Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 59 (2010) 1723 – 1729
Metabolism
Clinical and Experimental
www.metabolismjournal.com

# Plasma myeloperoxidase is inversely associated with endothelium-dependent vasodilation in elderly subjects with abnormal glucose metabolism

Leonard P. van der Zwan[a], Tom Teerlink[a], Jacqueline M. Dekker[b], Ronald M.A. Henry[c], Coen D.A. Stehouwer[c], Cornelis Jakobs[a], Robert J. Heine[d], Peter G. Scheffer[a,*]

[a]*Metabolic Laboratory, Department of Clinical Chemistry, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands*
[b]*EMGO institute, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands*
[c]*Department of Internal Medicine, Maastricht University Medical Center, Maastricht, The Netherlands*
[d]*Department of Endocrinology and Diabetes Center, VU University Medical Center, PO Box 7057, 1007 MB Amsterdam, The Netherlands*

Received 1 December 2009; accepted 14 April 2010

**Abstract**

Myeloperoxidase (MPO), a biomarker related to inflammation, oxidative stress, and nitric oxide scavenging, has been shown to impair endothelium-dependent vasodilation. Because elevated hydrogen peroxide concentrations in diabetic vessels may enhance MPO activity, we hypothesized that a stronger association of MPO with flow-mediated dilation (FMD) may be found in subjects with abnormal glucose metabolism. Myeloperoxidase concentrations were measured in EDTA plasma samples from participants of a population-based cohort study, including 230 subjects with normal glucose metabolism and 386 with abnormal glucose metabolism. Vascular function was expressed as FMD and nitroglycerin-mediated dilation of the brachial artery. In subjects with abnormal glucose metabolism, MPO was negatively associated with FMD (−20.9 [95% confidence interval {CI}, −41.7 to −0.2] -$\mu$m change in FMD per SD increment of MPO). This association remained significant after adjustment for nitroglycerin-mediated dilation (−31.1 [95% CI, −50.0 to −12.3]) and was not attenuated after further adjustment for established risk factors. In subjects with normal glucose metabolism, MPO was not significantly associated with FMD (2.0 [95% CI, −16.0 to 20.0]). In conclusion, in subjects with abnormal glucose metabolism, plasma levels of MPO are inversely associated with endothelium-dependent vasodilation, possibly reflecting enhancement of MPO activity by vascular oxidative stress.


## 1. Introduction

Atherosclerosis is considered a disease involving inflammation, nitric oxide (NO) scavenging, and oxidative stress. Myeloperoxidase (MPO, EC 1.11.1.7) is a novel risk marker particularly useful to identify patients with acute cardiovascular disease (CVD) [1-5]. Myeloperoxidase is released upon activation of polymorphonuclear cells and monocytes and is important in host defense against pathogens by producing hypochlorous acid and other highly reactive antimicrobial compounds like hypobromous acid, cyanate, chloramines, and tyrosyl and hydroxyl radicals [6-9]. These highly reactive compounds may, by promoting oxidation of low-density lipoprotein (LDL) and impairing high-density lipoprotein's (HDL's) anti-inflammatory properties and reverse-cholesterol transport functions, be considered proatherogenic [6,9,10]. Nitric oxide serves as a substrate for peroxidases, and MPO may as such serve as a catalytic sink for NO [11]. Scavenging of NO by MPO-derived oxidants may further reduce the bioavailability of this powerful vasodilator. In agreement with this notion, in patients undergoing coronary angiography, heparin was shown to liberate vessel-associated MPO, increase NO availability, and improve endothelium-dependent vascular function [3]. An inverse association between MPO serum concentrations and brachial artery flow-mediated dilation (FMD) has been observed in a hospital-based population of whom 51% had CVD [12]. However, to the best of our knowledge, the relationship between MPO and vascular function in the general population has never been assessed. Notably, the activity of MPO depends on the presence of the

* Corresponding author. Tel.: +31 204443872; fax: +31 204443895.
*E-mail address:* p.scheffer@vumc.nl (P.G. Scheffer).

