Association of altered hemorheology with oxidative stress and inflammation in metabolic syndrome

Prajwal Gyawali, Ross S. Richards

School of Community Health, Charles Sturt University, Australia

Objective: We have shown increased whole blood viscosity (WBV), decreased erythrocyte deformability, and increased erythrocyte aggregation in metabolic syndrome (MetS) in our previous study. The objective of the study was to find out if the altered hemorheology shown in MetS in our previous study is associated with chronic inflammation and oxidative stress in the same subjects.
Methods: One hundred recruited participants were classified into three groups based on the number of the MetS components present following National Cholesterol Education Program, Adult Treatment Panel III definitions. WBV, erythrocyte aggregation, erythrocyte deformability, oxidative stress markers (erythrocyte reduced glutathione (GSH), superoxide dismutase (SOD), and urinary isoprostanes), inflammatory markers high-sensitivity C-reactive protein (hsCRP), and thrombotic marker D-dimer were measured. Data were analyzed by IBM SPSS 20 software.
Results: We found a significant association of altered hemorheology with chronic inflammation and oxidative stress in MetS. There was a linear increase in the level of hsCRP and a linear decrease in the level of SOD and GSH across the quartiles of erythrocyte aggregation. Similarly, the thrombotic marker D-dimer showed a linear increase and oxidative stress marker GSH showed a linear decrease trend across the quartiles of WBV.
Discussion: Alterations of hemorheology in MetS are probably due to the effect of chronic inflammation and oxidative stress. The negative effects of chronic inflammation and oxidative stress on the cardiovascular system could be due to the resulting altered hemorheology.

Keywords: Erythrocyte aggregation, Erythrocyte deformability, Glutathione, Metabolic syndrome, Whole blood viscosity

Uncorrected Proof

Introduction

Chronic low-grade inflammation has been hypothesized to play a role in the development of metabolic syndrome (MetS).[1] The American Heart Association has indicated that high-sensitivity C-reactive protein (hsCRP) measurements might provide information for a global risk assessment for coronary heart disease beyond that obtained from the established risk factors.[2] hsCRP has also been mooted as a marker for the primary prevention of cardiovascular diseases.[3] Oxidative stress, due to chronic inflammation, also contributes to the development of MetS.[4,5] Oxidative stress and inflammation play a major role in vascular disorders and circulation defects.[6,7] Alterations in macrovascular circulation and microcirculation are common in MetS,[8] and we have recently reviewed the effect of various hemorheological components on MetS.[9–11] In our recent study, we have shown altered hemorheology[12] among subjects with MetS when compared to healthy controls. Hemorheology is one of the major factors that could possibly alter the microcirculation.[13] The objective of the study, in this context, was to find out if the altered hemorheology shown in MetS in our previous study[12] is associated with chronic inflammation and oxidative stress in the same subjects.

Materials and methods

The detailed methodology and the principles of the instruments used in the study have been described elsewhere.[12] Briefly, 100 participants were recruited from a rural town of Australia from June–Dec 2013. Pregnant women, non-ambulatory patients, and children under 18 years of age were excluded from the study. Recruited participants were divided into three groups on the basis of absence or presence of MetS and its components. A modified National Cholesterol Education program Adult Treatment Panel-III (NCEP ATP-III) guideline was used to define MetS.[14] According to NCEP ATP-III guidelines, the individual is said to be in the state of MetS if he/she

Correspondence to: Prajwal Gyawali, School of Community Health, Charles Sturt University, Australia.
Email: clbioprajwal@gmail.com

© W. S. Maney & Son Ltd 2014
DOI 10.1179/1351000214Y.0000000120

fulfills three criteria from the following five criteria: waist circumference >102 cm in male and >88 cm in female; triglyceride (TG) ≥1.7 mmol/l or specific treatment; high-density lipoprotein cholesterol (HDL-C) <1.0 mmol/l in male and <1.3 mmol/l in female or specific treatment; blood pressure ≥130/85 mm Hg or previously diagnosed hypertension; and fasting glucose ≥5.6 mmol/l or previously diagnosed DM. Group I consists of the participants without any positive components of MetS (healthy controls); group II consists of the participants with one or two positive components; and group III consists of participants with three or more positive components. Participants in groups I and II are non-MetS whereas participants of group III are with MetS. The study was approved by the university human research ethics committee (2012/131). Anthropometric measurements (height, weight, and waist circumference) were obtained from the participants.

