration parameters in type II diabetics[1] and diabetics of both types,[2] while others using the same technique did not show any difference between normals and type I diabetics.[3] Several groups have shown that polymorphonuclear (PMN) and mononuclear (MN) cell filtration parameters also discriminate normals from type II diabetics,[4-6] but MacRury et al[7] did not find any difference in PMN filtration between type II diabetics and controls.

In a previous study[8] evaluating leukocyte filtration parameters in type I and II diabetics without macrovascular complications, we found that only the unfractionated leukocyte had a reduced filterability in comparison to normals. From this study, it also emerged that PMN membrane fluidity was not a distinguishing parameter. However, others noted a decrease of PMN membrane fluidity in type I diabetics[9] and an increase of MN leukocyte membrane fluidity in type II diabetics.[10]

We have recently studied the rheological and metabolic PMN parameters after chemotactic activation, and evaluated the behavior of these parameters in subjects with vascular atherosclerotic disease[11] and in type II diabetics with macrovascular complications (Caimi et al, 1998, unpublished data). Activation can reveal leukocyte abnormalities not found at rest,[12-14] and can simulate the variations present during in vivo activation.

The aim of this study was the evaluation of PMN filtration parameters and PMN membrane fluidity at baseline and after chemotactic activation in type I and II diabetics without macrovascular complications.

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Submitted August 13, 1997; accepted July 13, 1998.

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0026-0495/99/4801-0006$03.00/0

SUBJECTS AND METHODS

The study enrolled 21 type I diabetics (mean age, 29.0 ± 9.0 years; range, 17 to 47) and 16 type II diabetics (mean age, 57.1 ± 10.5 years; range, 44 to 77).

In type I diabetics, fasting blood glucose was 10.1 ± 4.1 mmol/L, glycated hemoglobin (HbA_{1c}) 7.7% ± 1.9%, serum total cholesterol 5.3 ± 0.7 mmol/L, and serum triglycerides 1.5 ± 0.6 mmol/L. All type I diabetics followed a controlled-carbohydrate diet and received three daily administrations of insulin.

In type II diabetics, fasting blood glucose was 9.3 ± 3.5 mmol/L, HbA_{1c} 7.3% ± 2.8%, serum total cholesterol 5.1 ± 1.1 mmol/L, and serum triglycerides 2.1 ± 1.0 mmol/L. All type II diabetics followed a controlled-carbohydrate diet and received oral hypoglycemic agents. The medication used was glibenclamide in nine patients (dose range, 7.5 to 15 mg/d) and gliclazide in seven patients (dose range, 120 to 240 mg/d). No patient used biguanides in the 6 months preceding the study.

In both groups, the absence of macrovascular complications was demonstrated both instrumentally (Doppler, echo-Doppler, electrocardiogram, and electroencephalogram) and by clinical criteria. Diagnosis and classification of diabetes mellitus was made according to the National Diabetes Data Group criteria.[15] Obese and hypertensive subjects were excluded from these two groups. In type I diabetics, the body mass index (BMI) was 22.4 ± 1.2 kg/m^2, systolic blood pressure (SBP) 115.2 ± 11.3 mm Hg, and diastolic blood pressure (DBP) 75.7 ± 7.8 mm Hg. In type II diabetics, the BMI was 24.2 ± 0.7 kg/m^2, SBP 130.3 ± 8.4 mm Hg, and DBP 83.7 ± 5.3 mm Hg.

Venous blood samples were drawn from patients in the fasting state and anticoagulated with EDTA-K3 (1.5 mg/mL). An unfractionated leukocyte suspension was prepared according to the method described by Mikita et al.[16] In the final preparation, leukocytes were suspended in Dulbecco's phosphate-buffered saline containing EDTA-K3 (1 mg/mL). Leukocytes were separated into MN and PMN cells[17] using a Ficoll-Hypaque medium with a density of 1.114 g/mL (Mono-Poly Resolving Medium; Flow Laboratories, Irvin, UK).