0026-0495/$ – see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2010.04.012

cosubstrate hydrogen peroxide. Production of reactive oxygen species, including superoxide and its dismutation product hydrogen peroxide, is increased in diabetic vessels and is important in the pathogenesis of diabetic vascular complications [13,14]. Consequently, MPO may amplify hyperglycemia-induced endothelial dysfunction. Therefore, the aim of our study was to assess in the general population if the plasma concentration of MPO is associated with endothelium-dependent vasodilation and, if so, whether this association is modified by glucose metabolism status.

## 2. Materials and methods

### 2.1. Subjects

The present study was conducted in the Hoorn Study follow-up examination [15] and the Hoorn Screening Study [16], which are population-based studies in a white population. From the 822 participants, we excluded subjects with missing data on primary variables of interest (n = 72) and subjects using lipid-lowering medication (n = 134) because these drugs may influence MPO and FMD values [17]. In total, 616 subjects (298 men and 318 women) remained, of whom 230 had normal glucose metabolism, 159 had impaired glucose metabolism, and 227 had type 2 diabetes mellitus according to the WHO-99 criteria [18]. Abnormal glucose metabolism (n = 386) was defined as either impaired glucose metabolism or type 2 diabetes mellitus. Measurement of vascular function and withdrawal of blood samples were done after an overnight fast. The local ethics committee approved the study, and all participants gave their written informed consent.

### 2.2. Biochemical analysis

A sandwich enzyme-linked immunosorbent assay (ELISA) (Mercodia, Uppsala, Sweden) was used to determine MPO concentrations in EDTA plasma, with intra- and interassay coefficients of variation of 3.3% and 5.0%, respectively [19]. Plasma C-reactive protein (CRP) concentrations were determined with a highly sensitive in-house sandwich ELISA [20]. Circulating plasma oxidized LDL (oxLDL) was determined by competitive ELISA (Mercodia). Oxidized LDL was expressed as the oxLDL to apolipoprotein (Apo) B-100 ratio to adjust for LDL particle number [21]. Glycated hemoglobin ($HbA_{1C}$) was analyzed by ion-exchange high-performance liquid chromatography (reference range, 4.3%-6.1%) on a modular monitoring system (Bio-Rad, Veenendaal, the Netherlands). Glucose was measured enzymatically (Roche, Mannheim, Germany), and insulin was determined by a 2-site immunoradiometric assay (Medgenix Diagnostics, Fleurus, Belgium). Total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglycerides were measured by standard enzymatic methods (Roche). Low-density lipoprotein cholesterol concentration was determined with a direct method by the N-geneous assay (GenZyme, Cambridge, MA). With this method, triglyceride concentrations up to 13.5 mmol/L do not interfere with measurement of LDL cholesterol. Apolipoprotein B-100 concentrations were determined nephelometrically using an Immage 800 immunochemistry system (Beckman Coulter, Fullerton, CA).

### 2.3. Vascular properties

Ultrasound examination of the right brachial artery was performed according to the guidelines of the International Brachial Artery Reactivity Task Force [22]. Baseline diameter, blood flow (peak systolic velocity), FMD, and nitroglycerin-mediated dilation (NMD) were determined by a single observer (RMAH) as previously described [23]. The intraobserver coefficients of variation were 4.3% for diameter, 14.7% for FMD, and 10.3% for NMD, respectively. Qualitatively satisfactory ultrasound examinations were obtained in 475 individuals (188 with normal glucose metabolism and 287 with abnormal glucose metabolism). Poor definition of the arterial wall due to obesity and inability to remain motionless due to musculoskeletal disorders were the main reasons for missing ultrasound data [23].

### 2.4. Other measurements

*Microalbuminuria* was defined as urinary albumin to creatinine ratio of at least 2.0 mg/mmol. *Prior CVD* was defined as Minnesota Code 1.1 to 1.3, 4.1 to 4.3, 5.1 to 5.3, or 7.1 on the electrocardiogram; coronary bypass operation or angioplasty; an ankle-brachial blood pressure index less than 0.9 in either leg; peripheral arterial bypass; or amputation for atherosclerotic disease. The Framingham risk score was calculated [24]. Data on smoking status and on ascorbic acid, retinol, and tocopherol daily intake were obtained by questionnaire [25].