Twenty milliliters of blood was taken from the participants for the analysis of erythrocyte aggregation (critical time and critical stress), erythrocyte deformability (EI_{max} and $SS_{1/2}$), whole blood viscosity (WBV) (shear stress 150/second), oxidative stress markers (erythrocyte superoxide dismutase (SOD) and erythrocyte-reduced glutathione (GSH)), inflammatory marker hsCRP, and thrombotic marker D-dimer. Erythrocyte deformability and aggregation measurements were carried out using a RheoScan-AnD 300 system (RheoMeditech Inc., Korea). Two indices were used to define erythrocyte aggregation: critical time and critical stress. The lesser the critical time, the faster the erythrocyte aggregation process and the higher the critical stress, the faster the erythrocyte aggregation.[15] Two measurement indices were used to define erythrocyte deformability: EI_{max} and $SS_{1/2}$.[16,17] EI_{max} is the maximum erythrocyte elongation index at infinite shear stress and $SS_{1/2}$ is the shear stress required for half of maximal deformation.

WBV measurement was carried out using a Brookfield DV-II + programmable viscometer (MA, USA), using a CP40 spindle at 37 °C. All the rheological measurements were performed within 2 hours of blood collection after adjusting EDTA anticoagulated whole blood to the hematocrit of 40%.

Inflammatory marker hsCRP and thrombotic marker D-dimer were measured on the day of collection in a commercial clinical pathology laboratory. GSH was measured by the 5,5′-di-thiobis-(2-nitrobenzoic acid) method on the same day of blood collection from the erythrocyte lysate (from the washed cells) after protein precipitation by metaphosphoric acids.[18] SOD was measured from the hemolysate using a commercially available kit (Cayman Chemical Company) using xanthine oxidase and tetrazolium salts. Hemolysate was prepared from packed red blood cells. One milliliter of packed red blood cells were mixed with 4 ml of ice-cold water and centrifuged at 1260 g for 9 minutes to obtain hemolysate. The hemolysate was stored in 1.5 ml Eppendorf tubes at −80°C in an ultra low-temperature freezer until tests were performed. Urinary 15-isoprostanes F_{2t} were measured using a commercially available kit (NWLSS™) and were expressed as ng of isoprostanes per mmol of urinary creatinine (Cayman Chemical Company). The urine sample was stored in 1.5 ml Eppendorf tubes at −80°C in an ultra low-temperature freezer until tests were performed.

Statistical analysis

Data were analyzed by IBM SPSS Statistics version 20. Normality of the data was tested by Shapiro–Wilk test. Kruskal–Wallis test was used to compare the median between three groups for non-normal data. Spearman's correlation was performed between hemorheological parameters and oxidative stress/inflammatory markers. Regression analysis was performed to find an association between MetS and hemorheology. Linear regression analysis was performed for prediction of hemorheology factors by inflammatory and oxidative stress parameters. Jonckheere trend test was used to analyze the trend of oxidative stress and inflammatory markers across the quartiles of critical stress, critical time, EI_{max}, and WBV. All the *P*-values were two-tailed, and those <0.05 were considered statistically significant.

Results

Baseline characteristics of the participants with and without MetS have been published in our previous report.[12] Demographic characteristics of the participants and mean values of MetS components in three different groups (groups I, II, and III) are shown in Table 1. hsCRP, D-dimer, SOD, glutathione, and isoprostanes were not normally distributed across the three different groups as assessed by Shapiro–Wilk test ($P < 0.05$). Hence, non-parametric tests were used. There were significant differences in the median value of hsCRP, D-dimer, SOD, glutathione, and isoprostanes across the three different studied groups (Table 2). Correlation of oxidative stress and inflammatory markers with the hemorheological parameters are shown in Table 3.