PMN Activation

After separation, part of the PMN cells were divided into several fractions, each of which had a concentration of 5 × 10^6 cells/mL. Each fraction was treated with two activating agents: 4-phorbol 12-myristate 13-acetate ([PMA] Sigma Chemical, St Louis, MO) and *N*-formyl-methionyl-leucyl-phenylalanine ([fMLP] Sigma Chemical). The activa-

tion was performed in vitro in accordance with the methods described by Yasui et al[18] and Masuda et al,[19] modified for the evaluation of PMN parameters as follows. The fractions of PMN suspension were treated with either 4.5 μmol/L PMA or 10^{-5} mol/L fMLP and incubated for either 5 minutes at 37°C or 15 minutes at 37°C. At the end of incubation, the activation was stopped by plunging the tubes into melting ice for a few minutes, and soon afterward, the PMN suspensions were centrifuged at 200 × *g* for 10 minutes at 20°C and resuspended in 1 mL Dulbecco's buffer containing EDTA-K3 (1 mg/mL).

At baseline and after 5 and 15 minutes of activation with PMA and fMLP, we evaluated PMN filtration parameters and PMN membrane fluidity.

PMN Filtration

PMNs were adjusted to a cell count of 3×10^6 mL. The filtration was performed using a St. George Filtrometer (Carri-Med, Dorking, UK).[20] Each cell suspension was tested against its cell-free suspension medium. Filters with a nominal pore size of 8 μm (Nucleopore, Cambridge, MA) were used. During filtration, the sample flowed in a horizontal tube under a constant pressure head of 4 cm H_2O. The data obtained are the means of three repeated measures. The parameters considered were the initial relative flow rate (IRFR) relative to the buffer solution, and the concentration of clogging particles (CP), which reflects the clogging of pores by a minority of rigid cells. These filtration parameters were evaluated by a microcomputer, displayed visually, and successively printed.

PMN Membrane Fluidity

PMNs were suspended in Dulbecco's buffer at a concentration of 4×10^6 cells/mL and labeled with 1-(4-[trimethylamino]phenyl)-6-phenyl-1,3,5-hexatriene ([TMA-DPH] Molecular Probes, Junction City, OR) previously dissolved in acetone. The labeling was performed as follows: 4 minutes of preincubation at 4°C followed by incubation for 20 minutes at 37°C, with a final probe concentration of 2 μmol/L. Fluorescence was measured at 37°C using a spectrophotofluorimeter (model LS5; Perkin-Elmer, Beaconsfield, UK) equipped with polarization accessories. The excitation wavelength was 360 nm and emission wavelength 430 nm. Examining the intensity of fluorescence with the polarizers oriented parallel and perpendicular to the plane of polarization, we calculated the fluorescence polarization degree (p), reflecting PMN membrane lipid fluidity.[21-23]

The same parameters were examined in a group of 19 normal subjects (12 men and seven women; mean age, 40.3 ± 7.3 years; range, 31 to 53). The mean fasting blood glucose was 4.91 ± 0.40 mmol/L, total serum cholesterol 4.77 ± 0.68 mmol/L, and serum triglycerides 1.11 ± 0.55 mmol/L. The BMI was 23.0 ± 1.2, SBP 119.2 ± 11.1 mm Hg, and DBP 79.2 ± 5.8 mm Hg.

Statistical Analysis

The results are expressed as the mean ± SD. At baseline, the difference between mean values for normals, type I diabetics, and type II diabetics was evaluated according to the one-way ANOVA model. The difference between mean values at baseline and after PMN activation was calculated according to Student's *t* test for paired data. The difference between the mean variation (Δ%) in normals, type I diabetics, and type II diabetics during PMN activation was evaluated according to the one-way ANOVA model.

RESULTS

No significant difference was evident in the baseline data for PMN filtration parameters between normals and type I or II diabetics. However, a significant difference was observed in PMN membrane fluidity, which was reduced in diabetics, particularly type II diabetics (Tables 1 and 2).