### 2.5. Statistical analysis

Data are presented as means and standard deviations or, if skewed, median and interquartile range. Skewed variables, that is, insulin, triglycerides, CRP, and vitamin intake, were $\log_e$ transformed before trend analyses and linear regression analyses. Statistical significance for linear trend was calculated by analysis of variance or by linear-by-linear $\chi^2$ tests. Differences in variables between normal glucose metabolism and abnormal glucose metabolism were tested by Mann-Whitney analyses. Associations of variables with MPO were tested by linear regression analyses adjusted for age and sex. Variables that were associated with MPO in these analyses ($P < .1$) were used as independent variables in a multivariable linear regression model to establish which variables were independently associated with MPO. In regression models for FMD, we considered age, sex, baseline diameter, and the increase in peak systolic velocity as standard correction variables. Regression models for NMD were adjusted for age, sex, baseline diameter, and glucose tolerance status. Effect modification by glucose metabolism

status was investigated by including appropriate interaction terms in the models. Data were analyzed using SPSS software, version 15 (SPSS, Chicago, IL). A 2-tailed $P$ value < .05 was considered to indicate statistical significance.

## 3. Results

### 3.1. Subjects characteristics

Subject characteristics by tertiles of MPO are shown in Table 1. Age, fasting glucose, $HbA_{1C}$, insulin, free fatty acids, CRP, waist circumference, blood pressure, antihypertensive medication, prior CVD, and Framingham risk score increased across tertiles of MPO. In line with the increasing trends of variables related to glucose metabolism, the mean MPO concentration was higher in subjects with abnormal glucose metabolism than in subjects with normal glucose metabolism (60.2 ± 19.8 vs 57.3 ± 18.2 μg/L, $P$ = .025). Higher tertiles of MPO were significantly associated with a lower daily intake of ascorbic acid (Table 1).

### 3.2. Correlates of plasma MPO concentration

Age- and sex-adjusted analyses revealed positive significant associations between MPO and age, glucose, $HbA_{1C}$, insulin, CRP, and blood pressure (Table 2). Myeloperoxidase was negatively associated with daily ascorbic acid intake. Myeloperoxidase was not significantly associated with sex, oxLDL/Apo B-100 ratio, Apo B-100, plasma lipids, serum albumin, waist circumference, smoking, retinol and tocopherol intake, microalbuminuria, and prior CVD. In a multivariable linear regression model, the only significant independent predictive variables were CRP (standardized $\beta$, 0.20; $P$ < .001) and ascorbic acid intake (standardized $\beta$, −0.11; $P$ = .007). The proportion of variance ($R^2$) explained by this model was 0.086. Separate regression models for subjects with normal and abnormal glucose metabolism yielded essentially the same results as obtained for the entire cohort (data not shown).

### 3.3. MPO and vascular function

Initial analyses showed that the relation between MPO and FMD was modified by an abnormal glucose metabolism status ($P_{interaction}$ < .05), but did not differ between subjects with impaired glucose metabolism and type 2 diabetes mellitus ($P_{interaction}$ = .50). Hence, the association between MPO and FMD was studied separately for the normal glucose metabolism group and the abnormal glucose metabolism group.