Post-hoc analysis of median comparison among three groups

Pairwise comparisons were performed using Dunn's (1964) procedure with a Bonferroni correction for multiple comparisons. Post-hoc pairwise comparisons revealed that hsCRP level was significantly different

Uncorrected Proof

Table 1 Demographic characteristic of the participants and mean values (±SD) of MetS components in three different groups

Parameters	Group I ($n = 31$)	Group II ($n = 33$)	Group III ($n = 36$)	*P*-value
Age (years)	45.4 ± 8.9	56.52 ± 12.9	63.0 ± 12.1	<0.0005
Sex (male/female)	10/21	19/14	24/12	—
Waist circumference (cm)	81.16 ± 8.4	95.79 ± 13.23	110.25 ± 15.46	<0.0005
Height (cm)	166.0 ± 9.0	167.9 ± 6.2	170.8 ± 8.2	0.050
Weight (kg)	67.63 ± 9.27	76.25 ± 13.32	90.14 ± 16.74	<0.0005
BMI (kg/m^2)	24.49 ± 2.29	27.02 ± 4.66	30.89 ± 5.50	<0.0005
Systolic blood pressure (mm Hg)	116.2 ± 9.8	127.5 ± 15.1	142.5 ± 19.1	<0.0005
Fasting blood glucose (mmol/l)	6.92 ± 2.64	6.06 ± 1.97	4.93 ± 0.41	<0.0005
HDL-C (mmol/l)	1.45 ± 0.27	1.43 ± 0.43	1.09 ± 0.22	<0.0005
Triglyceride (mmol/l)	1.09 ± 0.34	1.37 ± 0.62	2.06 ± 0.85	<0.0005
Current smokers (*n*)	1	4	7	—
Antidiabetic medications	—	11	22	—
Antihypertensive medications	—	13	29	—
Antilipidemic medications	—	3	12	—

Table 2 Median values (interquartile range) of oxidative stress and inflammatory markers in three different groups

Parameters	Group I	Group II	Group III	*P*-value
hs-CRP (mg/l)	0.44 (0.96)	1.25 (3.78)	2.33 (5.27)	0.001
D-dimer (μg/ml)	0.36 (0.23)	0.39 (0.17)	0.53 (0.33)	0.003
SOD (U/ml)	630.05 (206.8)	587.69 (145.78)	524.79 (147.18)	0.021
Glutathione (mg/dl)	65.1 (9.0)	61.80 (13.0)	52.0 (10.0)	<0.0005
Urinary isoprostanes (ng/mmol)	65.18 (88.94)	144.09 (167.21)	165.71 (195.21)	0.001

between groups I and III ($P = 0.001$) and between groups II and III ($P = 0.015$). Glutathione level was significantly different between groups I and III ($P < 0.0005$) and between groups I and II ($P = 0.001$). In the case of urinary isoprostanes level ($P = 0.001$), SOD level ($P = 0.018$), and D-dimer level ($P = 0.003$), post-hoc pairwise statistics revealed the significant difference between groups I and III only.

The WBV, EI_{max}, critical stress, and critical time were divided into four groups (quartiles) using twenty-fifth, fiftieth, and seventy-fifth percentiles. Since, in our previous study, we could not demonstrate significant difference in the value of $SS_{1/2}$ among three different groups,[12] this marker was not analyzed by the Jonckheere trend test. The Jonckheere trend test was used to analyze the trend of oxidative stress and inflammatory markers across the quartiles. There was a linear increase in the level of hsCRP (P trend < 0.0005), D-dimer (P trend = 0.002) and urinary isoprostanes (P trend = 0.007) and a linear decrease in the level of SOD (P trend = 0.035) and glutathione (P trend = 0.004) across the quartiles of critical stress. On the contrary, there was a linear decrease in the level of hsCRP (P trend < 0.0005), D-dimer (P trend = 0.021), and urinary isoprostanes (P trend = 0.016) and a linear increase in the level of SOD (P trend = 0.036) and glutathione (P trend = 0.001) across the quartiles of critical time. Similarly, D-dimer (P trend = 0.049) and urinary isoprostanes (P trend = 0.015) showed a linear increase, and glutathione showed a linear decrease (P trend = 0.005) across the quartiles of WBV whereas hsCRP (P trend = 0.147) and SOD (P trend = 0.542) did not follow any significant trend across the quartiles. Among the markers analyzed, only urinary isoprostanes showed a significant decreasing trend across the quartiles of EI_{max} (P trend = 0.005). Similarly, regression analysis showed that oxidative stress and inflammatory markers significantly predicted erythrocyte aggregation (Table 4).