After PMN activation with PMA, a decrease in the IRFR and an increase in CP was observed in normals, type I diabetics, and type II diabetics. These variations, as compared with basal values, were found at both 5 and 15 minutes. In normals, no significant variation was present in PMN membrane fluidity. In type I diabetics, a constant and significant decrease in PMN membrane fluidity was found. In type II diabetics, no variation was noted in PMN membrane fluidity (Table 1).

After PMN activation with fMLP, a decrease in the IRFR and an increase in CP were evident in normals, type I diabetics, and type II diabetics. These variations, in comparison to basal values, were present at both 5 and 15 minutes. In normals, no significant variation was evident in PMN membrane fluidity. In type I diabetics, a decrease in PMN membrane fluidity was found. In type II diabetics, no variation was observed in PMN membrane fluidity (Table 2).

After PMN activation with PMA, in comparison to basal values, the Δ% for the IRFR was significantly different between normals, type I diabetics, and type II diabetics at 5 minutes (normals, −39.3 ± 24.7; type I diabetics, −63.3 ± 20.9; type II diabetics, −62.0 ± 21.0; F = 5.453, $P = .0075$) and 15 minutes (normals, −38.4 ± 25.8; type I diabetics, −72.2 ± 16.1; type II diabetics, −60.2 ± 29.0; F = 9.318, $P = .004$). At neither point in time were the values for the Δ% of PMN membrane fluidity significantly different between normals and type I or II diabetics.

After PMN activation with fMLP, in comparison to basal values, the Δ% of the IRFR was significantly different between normals and type I and II diabetics at 5 minutes (normals, −13.6 ± 12.5; type I diabetics, −31.7 ± 21.4; type II diabetics, −26.8 ± 13.2; F = 4.563, $P = .0156$) and 15 minutes (normals, −6.9 ± 9.1; type I diabetics, −27.8 ± 27.2; type II diabetics, −24.2 ± 23.1; F = 3.902, $P = .0279$). At neither point in time were the values for the Δ% of PMN membrane fluidity significantly different between normals and type I or II diabetics.

Table 1. PMN Filtration Parameters and Membrane Fluidity (expressed as TMA-DPH polarization degree) in Normal Subjects, Type I Diabetics, and Type II Diabetics at Baseline and After Activation With PMA (mean ± SD)

Parameter	Baseline	5 Minutes	15 Minutes
Normals			
IRFR	0.954 ± 0.098	0.601 ± 0.284†	0.589 ± 0.255†
CP (×10⁵/mL)	1.641 ± 0.768	8.134 ± 1.728†	9.075 ± 0.953†
TMA-DPH	0.324 ± 0.026*	0.339 ± 0.023	0.334 ± 0.028
Type I diabetics			
IRFR	0.901 ± 0.112	0.327 ± 0.179†	0.250 ± 0.144†
CP (×10⁵/mL)	1.666 ± 0.970	7.003 ± 1.580†	7.952 ± 1.257†
TMA-DPH	0.344 ± 0.012*	0.351 ± 0.007†	0.356 ± 0.007†
Type II diabetics			
IRFR	0.918 ± 0.099	0.351 ± 0.205†	0.371 ± 0.278†
CP (×10⁵/mL)	1.552 ± 0.570	7.506 ± 2.219†	8.132 ± 2.188†
TMA-DPH	0.355 ± 0.032*	0.349 ± 0.024	0.352 ± 0.027

*F = 6.830, $P < .01$ (1-way ANOVA).

†$P < .001$ *v* baseline (Student's *t* test).