Brachial artery characteristics of the study population are shown in Table 3. At baseline and after both NMD and FMD, the brachial artery diameter was larger in subjects with abnormal glucose metabolism compared with subjects with

Table 1
Subject characteristics overall and by tertiles of plasma MPO

| Variable<br>MPO | Unit<br>μg/L | Overall | 1st tertile<br><50.6 | 2nd tertile<br>50.6-62.7 | 3rd tertile<br>>62.7 | $P_{trend}$ |
|---|---|---|---|---|---|---|
| n | | 616 | 206 | 206 | 204 | |
| Age | y | 69.0 (7.3) | 68.4 (7.0) | 68.5 (6.9) | 70.2 (7.7) | .014 |
| Sex, male | % | 48 | 54 | 42 | 49 | .32 |
| Fasting glucose | mmol/L | 6.4 (1.5) | 6.3 (1.5) | 6.3 (1.3) | 6.7 (1.7) | <.001 |
| $HbA_{1C}$ | % | 6.1 (0.8) | 5.9 (0.7) | 6.1 (0.7) | 6.2 (0.9) | <.001 |
| Insulin[a] | pmol/L | 60 (42-88) | 57 (43-78) | 59(39-86) | 67 (44-99) | .003 |
| Abnormal glucose metabolism | % | 63 | 60 | 59 | 70 | .039 |
| OxLDL/Apo B-100 | U/g | 63.4 (9.0) | 63.6 (9.1) | 62.7 (9.0) | 63.8 (9.1) | .86 |
| Apo B-100 | g/L | 1.04 (0.23) | 1.04 (0.25) | 1.04 (0.22) | 1.05 (0.23) | .89 |
| LDL cholesterol | mmol/L | 3.8 (0.9) | 3.8 (0.9) | 3.8 (0.8) | 3.7 (0.9) | .67 |
| HDL cholesterol | mmol/L | 1.39 (0.41) | 1.35 (0.40) | 1.47 (0.42) | 1.35 (0.39) | .96 |
| Triglycerides[a] | mmol/L | 1.3 (1.0-1.8) | 1.3 (1.0-1.7) | 1.3 (1.0-1.7) | 1.4 (1.0-2.0) | .10 |
| Free fatty acids | mmol/L | 0.56 (0.24) | 0.53 (0.21) | 0.59 (0.24) | 0.58 (0.26) | .034 |
| CRP[a] | mg/L | 2.3 (1.1-4.8) | 1.7 (0.9-2.9) | 1.9 (1.1-4.3) | 3.8 (2.0-7.3) | <.001 |
| Serum albumin | g/L | 41.5 (2.9) | 41.6 (2.6) | 41.7 (3.1) | 41.2 (3.0) | .18 |
| Waist circumference | cm | 96 (13) | 94 (11) | 96 (12) | 98 (14) | .007 |
| Systolic blood pressure | mm Hg | 142 (21) | 140 (20) | 141 (21) | 146 (21) | .002 |
| Diastolic blood pressure | mm Hg | 83 (11) | 82 (10) | 83 (11) | 84 (11) | .029 |
| Antihypertensives | % | 33 | 29 | 29 | 43 | .003 |
| Current smoking | % | 16 | 11 | 21 | 17 | .12 |
| Retinol intake[a] | mg/d | 541 (393-869) | 581 (392-904) | 549 (412-878) | 496 (366-766) | .34 |
| Ascorbic acid intake[a] | mg/d | 97 (72-132) | 107 (79-144) | 96 (72-134) | 89 (68-124) | .001 |
| Tocopherol intake[a] | mg/d | 10.9 (8.3-14.6) | 11.2 (8.6-14.9) | 11.1 (8.5-14.4) | 9.9 (7.6-14.5) | .050 |
| Microalbuminuria | % | 15 | 12 | 13 | 19 | .06 |
| Prior CVD | % | 45 | 42 | 40 | 52 | .042 |
| Framingham risk score | | 10.1 (3.5) | 9.7 (3.3) | 9.9 (3.4) | 10.7 (3.8) | .005 |

Values are displayed as means (SD), medians (interquartile range), or percentages. $P$ for trend values were age and sex adjusted.

[a] Variables were $\log_e$ transformed before linear trend analysis.