Table 3 Spearman's correlation of hemorheological parameters with inflammatory and oxidative stress markers

Parameters	Critical time (seconds)	Critical stress (Pa)	EI_{max}	$SS_{1/2}$	WBV (mPa/second)
hsCRP (mg/l)					
r	−0.517	0.450	−0.126	0.039	0.120
P-value	0.000	0.000	0.211	0.701	0.233
D-dimer (μg/ml)					
r	−0.262	0.314	0.011	−0.175	0.171
P-value	0.009	0.002	0.914	0.083	0.092
SOD (U/ml)					
r	0.241	−0.198	−0.075	0.240	−0.093
P-value	0.016	0.048	0.458	0.016	0.355
Glutathione (mg/dl)					
r	0.336	−0.234	0.167	0.018	−0.251
P-value	0.001	0.019	0.097	0.860	0.012
Urinary isoprostanes (ng/mmol)					
r	−0.288	0.257	−0.280	0.105	0.225
P-value	0.004	0.010	0.005	0.299	0.024

Discussion

Oxidative stress is defined as an imbalance between prooxidant and antioxidant factors in favor of the prooxidants.[19] In order to look for evidence of oxidative damage *in vivo*, erythrocytes from patients

Uncorrected Proof

Table 4 ***P*-values (regression coefficient R^{2*}) of linear regression analysis for prediction of hemorheological factors by inflammatory and oxidative stress parameters**

Predictors (continuous variable)	Outcome variables				
	Critical time	Critical stress	EI_{max}	$SS_{1/2}$	WBV
hs-CRP (mg/l)	<0.0005 (0.199)	<0.0005 (0.187)	0.110	0.381	0.682
D-dimer (μg/ml)	0.358	0.226	0.943	0.201	0.577
SOD (U/ml)	0.009 (0.068)	0.078 (0.031)	0.720	0.016 (0.058)	0.273
Glutathione (mg/dl)	0.004 (0.080)	0.003(0.086)	0.106	0.516	0.043(0.041)
Urinary isoprostanes (ng/mmol)	0.005 (0.076)	0.018 (0.056)	0.003 (0.086)	0.354	0.005 (0.077)

*Regression coefficient is shown only for the parameters with *P*-value <0.05.

with MetS, with only one or two components of MetS and healthy control subjects were assessed for erythrocyte GSH, SOD, and urinary isoprostanes in the present study. This study shows the increased oxidative stress in MetS groups (group III) when compared to healthy controls (group I) (Table 2), suggesting that the erythrocyte antioxidant defense mechanism is exhausted in MetS due to overproduction of reactive oxygen species. These observations are consistent with studies that have highlighted oxidative stress as a pathophysiologic component of MetS.[4,5]Increased oxidative stress has been suggested as a prior event in the development of MetS,[20] and it is likely to be a common second-level abnormality in MetS.[21]

Erythrocyte GSH and SOD levels were decreased in MetS group when compared to healthy controls. The ubiquitous nature of the erythrocyte and the fact that it is an expendable cell (replaced on average every 120 days) make it an ideal cell to protect other body tissues from free radical damage even at the expense of their own structure and function.[22] In carrying out their role of free radical scavenging, erythrocytes become damaged by oxidation, which consumes endogenous-reducing substances thereby decreasing the level of erythrocyte antioxidant GSH and SOD. Reactive oxygen species superoxide anion crosses the erythrocyte membrane through specific superoxide channel and is neutralized by the antioxidant SOD inside the erythrocyte.[22,23] SOD is inactivated by its own product hydrogen peroxide.[24] Hydrogen peroxide is broken down to water and oxygen by the enzyme catalase and glutathione peroxidase, the latter of which consumes GSH. Similar to our study, Koziróg *et al.* showed lower erythrocyte activity of SOD, GSH, and catalase in MetS subjects when compared to healthy controls.[25]

Oxidative stress causes damage to critical biomolecules including DNA, lipids, and proteins.[26] The increased concentration of urinary isoprostanes seen in the MetS group in the present study is due to the damage caused by free radicals. This observation is consistent with that of Tsai *et al.* who reported elevated urinary isoprostanes level in MetS group when compared to healthy controls.[27] Similarly, the present study shows an increased inflammation level demonstrated by increased hsCRP level in the MetS group compared to healthy controls. Adipocytes in obese patients with MetS release high amounts of tumor necrosis factor-alpha and interleukin-6 into the circulation, which stimulate the production of hsCRP by the liver and induce insulin resistance.[28] Several studies in the past have confirmed this association of hsCRP with MetS[3,29,30] and altered hemorheology.[31]

The present study provides the evidence that oxidative stress and chronic inflammation is present in MetS. This study further investigated to what extent these two parameters affect hemorheology. The altered hemorheology in MetS that was demonstrated in our previous study[12] seems to be associated with the chronic inflammation and oxidative stress. This study was conducted among patients that take anti-inflammatory drugs. Nevertheless, hsCRP showed a linear increase across the quartiles of critical stress, linear decrease across the quartiles of critical time, and significantly predicted critical stress and critical time. It seems likely that increased erythrocyte aggregation is due to the presence of increased adhesive proteins (such as inflammatory cytokines and acute phase proteins) in their milieu in MetS.