Table 2. PMN Filtration Parameters and Membrane Fluidity (expressed as TMA-DPH polarization degree) in Normal Subjects, Type I Diabetics, and Type II Diabetics at Baseline and After Activation With fMLP (mean ± SD)

Parameter	Baseline	5 Minutes	15 Minutes
Normals			
IRFR	0.954 ± 0.098	0.831 ± 0.158*	0.877 ± 0.101*
CP ($\times 10^5$/mL)	1.641 ± 0.768	4.027 ± 2.250*	3.538 ± 1.873*
TMA-DPH	0.324 ± 0.026§	0.334 ± 0.026	0.326 ± 0.035
Type I diabetics			
IRFR	0.901 ± 0.112	0.605 ± 0.166†	0.629 ± 0.215†
CP ($\times 10^5$/mL)	1.666 ± 0.970	3.981 ± 1.695†	3.585 ± 2.046†
TMA-DPH	0.344 ± 0.012§	0.348 ± 0.010‡	0.349 ± 0.017‡
Type II diabetics			
IRFR	0.918 ± 0.099	0.678 ± 0.166†	0.690 ± 0.224*
CP ($\times 10^5$/mL)	1.552 ± 0.570	3.975 ± 1.899†	3.508 ± 2.000‡
TMA-DPH	0.355 ± 0.032§	0.354 ± 0.028	0.354 ± 0.029

*$P < .01$ *v* baseline (Student's *t* test).
†$P < .001$ *v* baseline (Student's *t* test).
‡$P < .05$ *v* baseline (Student's *t* test).
§See Table 1.

DISCUSSION

In this study, in agreement with our previous results[8] and those of MacRury et al,[7] PMN filtration parameters do not distinguish normals from type I or II diabetics. We noted a slight but significant difference in PMN membrane fluidity between normals and type I and II diabetics. This agrees with the observation by Kantar et al[9] in evaluating PMN membrane fluidity in type I diabetics, and contrasts with the observation by Tong et al[10] in examining MN leukocyte membrane fluidity in type II diabetics.

In normals and type I and II diabetics, after chemotactic activation with PMA and fMLP, a significant variation in PMN filtration parameters was observed. This variation was present at both 5 and 15 minutes in comparison to basal values. With either stimulating agent, the change in the IRFR in type I and II diabetics at both observation points was greater than in normals, while the changes in CP in type I and II diabetics were not statistically different from those present in normals. In this study, the trend of PMN filtration after activation was different from that previously noted by us (unpublished data) in type II diabetics with macrovascular complications, in whom we found a lower variation in CP after PMN activation with PMA versus normals. Initially, this may have seemed attributable to the PMN metabolic abnormality present in diabetes mellitus,[24-26] which is characterized principally by an inhibition of glycolysis, secondary to reduced phosphofructokinase activity, an increase of the hexosomonophosphate shunt, and intracellular sorbitol accumulation. This observation does not confirm such a hypothesis. It shows that the altered PMN metabolic pattern found in diabetes mellitus, although leading to lower activation, expressed as a reduced chemiluminescence and superoxide anion response,[27-30] does not directly influence PMN flow properties. These properties, after activation, seem especially dependent on the f-actin/g-actin dynamic balance.[31,32] In diabetics, after activation, the depolymerization of f-actin to g-actin may be slower than in normals, as hypothesized by Pecsvarady et al.[6]

In agreement with others[9,18,19,33,34] who found a postactivation decrease of PMN membrane fluidity, we expected to find a reduction of this parameter in normals and type I and II diabetics. In type I diabetics, we indeed found a decrease in PMN membrane fluidity, while no significant variation of this microrheological parameter was observed in type II diabetics and normals. In this latter group, the data may be explained if we consider that the standard deviation for the degree of TMA-DPH polarization is greater than that observed in type I diabetics, and thus, the increase found in normals during PMN activation was not statistically significant.

After PMN activation with either of the chemoattractant agents, the Δ% of PMN membrane fluidity was not statistically different between normals and diabetics of either type, whereas our results clearly show that in type I diabetics this microrheological determinant during activation with PMA and fMLP, in comparison to baseline, significantly changes at 5 and 15 minutes. This trend may be the basis of a specific metabolic pattern in PMNs of type I diabetics, which is revealed after in vitro activation with both agents. This hypothesis may be plausible, considering that in type I and II diabetics fasting blood glucose and HbA_{1c} levels were similar.

