Decreased platelet nitric oxide contributes to increased circulating monocyte-platelet aggregates in hypertension

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Aims
The aim of this study was to determine the effect of blood pressure (BP) on platelet nitric oxide (NO) signalling and on formation of circulating monocyte-platelet aggregates (MPA), as well as the role of platelet NO in modulating MPA in hypertension.

Methods and results
We first examined platelet NO signalling in 23 untreated hypertensive (UH) and 23 normotensive (NT) subjects. Platelets from hypertensives exhibited reduced NO synthase activation by albuterol or collagen, as well as suppressed basal and stimulated NO-attributable cyclic guanosine-3',5'-monophosphate, compared with NT. In a second study, comprising 106 subjects with a wide BP range, circulating MPA showed a strong positive correlation with BP. On multiple regression analysis, using a model incorporating systolic BP (SBP), diastolic BP, age, lipids, gender, and smoking status, the only independent predictor of MPA was SBP. Nitric oxide synthase inhibition with NG-monomethyl-L-arginine increased MPA in NT but not in hypertensives, whereas the NO donor spermine NONOate (SNO) decreased MPA in NT but not in hypertensives. Platelet P-selectin expression was higher in hypertensives than in NT, and its expression was suppressed by SNO in NT only.

Conclusion
Platelet NO production and responsiveness are suppressed with raised BP, and this may contribute to the increase in platelet P-selectin and hence in circulating MPA in hypertension.

Keywords
Platelets • Monocytes • Monocyte-platelet aggregates • Nitric oxide • Blood pressure

Introduction
Hypertension is associated with increased risk of atherothrombotic complications, predominantly coronary heart disease and stroke, and the risk of these increases continuously with increasing blood pressure (BP) levels. Platelets are involved in the pathogenesis not only of arterial thrombosis but also of atherosclerosis itself. When the platelet is activated, P-selectin rapidly translocates to the plasmalemma, where it can interact with P-selectin glycoprotein ligand-1 (PSGL-1) on leucocytes. These interactions occur primarily between platelets and monocytes although also, to a lesser extent, between platelets and neutrophils. The resulting monocyte-platelet aggregates (MPA) represent a sensitive early marker of platelet activation in several clinical settings and may also in themselves mediate vascular inflammation, atherosclerosis, and thrombosis.

Platelet abnormalities indicative of increased platelet activation have been described with increasing BP. Therefore, platelet activation may provide an important link between raised BP and atherothrombotic disease. Nitric oxide (NO), produced either by endothelial cells or by platelets themselves, inhibits platelet adhesion, aggregation, recruitment, and formation of leucocyte-platelet aggregates. Whether platelet responsiveness to NO is decreased in hypertension is currently unknown; nor is it known whether MPA are increased in hypertension, and whether this may relate to altered NO signalling in platelets.
We hypothesized that in essential hypertension, platelet NO biosynthesis and/or responsiveness is impaired, giving rise to increased platelet activation. The aims of this study were therefore first to investigate whether platelet L-arginine/NO signalling is impaired in hypertensive patients, and the mechanisms underlying this; and secondly to ascertain whether such impairment translates into functional consequences relating to platelet function, specifically the expression of P-selectin on platelets and formation of MPA.

Methods

Platelet nitric oxide biosynthesis and signalling

Twenty-three untreated hypertensive subjects (UH) (BP: 151.6 ± 1.8/92.8 ± 1.7 mmHg) and 23 normotensive controls (NT) (BP: 117.9 ± 2.3/74.8 ± 1.8 mmHg) closely matched for age and sex, were recruited. Untreated hypertensive subjects had recently diagnosed mild essential hypertension (Grade 1) as defined by the British Hypertension Society criteria (BP: 140–159 mmHg systolic and/or 90–99 mmHg diastolic).

Over a period of 6 months, UH were recruited sequentially from the Hypertension Clinics at Guy’s and St Thomas’ Hospitals, London, and NT were recruited from the departmental database of healthy volunteer subjects. Blood pressure was measured by sphygmomanometry. No subject was on regular treatment with aspirin, non-steroidal anti-inflammatory or other anti-platelet medication, or on lipid-lowering therapy, and none had taken any such medication for at least 2 weeks prior to study. All subjects were clinically healthy, with no evidence on history or physical examination of cardiovascular disease (other than hypertension in the case of UH) or other significant co-morbidity. Furthermore, all UH were studied within a few days of their clinic visit, in order to avoid any delay in commencing anti-hypertensive therapy if clinically indicated. The study was approved by the St Thomas’ Hospital Research Ethics Committee, and all subjects gave written informed consent.