Erythrocyte GSH and SOD showed negative correlation with erythrocyte aggregation and WBV whereas erythrocyte SOD showed negative correlation with erythrocyte aggregation (Table 3). The data appear to support the proposition that the increased erythrocyte aggregation is also due to the oxidative damage of erythrocytes. The labile groups in the proteins and lipids of the erythrocyte's cytoskeleton are oxidized due to the decreased antioxidant level, and this oxidative modification of membrane proteins and lipids possibly increases the tendency of 'damaged' erythrocytes to adhere with other erythrocytes thereby increasing erythrocyte aggregation. Supporting this, Richards and Nwose[32] observed increased erythrocyte oxidative stress with increased WBV in different stages of diabetes pathogenesis. Abnormal WBV was linked to oxidative stress in 76% of the apparently healthy controls.[32] It was shown that intravenous infusion of reduced glutathione significantly reduced blood viscosity and erythrocyte aggregation in atherosclerotic

Uncorrected Proof

patients[33] supporting the observations of the present study that the depletion of the antioxidant is associated with alterations in hemorheological profile.

The present study demonstrated a positive correlation of urinary isoprostanes with WBV and erythrocyte aggregation, and a negative correlation with erythrocyte deformability (Table 3). Unfavorable alteration in erythrocyte lipid membrane fluidity in MetS participants has been reported by Kowalczyk *et al*.[34] MDA concentration in erythrocyte of MetS was shown to be higher when compared to healthy controls. Supporting the findings of the present study, membrane lipid peroxidation was considered a possibility for altered membrane properties.[34]

Regression analysis in the present study showed that all of the oxidative stress markers and hsCRP significantly predicted erythrocyte aggregation parameters and urinary isoprostanes predicted all of the hemorheological parameters except $SS_{1/2}$. This reveals that oxidative stress and chronic inflammation influence blood rheological profile. However, regression coefficient indicates that the influence is not big. Though the present study suggests some association between rheological parameters and oxidative stress/chronic inflammation, large studies should be undertaken to see how big the effect is.

Yedgar *et al*. have suggested that the inflammation and oxidative stress may alter the erythrocyte flow due to altered rheological properties and may lead to vascular occlusion.[35] In the present study, D-dimer is positively correlated with the erythrocyte aggregation. The possible vascular occlusion and slow flow due to the aggregates could enhance endothelial dysfunction and activate the coagulation system. Increased concentration of D-dimer has been associated with coronary heart diseases.[36] Another plausible mechanism suggested by studies demonstrating an effect by free radicals on fibrinogen molecules is that a shift in the oxidant/antioxidant dynamic balance could lead to increased *in vivo* oxidation of the fibrinogen molecule promoting its conversion to fibrin and ultimately D-dimer.[37,38]

Recently, Toth *et al.* have shown the effect of hemorheological factors in cardiovascular medicine.[39] The findings of the present study highlight the cause that leads to alterations in hemorheology and the effect of these alterations in the cardiovascular system through increased D-dimer. Moreover, recently, we have shown the positive correlation of increased WBV and erythrocyte aggregation with peripheral arterial atherosclerotic marker: toe brachial pressure index.[12] The possible effect of hemorheological factors in cardiovascular medicine has begun to be noticed for several years now.[13,39,40] Taken together, the findings of the present study and our previous study would seem to suggest that the negative effect of inflammatory and oxidant molecules seen in the cardiovascular system could be due to the resulting altered hemorheology.

Conclusion

Inflammation is increased and antioxidant capacity is decreased in MetS. Erythrocyte aggregation, WBV, and erythrocyte deformability showed significant association with one or more of the markers analyzed. Alterations of hemorheology in MetS are probably due to the effect of chronic inflammation and oxidative stress. The negative effect of chronic inflammation and oxidative stress in the cardiovascular system could be due to the resulting altered hemorheology.