Keeping in mind the relationship between leukocyte count and insulin resistance in healthy men,[35,36] the relationship between mononuclear leukocyte membrane fluidity and insulin resistance in type II diabetics,[10,37] and the influence of cell membrane physical properties on insulin activity,[38] we believe that further investigation of PMN rheology in diabetes mellitus, both at baseline and after activation, is needed.

ACKNOWLEDGMENT

The authors wish to thank S. La Barbera for technical assistance.

REFERENCES

1. Ernst E, Matrai A: Altered red and white blood cell rheology in type II diabetes. Diabetes 35:1412-1415, 1986
2. Vermes I, Steinmetz ET, Zeyen LJJM, et al: Rheological properties of white blood cells are changed in diabetic patients with microvascular complications. Diabetologia 30:434-436, 1987
3. Juhan-Vague I, Roul C, Rousset B, et al: Study of red blood cells (RBC) and white blood cells (WBC) filtrability in uncontrolled type I (insulin-dependent) diabetic patients with the St George's filtrometer. Clin Hemorheol 7:427, 1987 (abstr)
4. Moessmer G, Wenby RB, Gray DS, et al: Micropore filtration behavior of RBC and WBC from obese, non–insulin-dependent diabetics. Clin Hemorheol 9:501, 1989 (abstr)
5. Perego MA, Danese C, Volpe MS, et al: Rheological leukocyte indexes in diabetes mellitus type II. Ann Cardiol Angeiol (Paris) 4:A75-A80, 1992
6. Pecsvarady Z, Fisher TC, Darwin CH, et al: Decreased polymorphonuclear leukocyte deformability in NIDDM. Diabetes Care 17:57-63, 1994
7. MacRury SM, Lennie SE, Paterson KR, et al: Deformability of leucocyte subpopulations in type 1 (insulin-dependent) and type 2 (non–insulin-dependent) diabetic patients. Clin Hemorheol 14:539-544, 1994
8. Caimi G, Lo Presti R, Montana M, et al: Rheological and metabolic leucocyte determinants in diabetes mellitus. Clin Hemorheol 15:53-60, 1995
9. Kantar A, Giorgi PL, Curatola G, et al: Alterations in membrane

fluidity of diabetic polymorphonuclear leukocytes. Biochem Med Metab Biol 46:422-426, 1991

10. Tong P, Thomas T, Berrish T, et al: Cell membrane dynamics and insulin resistance in non–insulin-dependent diabetes mellitus. Lancet 345:357-358, 1995

11. Caimi G, Lo Presti R, Ferrara F, et al: Vascular atherosclerotic disease: Behaviour of the polymorphonuclear leukocyte (PMN) filtration parameters, PMN membrane fluidity and PMN cytosolic Ca^{2+} content after chemotactic activation. Clin Hemorheol 16:523-531, 1996

12. Nash GB, Jones JG, Mikita J, et al: Effects of preparative procedures and of cell activation on flow of white cells through micropore filters. Br J Haematol 70:171-176, 1988

13. Pecsvarady Z, Fisher TC, Fabok A, et al: Kinetics of granulocyte deformability following exposure to chemotactic stimuli. Blood Cells 18:333-352, 1992

14. Inoue S, Lan Y, Muran J, et al: Reduced hydrogen peroxide production in neutrophils from patients with diabetes. Diabetes Res Clin Pract 33:119-127, 1996

15. National Diabetes Data Group: Classification and diagnosis of diabetes mellitus and other categories of glucose intolerance. Diabetes 28:1039-1057, 1979

16. Mikita J, Nash G, Dormandy J: A simple method of preparing white blood cells for filterability testing. Clin Hemorheol 6:635-639, 1986

17. Lennie SE, Lowe GDO, Barbenel JC, et al: Filterability of white blood cell subpopulations, separated by an improved method. Clin Hemorheol 7:811-816, 1987

18. Yasui K, Masuda M, Matsuoka T, et al: Abnormal membrane fluidity as a cause of impaired dynamics of chemoattractant receptors on neonatal polymorphonuclear leukocytes: Lack of modulation of the receptors by a membrane fluidizer. Pediatr Res 24:442-446, 1988