Venepuncture was performed using a light tourniquet and a Butterfly® 19 G needle in an antecubital vein. One hundred millilitres of whole blood was collected into trisodium citrate (0.38% final concentration), centrifuged (145 g, 10 min, 22 °C), and the resultant platelet-rich plasma applied to a freshly washed Sepharose CL-2B gel column. Gel-filtered platelets (GFP) were eluted and collected as described. Platelet count was measured in the eluate using a Coulter counter (Beckman Coulter, Gen.S System 2). Platelet NO synthase (NOS) activity and NO-attributable cyclic guanosine-3’5’-monophosphate (cGMP) were measured in GFP, basally and in response to albuterol (10 μmol/L) or collagen (8 μg/mL), from the conversion of L-[3H]arginine to L-[3H]citrulline and by radiomunnoassay, respectively, as described. In a subset of these subjects (10 UH and 10 NT, closely matched for age and sex), expression of NOS-3 and of soluble guanylyl cyclase (sGC), as well as Ser1177 and Ser631 phosphorylation of NOS-3, were determined in GFP by western blotting as outlined in the Supplementary material online; and in these same subjects, plasma concentrations of symmetric dimethylarginine (SDMA), asymmetric dimethylarginine (ADMA), and arginine were quantified as described.

Measurement of monocyte-platelet aggregates and of platelet P-selectin expression

A cohort of 106 subjects was studied (mean age: 45.9 ± 1.1 years; 72 men, 34 women; BP range: 100–170 mmHg systolic and 65–104 mmHg diastolic). This comprised 17 UH (BP: 153.9 ± 2/96.3 ± 1.7 mmHg; age: 48.6 ± 3.0 years; 11 men, 6 women), 21 hypertensives on antihypertensive drug treatment (TH) (BP: 134.8 ± 2.2/88.1 ± 1.9 mmHg; age: 48.7 ± 2.7 years; 17 men, 4 women), 16 untreated pre-hypertensives (PH) (BP: 137.5 ± 1.4/88.4 ± 1.7 mmHg; age: 44.6 ± 2.4 years; 10 men, 6 women), and 52 NT (BP: 117.8 ± 1.3/77.5 ± 0.9 mmHg; age: 44.4 ± 1.7 years; 34 men, 18 women), as defined by the British Hypertension Society criteria. Over a period of 18 months, hypertensive subjects were recruited sequentially from the Hypertension Clinics at Guy’s and St Thomas’ Hospitals, London, and PH and NT were similarly recruited from the departmental database of healthy volunteer subjects. Blood pressure was measured by sphygmomanometry. Treated hypertensives were receiving one antihypertensive agent only (seven were treated with a calcium antagonist, four with an angiotensin-converting-enzyme inhibitor, four with an angiotensin receptor blocker, two with a β-blocker, and four with a thiazide diuretic); in some cases, these patients had achieved target BP levels, and in other cases, their BP remained suboptimally controlled. Framingham’s cardiovascular risk score was calculated as described previously. No subject was on regular treatment with aspirin, non-steroidal anti-inflammatory or other anti-platelet medication, and none had taken any such medication for at least 2 weeks prior to study. No subject was recruited who was on lipid-lowering therapy. All subjects were clinically healthy, with no evidence on history or physical examination of cardiovascular disease (other than hypertension) or other significant co-morbidity. Furthermore, all patients recruited from the Hypertension Clinics were studied within a few days of their clinic visit, in order to avoid any delay in commencing or altering antihypertensive therapy if clinically indicated. The study was approved by the St Thomas’ Hospital Research Ethics Committee, and all subjects gave written informed consent.

Measurements of MPA in whole blood and of platelet P-selectin expression were performed as described in the Supplementary material online.

Statistical analysis

All data were expressed as mean ± SD and were found to be distributed normally. Statistical analyses were performed in GraphPad Prism or Minitab. Within- and between-group comparisons were made using one- or two-way ANOVA with or without repeated measures, as appropriate.