Disclaimer statements

Contributors None.

Funding None.

Conflicts of interest None.

Ethics approval Obtained from HREC and CSU.

References

1 Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005;111:1448–54.
2 Pearson TA, Mensah GA, Alexander RW, Anderson JL, Cannon RO, Criqui M, *et al.* Markers of inflammation and cardiovascular disease application to clinical and public health practice: a statement for healthcare professionals from the centers for disease control and prevention and the American heart association. Circulation 2003;107:499–511.
3 Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391–97.
4 Furukawa S, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, *et al.* Increased oxidative stress in obesity and its impact on metabolic syndrome. The Journal of Clinical Investigation 2004;114:1752–61.
5 Sankhla M, Sharma TK, Mathur K, Rathor JS, Butolia V, Gadhok AK, *et al.* Relationship of oxidative stress with obesity and its role in obesity induced metabolic syndrome. Clinical Laboratory 2012;58:385–92.
6 Hansson GK. Mechanisms of disease: inflammation, atherosclerosis, and coronary artery disease. The New England Journal of Medicine 2005;352:1685–95 + 26.
7 Cai H, Harrison DG. Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circulation Research 2000;87:840–44.
8 Czernichow S, Greenfield JR, Galan P, Jellouli F, Safar ME, Blacher J, *et al.* Macrovascular and microvascular dysfunction in the metabolic syndrome. Hypertension Research: Official Journal of the Japanese Society of Hypertension 2010;33:293–97.
9 Gyawali P, Richards RS, Hughes DL, Tinley P. Erythrocyte aggregation and metabolic syndrome. Clinical Hemorheology and Microcirculation 2014;57:73–83.
10 Gyawali P, Richards RS, Nwose EU. Erythrocyte morphology in metabolic syndrome. Expert Review of Hematology 2012;5:523–31.
11 Gyawali P, Richards RS, Nwose EU, Bwititi PT. Whole-blood viscosity and metabolic syndrome. Clinical Lipidology 2012;7:709–19.
12 Gyawali P, Richards RS, Tinley P, Nwose EU. Hemorheology, ankle brachial pressure index (abpi) and toe brachial pressure