19. Masuda M, Komiyama Y, Murakami T, et al: Difference in changes of membrane fluidity of polymorphonuclear leukocytes stimulated with phorbol myristate acetate and formyl-methionyl-leucyl-phenylalanine: Role of excited oxygen species. J Leukoc Biol 47:105-110, 1990

20. Dormandy J, Flute P, Matrai A, et al: The new St. George's blood filtrometer. Clin Hemorheol 5:975-983, 1985

21. Donner M, Stoltz JF: Comparative study on fluorescent probes distributed in human erythrocyte and platelet. Biorheology 22:385-397, 1985

22. Donner M, Muller S, Stoltz JF: Molecular rheology of white blood cells. Rev Port Hemorreol 3:235-247, 1989

23. Shinitzky M, Barenholtz Y: Fluidity parameters of lipids regions determined by fluorescence polarization. Biochim Biophys Acta 515:367-394, 1978

24. Qvist R, Larkins RG: Decreased stimulated glucose oxidation and iodination by polymorphonuclear leukocytes from insulin-treated diabetic subjects. Diabetes 30:256-262, 1981

25. Esmann V: The polymorphonuclear leukocyte in diabetes mellitus. J Clin Chem Clin Biochem 21:561-567, 1983

26. Wilson RM, Tomlinson DR, Reeves WG: Neutrophil sorbitol production impairs oxidative killing in diabetes. Diabet Med 4:37-40, 1987

27. Shah SV, Wallin JD, Eilen SD: Chemiluminescence and superoxide anion production by leukocytes from diabetic patients. J Clin Endocrinol Metab 57:402-409, 1983

28. Wierusz-Wysocka B, Wysocki H, Siekirka H, et al: Evidence of polymorphonuclear neutrophils (PMN) activation in patients with insulin-dependent diabetes mellitus. J Leukoc Biol 42:519-523, 1987

29. Sato N, Shimizu H, Suwa K, et al: MPO activity and generation of active O_2 species in leukocytes from poorly controlled diabetic patients. Diabetes Care 15:1050-1052, 1992

30. Gallacher SJ, Thomson G, Fraser WD, et al: Neutrophil bactericidal function in diabetes mellitus: Evidence for association with blood glucose control. Diabet Med 12:916-920, 1995

31. Sklar LA, Omann GM, Painter RG: Relationship of actin polymerization and depolymerization to light scattering in human neutrophils: Dependence on receptor occupancy and intracellular Ca^{++}. J Cell Biol 101:1161-1166, 1985

32. Wallace PJ, Packman CH, Wersto RP, et al: The effects of sulphydryl inhibitors and cytochalasin on the cytoplasmic and cytoskeletal actin of human neutrophils. J Cell Physiol 132:325-330, 1987

33. Stocker R, Winterhalter KH, Richter C: Increased fluorescence polarization of 1,6 diphenyl 1,3,5 hexatriene in the phorbol myristate acetate–stimulated plasma membrane of human neutrophils. FEBS Lett 144:199-203, 1982

34. Fiorini R, Curatola G, Bertoli E, et al: Changes of fluorescence anisotropy in plasma membrane of human polymorphonuclear leukocytes during the respiratory burst phenomenon. FEBS Lett 273:122-126, 1990

35. Jeppesen JL, Maheux P, Facchini FS: White blood cell count and insulin resistance in healthy nonsmoking men. Am Heart J 127:459-461, 1994

36. Targher G, Seidell JC, Tonoli M, et al: The white blood cell count: Its relationship to plasma insulin and other cardiovascular risk factors in healthy male individuals. J Intern Med 239:435-441, 1996

37. Devynck M-A: Do cell membrane dynamics participate in insulin resistance? Lancet 345:336-337, 1995

38. Kinnunen PKJ: On the principles of functional ordering in biological membranes. Chem Phys Lipids 57:375-399, 1991