In examining the correlation between MPA and BP, since our subjects were recruited from two separate sources (hypertensives from our Hypertension Clinic and PH and NT from our database of healthy volunteers), we examined the possibility that the relationship found between MPA and BP was influenced by source of subject. In our multiple regression model, we found that subject source was not associated with MPA after adjusting for systolic BP (SBP). We also tested for the presence of a possible interaction between SBP and subject source, in order to determine whether the slope of the relationship between MPA and SBP differed according to subject source. No such interaction was seen. In subsequent analysis, we therefore investigated the relationship between MPA and BP in the group of subjects taken as a whole. Associations were analysed by least squares regression analysis and multiple regression analysis. In all cases, P < 0.05 (two-tailed) was considered significant. All the above assays had intra- and interassay coefficients of variation <10%. Moreover, preliminary studies had shown that platelet NOS activity, cGMP, and circulating MPA levels were highly reproducible within the same individual when measured on different days (again with coefficients of variation <10%).
Results

Platelet-derived nitric oxide production is impaired in hypertension

Platelet NO signalling was examined in 23 UH and 23 NT closely matched for age and sex (‘study group 1’). Their characteristics are shown in the Supplementary material online, Table S1. Apart from BP levels, these subjects exhibited very similar characteristics (ethnicity, smoking status, body mass index, lipid profile, glucose, HbA1c, renal profile, and platelet count).

Basal platelet NOS activity was not different between NT and UH (60 ± 20 vs. 50 ± 10 femol L-citrulline/10^8 platelets, respectively, P = 0.45). In NT, stimulation of platelets with either albuterol (10^{-5} mol/L) or collagen (8 μg/mL) elicited an increase in NOS activity from basal, as we have described previously; however, this did not occur in UH (see Supplementary material online, Figure S1A).

Platelet NO-attributable cGMP was calculated from the difference in cGMP in the absence and presence of N^G-monomethyl-L-arginine (L-NMMA). In NT, NO-attributable cGMP was increased by either albuterol (10^{-5} mol/L) or collagen (8 μg/mL), again as described previously and in line with the results from platelet NOS assay. In contrast, in UH, NO-attributable cGMP was undetectable at baseline and did not increase in response to either agonist (see Supplementary material online, Figure S1B).

Western blotting revealed no detectable difference in NOS-3 expression, either at baseline or after stimulation with albuterol and collagen, between NT and UH (see Supplementary material online, Figure S2A). Nor was sGC expression different between NT or UH, either at baseline or following treatment with either agonist (see Supplementary material online, Figure S2B). Expression of these was expressed as a ratio to that of the housekeeping protein α-tubulin, whose expression did not differ between UH and NT and was not affected by albuterol or collagen (see Supplementary material online, Figure S2C). We also quantified phosphorylation of NOS-3 in platelets from these subjects, at serine residues 1177 and 633, since phosphorylation at either residue (by various kinases, including protein kinase A and Akt) can activate NOS-3. We found that neither phosphoserine-1177-NOS-3 nor phosphoserine-633-NOS-3 differed between UH and NT at baseline, and neither increased after stimulation with either agonist in either group (see Supplementary material online, Figure S2D and E).

Plasma asymmetric dimethylarginine is increased in hypertension

We considered that differences in platelet NO synthesis between NT and UH might be explained, at least in part, by differences in levels of endogenous NOS inhibitors. We therefore measured plasma levels of the endogenous NOS inhibitor ADMA, as well as those of SDMA (which does not affect NOS) and of their precursor arginine. Asymmetric dimethylarginine levels were significantly greater in UH than in NT, with no differences observed in either SDMA or arginine levels or in plasma creatinine (see Supplementary material online, Figure S3).

Circulating monocyte-platelet aggregates correlate directly with blood pressure levels

To examine the relationship between BP and levels of circulating MPA, a cohort of 106 subjects with a wide range of BP was studied (‘study group 2’). Their characteristics as a whole are shown in the Supplementary material online, Table S2, and broken down by the subgroups (NT, TH, PH, and UH) in the Supplementary material online, Table S3. No differences were present in characteristics (age, sex distribution, ethnicity, lipid profile, glucose, HbA1c, renal profile, and platelet and white cell counts) between the subgroups of UH, TH, PH, and NT; and all hypertensives had combined systolic and diastolic hypertension, with no subject exhibiting isolated systolic hypertension. Heart rate was also not different between any of the subgroups, rendering it unlikely that differences in sympathetic activity were present.

Monocyte-platelet aggregates were identified by double immunofluorescence staining for both monocyte (CD14) and platelet (CD42b) antigens (Figure 1A), and quantified by double immunofluorescence flow cytometry. We confirmed the importance of interaction between platelet-expressed P-selectin and monocyte-expressed PSGL-1 in the formation of MPA, since in preliminary experiments, co-incubation of blood with anti-PSGL-1 antibody virtually abolished MPA (Figure 1B).