Uncorrected Proof

index (tbpi) in metabolic syndrome. Microvascular Research 2014;95:31–36.
13 Baskurt OK, Meiselman HJ. Blood rheology and hemodynamics. Seminars in the Thrombosis and Hemostasis 2003;29: 435–50.
14 Grundy SM, Brewer HB, Jr, Cleeman JI, Smith C, Jr, Lenfant C. Definition of metabolic syndrome: report of the national heart, lung, and blood institute/American heart association conference on scientific issues related to definition. Circulation 2004;109: 433–38.
15 Hou JX, Shin S. Transient microfluidic approach to the investigation of erythrocyte aggregation: comparison and validation of the method. Korea–Australia Rheology Journal 2008;20:253–60.
16 Baskurt OK, Hardeman MR, Uyuklu M, Ulker P, Cengiz M, Nemeth N, *et al.* Parameterization of red blood cell elongation index – shear stress curves obtained by ektacytometry. Scandinavian Journal of Clinical and Laboratory Investigation 2009;69:777–88.
17 Simmonds MJ, Minahan CL, Serre KR, Gass GC, Marshall-Gradisnik SM, Haseler LJ, *et al.* Preliminary findings in the heart rate variability and haemorheology response to varied frequency and duration of walking in women 65–74 yr with type 2 diabetes. Clinical Hemorheology and Microcirculation 2012;51: 87–99.
18 Nwose EU, Richards RS, McDonald S, Jelinek HF, Kerr PG, Tinley P. Assessment of diabetic macrovascular complications: a prediabetes model. British Journal of Biomedical Science 2010;67:59–66.
19 Sies H. Oxidative stress: oxidants and antioxidants. Experimental Physiology 1997;82:291–95.
20 Roberts CK, Sindhu KK. Oxidative stress and metabolic syndrome. Life Sciences 2009;84:705–12.
21 Grattagliano I, Palmieri VO, Portincasa P, Moschetta A, Palasciano G. Oxidative stress-induced risk factors associated with the metabolic syndrome: a unifying hypothesis. The Journal of Nutritional Biochemistry 2008;19:491–504.
22 Richards RS, Roberts TK, McGregor NR, Dunstan RH, Butt HL. The role of erythrocytes in the inactivation of free radicals. Medical Hypotheses 1998;50:363–67.
23 Lynch RE, Fridovich I. Permeation of the erythrocyte stroma by superoxide radical. Journal of Biological Chemistry 1978;253: 4697–99.
24 Salo DC, Lin SW, Pacifici RE, Davies KJ. Superoxide dismutase is preferentially degraded by a proteolytic system from red blood cells following oxidative modification by hydrogen peroxide. Free Radical Biology & Medicine 1988;5:335–39.
25 Koziróg M, Poliwczak AR, Duchnowicz P, Koter-Michalak M, Sikora J, Broncel M. Melatonin treatment improves blood pressure, lipid profile, and parameters of oxidative stress in patients with metabolic syndrome. Journal of Pineal Research 2011;50:261–66.
26 Floyd RA, Carney JM. Free radical damage to protein and DNA: mechanisms involved and relevant observations on brain undergoing oxidative stress. Annals of Neurology 1992;32:S22–S27.
27 Tsai IJ, Croft KD, Mori TA, Falck JR, Beilin LJ, Puddey IB, *et al.* 20-HETE and F2-isoprostanes in the metabolic syndrome: the effect of weight reduction. Free Radical Biology & Medicine 2009;46:263–70.
28 Pickup JC, Mattock MB, Chusney GD, Burt D. NIDDM as a disease of the innate immune system: association of acute-phase reactants and interleukin-6 with metabolic syndrome X. Diabetologia 1997;40:1286–92.
29 Sigdel M, Kumar A, Gyawali P, Shrestha R, Tuladhar ET, Jha B. Association of high sensitivity C-reactive protein with the components of metabolic syndrome in diabetic and non-diabetic individuals. Journal of Clinical and Diagnostic Research 2014;8: CC11–13.
30 Shrestha R, Jha SC, Khanal M, Gyawali P, Yadav BK, Jha B. Association of cardiovascular risk factors in hypertensive subjects with metabolic syndrome defined by three different definitions. JNMA Journal of Nepal Medical Association 2011;51: 157–63.
31 Fusman G, Mardi T, Justo D, Rozenblat M, Rotstein R, Zeltser D, *et al.* Red blood cell adhesiveness/aggregation, C-reactive protein, fibrinogen, and erythrocyte sedimentation rate in healthy adults and in those with atherosclerotic risk factors. The American Journal of Cardiology 2002;90:561–63.
32 Richards RS, Nwose EU. Blood viscosity at different stages of diabetes pathogenesis. British Journal of Biomedical Sciences 2010;67:67–70.
33 Coppola L, Grassia A, Giunta R, Verrazzo G, Cava B, Tirelli A, *et al.* Glutathione (GSH) improved haemostatic and haemorheological parameters in atherosclerotic subjects. Drugs under Experimental and Clinical Research 1991;18:493–98.
34 Kowalczyk E, Kowalski J, Błaszczyk J, Gwoździński L, Ciećwierz J, Sienkiewicz M. Estimation of cell membrane properties and erythrocyte red-ox balance in patients with metabolic syndrome. Molecular Biology Reports 2012:1–6.
35 Yedgar S, Koshkaryev A, Barshtein G. The red blood cell in vascular occlusion. Pathophysiology of Haemostasis and Thrombosis 2002;32:263–68.
36 Lowe GDO, Rumley A. Use of fibrinogen and fibrin D-dimer in prediction of arterial thrombotic events. Thrombosis and Haemostasis 1999;82:667–72.
37 Becatti M, Marcucci R, Bruschi G, Taddei N, Bani D, Gori AM, *et al.* Oxidative modification of fibrinogen is associated with altered function and structure in the subacute phase of myocardial infarction. Arteriosclerosis Thrombosis, and Vascular Biology 2014.
38 Martinez M, Weisel JW, Ischiropoulos H. Functional impact of oxidative posttranslational modifications on fibrinogen and fibrin clots. Free Radical Biology & Medicine 2013;65:411–18.
39 Toth A, Papp J, Rabai M, Kenyeres P, Marton Z, Kesmarky G, *et al.* The role of hemorheological factors in cardiovascular medicine. Clinical Hemorheology and Microcirculation 2014; 56:197–204.
40 Popel AS, Johnson PC. Microcirculation and hemorheology. Annual Review of Fluid Mechanics 2005;37:43.

Uncorrected Proof