A highly significant positive correlation was found between circulating MPA and both SBP and diastolic BP (DBP) (Figure 1C and D). Monocyte-platelet aggregates correlated more closely with SBP (r = 0.56, P < 0.0001) than with DBP (r = 0.44, P < 0.0001). Figure 1E shows the breakdown of MPA levels according to the subgroups (NT, TH, PH, and UH).

Since raised BP often clusters with other cardiovascular risk factors, we also examined for the presence of a possible relationship between MPA levels and other risk factors. No relationship was observed between MPA and total cholesterol, cholesterol subfractions (high- and low-density lipoprotein cholesterol), or triglycerides; similarly, no correlation was found between MPA and Framingham’s risk score. On the other hand, a weak correlation was observed between MPA and age (r = 0.1, P < 0.01). In a multiple regression model, we included SBP, DBP, age, total cholesterol, cholesterol subfractions, triglycerides, gender, and smoking status. The only significant independent predictor of circulating MPA levels was SBP (β = 0.553, P < 0.001). Moreover, there was no effect of antihypertensive treatment on the observed correlation.
increase in MPA when compared with corresponding control incubation, from $35.3 \pm 1.2$ to $43.5 \pm 1.2\%$ ($P < 0.0001$). In contrast, incubation with the selective NOS-2 inhibitor 1400W at two different concentrations (0.05 and 1 mmol/L), previously shown to strongly and specifically inhibit NOS-2 activity in leucocytes,16,17 did not affect MPA levels (data not shown).

We wished to determine whether the increase in MPA with BP may be explained, at least in part, by a decrease in platelet NO signalling with increasing BP. We therefore performed subgroup analysis to ascertain whether L-NMMA had a differential effect on MPA levels in whole blood, in NT when compared with UH. We found that indeed, L-NMMA increased MPA in blood from NT, but not in that from UH (Figure 2A).

To ascertain whether the decreased ability of L-NMMA to increase MPA in blood from hypertensive subjects may relate purely to the higher basal level of MPA in these subjects such that, at such a higher basal level, it may not be possible for MPA to increase further (to L-NMMA) or to decrease (to SNO). To exclude this possibility, in a group of 11 NT subjects, blood was treated with ADP (1 mmol/L) for 5 min, and the effect of L-NMMA (0.1 mmol/L) or SNO (10 mmol/L) examined. Adenosine diphosphate increased MPA in these NT subjects, to levels comparable to (or even higher than) those seen in UH. Nonetheless, co-incubation with L-NMMA increased, and with SNO decreased, MPA levels in these subjects (Figure 2B).

Since platelet P-selectin is a crucial determinant of MPA formation, in a subset of our cohort, we compared platelet P-selectin expression between UH (n = 9) and NT (n = 9), and examined the effect of SNO on its expression within each of these groups. Platelet P-selectin was higher in UH when compared with NT and, whereas SNO decreased the expression of P-selectin in NT, it failed to do so in UH (Figure 2C).

**Discussion**

In this study, we found that basal platelet NOS activity was not different between NT and UH. In contrast, whereas both albuterol

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**Figure 1** (A) Visualization of monocyte-platelet aggregates by immunofluorescence confocal microscopy. Monocytes were stained with phycoerythrin (PE)-labelled anti-CD14 and platelets with fluorescein isothiocyanate (FITC)-labelled anti-CD42b. The overlay shows double-stained aggregates. (B) Quantification of monocyte-platelet aggregates by double immunofluorescence flow cytometry. The double-positive (for CD14 and CD42b) population is represented by events in the top right-hand quadrant. Co-incubation of blood for 2 h with anti-P-selectin glycoprotein ligand-1 antibody (20 μg/mL: Santa Cruz Biotechnology Inc.) results in a marked decrease in measured monocyte-platelet aggregates (percentages shown in the figure), confirming the importance of P-selectin/P-selectin glycoprotein ligand-1 interaction in monocyte-platelet aggregates formation. (C–E) Relationship between monocyte-platelet aggregates and blood pressure. Correlations are shown for both systolic blood pressure (C) and diastolic blood pressure (D). Also shown are monocyte-platelet aggregate levels according to blood pressure status by the British Hypertension Society criteria (E). NT, normotensives; TH, treated hypertensives; PH, pre-hypertensives; UH, untreated hypertensives. n = 106.
and collagen increased platelet NOS activity in NT, they failed to do so in UH. Moreover, NO-attributable cGMP was also not detectable in UH not only upon stimulation but also under baseline conditions. These experiments suggest that platelet NO biosynthesis is impaired in hypertensives, and especially so under conditions of stimulation.

The above findings could not be explained by differences in platelet expression of NOS-3 or sGC between NT and UH, nor by differences in the ability of NOS-3 to undergo phosphorylation at serine-1177 or serine-633; indeed, neither agonist increased phosphorylation of these residues, even in NT. Although NOS-3 is a Ca\(^{2+}\)-dependent enzyme, we also considered it unlikely that the findings could be explained by differences in intraplatelet Ca\(^{2+}\) handling, since intraplatelet Ca\(^{2+}\) in hypertension if anything may be increased rather than decreased,\(^{18}\) and since we have previously demonstrated no effect of albuterol or collagen on intraplatelet Ca\(^{2+}\).\(^{13}\) We therefore investigated the possibility that platelet NO production may be suppressed due to increase levels of endogenous inhibitors of NO formation in hypertensives, with a predominant effect on stimulated NOS activity. We found that indeed, plasma ADMA levels were higher in UH compared with NT, with no change in levels of SDMA (which has no effect on NOS) or of arginine. Although it is not possible to say with certainty what levels of ADMA would be present within platelets, they are likely to be considerably higher than in plasma due to active uptake (alongside L-arginine and other cationic amino acids) by the y\(^{+}\)L transporter.\(^{19}\) Our data confirm previous reports of increased ADMA levels in essential hypertension, which have been linked with decreased excretion of NO metabolites in urine,\(^{20}\) as well as with impaired endothelium-dependent responses.\(^{21}\)

Another important finding from the present study is that MPA in blood are closely related to BP levels, particularly SBP. The partial correlation coefficient (\(\beta = 0.553\)) indicates that 30\% of the observed variance in MPA is attributable to SBP. No independent relationship was found to other traditional cardiovascular risk factors, or indeed to Framingham's cardiovascular risk score; although a weak correlation was seen with age, this is likely accounted for by the increase in BP seen with age, since on multiple regression analysis, we found SBP to be the only independent predictor of MPA. Nor was any effect seen of antihypertensive treatment on the observed correlation, and MPA levels in the treated hypertensives were closely clustered around the regression line relating MPA to SBP and DBP, suggesting that BP itself could be sufficient to determine MPA levels.

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**Figure 2** (A) Effect of N\(^{G}\)-monomethyl-L-arginine (0.1 mmol/L) on monocyte-platelet aggregates in normotensives (n = 45) and untreated hypertensives (n = 46) subgroups. Open bars indicate N\(^{G}\)-monomethyl-L-arginine absent and filled bars represent N\(^{G}\)-monomethyl-L-arginine present. ***p < 0.001 when compared with the absence of N\(^{G}\)-monomethyl-L-arginine. (B) Correlation between nitric oxide-inhibitable monocyte-platelet aggregates and systolic blood pressure (n = 29). (C) Correlation between nitric oxide-inhibitable monocyte-platelet aggregates and diastolic blood pressure (n = 29). (D) Effect of spermine NONOate (10 \(\mu\)mol/L) on monocyte-platelet aggregates in normotensives (n = 16) and untreated hypertensives (n = 13) subgroups. Open bars indicate spermine NONOate absent and filled bars represent spermine NONOate present. ***p < 0.001 when compared with the absence of spermine NONOate. (E) Effect of N\(^{G}\)-monomethyl-L-arginine (0.1 mmol/L) and of spermine NONOate (10 \(\mu\)mol/L) on monocyte-platelet aggregates, following ADP (1 \(\mu\)mol/L) stimulation, in normotensives (n = 11). Open bars indicate N\(^{G}\)-monomethyl-L-arginine or spermine NONOate absent and filled bars represent N\(^{G}\)-monomethyl-L-arginine or spermine NONOate present. ***p < 0.001 when compared with the absence of N\(^{G}\)-monomethyl-L-arginine or spermine NONOate. (F) Percentage of platelets expressing P-selectin, and the effect of spermine NONOate (10 \(\mu\)mol/L) on P-selectin expression in normotensives (n = 9) and untreated hypertensives (n = 9) subgroups. Open bars indicate spermine NONOate absent and filled bars represent spermine NONOate present. *p < 0.05 when compared with normotensives, **#p < 0.01 when compared with the absence of spermine NONOate.
We have previously shown that platelet-derived NO exerts an important modulating effect on MPA.\textsuperscript{13} This is evidenced again in this study both by the increase in MPA observed when blood is incubated with the NOS inhibitor L-NMMA and by the decrease in MPA when blood is treated with the NO donor SNO, in NT. In contrast, hypertensives exhibited a blunted response to L-NMMA, consistent with a decreased availability of platelet-derived NO. Moreover, the ability of SNO to decrease MPA numbers in vitro decreased with increasing BP, suggesting that platelet responsiveness to exogenous NO is inversely related to BP. We also found that platelets from essential hypertensive subjects express P-selectin to a greater degree than NT, consistent with previous reports.\textsuperscript{7,22,23} Since interaction of platelet P-selectin with monocyte-expressed PSGL-1 is essential to the formation of MPA, BP-related alterations in platelet expression of P-selectin can explain the effect of BP on MPA formation. How pressure may regulate platelet P-selectin expression, however, remains unclear. Taken together, these BP-related changes in platelet NO signalling may in large part contribute to the increase in circulating MPA observed with increasing BP, and this in turn may contribute to the observed increase in atherothrombotic complications in the context of raised BP.

Our data suggest that raised BP gives rise to changes in platelet function, especially with regard to NO synthesis and responsiveness. The reverse may in fact be the case that subjects who are predisposed to develop hypertension possess dysfunctional circulating platelets and that platelet dysfunction in these subjects—especially as regards NO signalling—gives rise to pathological effects on the vessel wall: initially vasoconstriction followed in the longer term by structural changes, thereby causing raised BP. However, we consider this to be unlikely, since patients who were on drug therapy for their hypertension had circulating MPA that were commensurate with their BP level, implying that BP is causal at least so far as MPA are concerned. Indeed, shear stress and increased pressure both have important effects on cytoskeletal organization, and we have recently demonstrated that the actin cytoskeleton has important regulatory effects on NOS-3 activity in platelets.\textsuperscript{24} We therefore postulate that raised BP may suppress platelet NO biosynthesis through effects on the interaction of NOS-3 with actin and/or other elements of the cytoskeleton. This requires clarification in future studies.

Our study has potential limitations. First, the NT and PH subjects were recruited from a different source than those with hypertension. In our multiple regression model, we found that subject source was not associated with MPA after adjusting for SBP. We also found no evidence of interaction between SBP and subject source. Nevertheless, we cannot completely exclude the possibility that the subjects from our database of volunteer subjects and those recruited from the Hypertension Clinics differed in respects other than their BPs, which might in themselves give rise to differences in NO signalling and/or MPA formation. Secondly, it is possible that the antihypertensive agents taken by the treated hypertensive group might in themselves have important effects on MPA formation. However, as discussed above, MPA levels in the treated hypertensives were closely clustered around the regression line relating MPA to SBP and DBP, with no effect seen of antihypertensive treatment on these correlations.

Nevertheless, again we cannot completely discount this possibility, and in future studies, it would be useful to study large numbers of hypertensives treated with different classes of antihypertensive drugs, in order to ascertain whether the different antihypertensive drug classes exert differential effects on platelet NO signalling and/or MPA formation.

Platelets produce NO, but until recently its role if any has been unclear; recent evidence, however, suggests that it plays an important role in modulating platelet function, as has been recently reviewed,\textsuperscript{25} and one of its important roles is in modulating the formation of circulating MPA. Circulating MPA are a sensitive and specific marker of platelet activation, and much evidence now exists to suggest that these particles play an important pathological role in the development of atherosclerosis and its complications. Atherosclerotic cardiovascular disease is more common in hypertension, and there is a direct relationship between thrombotic complications of atherosclerosis (myocardial infarction and stroke) and BP throughout the spectrum of BP, from NT to hypertensive. The present study demonstrates that platelet NO production and responsiveness are both impaired in hypertension, that circulating MPA increase in direct relation to SBP, and that impairment of platelet NO signalling may underlie, at least partially, the BP-related increase in MPA. It is also likely that endothelium-derived NO has a major effect on platelet activation and MPA formation, and this may also play an important role in hypertension. The relative contributions of platelet and endothelial NO in this regard remain to be determined.

Supplementary material

Supplementary material is available at European Heart Journal online.

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