Table 2
Age- and sex-adjusted associations with MPO

| Variable | Unit | Unstandardized $\beta$ | $P$ value |
|---|---|---|---|
| Age | y | 0.29 (0.08 to 0.50) | .006 |
| Sex | Male vs female | 0.02 (−3.02 to 3.07) | .99 |
| Fasting glucose | mmol/L | 1.30 (0.32 to 2.22) | .010 |
| $HbA_{1C}$ | % | 2.94 (0.97 to 4.91) | .003 |
| Insulin[a] | pmol/L | 3.26 (0.45 to 6.07) | .023 |
| Abnormal glucose metabolism | Yes vs no | 2.72 (−0.42 to 5.86) | .09 |
| OxLDL/Apo B-100 | U/g | −0.01 (−0.17 to 0.16) | .96 |
| Apo B-100 | g/L | −1.46 (−8.06 to 5.13) | .66 |
| LDL cholesterol | mmol/L | −0.71 (−2.46 to 1.05) | .43 |
| HDL cholesterol | mmol/L | −0.32 (−4.40 to 3.75) | .88 |
| Triglycerides[a] | mmol/L | 0.63 (−2.72 to 3.98) | .71 |
| Free fatty acids | mmol/L | 5.32 (−1.49 to 12.12) | .13 |
| CRP[a] | mg/L | 3.91 (2.59 to 5.22) | <.001 |
| Serum albumin | g/L | −0.28 (−0.82 to 0.25) | .30 |
| Waist circumference | cm | 0.13 (−0.003 to 0.25) | .057 |
| Systolic blood pressure | mm Hg | 0.09 (0.02 to 0.17) | .019 |
| Diastolic blood pressure | mm Hg | 0.17 (0.03 to 0.31) | .016 |
| Current smoking | Yes vs no | 2.86 (−1.28 to 7.00) | .18 |
| Retinol intake[a] | mg/d | −0.57 (−3.21 to 2.08) | .67 |
| Ascorbic acid intake[a] | mg/d | −5.22 (−8.55 to −1.90) | .002 |
| Tocopherol intake[a] | mg/d | −2.30 (−6.03 to 1.43) | .23 |
| Microalbuminuria | Yes vs no | −1.74 (−6.11 to 2.64) | .44 |
| Prior CVD | Yes vs no | 1.92 (−1.29 to 5.13) | .24 |

Age-and sex-adjusted regression coefficients are expressed as change in MPO (in micrograms per liter) per unit increase of the variable under consideration.
[a] Skewed variables were $\log_e$ transformed before analysis.

normal glucose metabolism. Absolute and relative changes in diameter after FMD, but not after NMD, were significantly smaller in individuals with abnormal glucose metabolism. Peak systolic velocity was higher at baseline, whereas the relative increase after reactive hyperemia was lower, in the abnormal glucose metabolism group.

Table 3
Brachial artery characteristics according to glucose tolerance status

| | Normal glucose metabolism (n = 188) | Abnormal glucose metabolism (n = 287) | $P$ value |
|---|---|---|---|
| *Diameter (μm)* | | | |
| Baseline | 4520 (759) | 4723 (741) | .002 |
| After FMD | 4713 (749) | 4888 (748) | .008 |
| After NMD | 4952 (773) | 5158 (733) | .003 |
| *Absolute change in diameter (μm)* | | | |
| After FMD | 194 (128) | 166 (162) | <.001 |
| After NMD | 448 (197) | 445 (223) | .69 |
| *Percentage change in diameter (%)* | | | |
| After FMD | 4.49 (3.13) | 3.62 (3.63) | <.001 |
| After NMD | 10.31 (5.30) | 9.85 (6.05) | .21 |
| *Peak systolic velocity (cm/s)* | | | |
| Baseline | 56 (12) | 59 (13) | .007 |
| After FMD | 107 (29) | 103 (22) | .55 |
| % Increase | 94 (49) | 81 (40) | .005 |

Values are expressed as mean (SD). Statistical significance was tested by Mann-Whitney analyses.

In the normal glucose metabolism group, mean FMD values did not significantly differ between subjects with MPO concentrations less than or greater than the median value (Fig. 1A). In contrast, FMD was significantly decreased at high MPO concentrations in the abnormal glucose metabolism group (Fig. 1B). The association between MPO and FMD was studied with multivariable linear regression analyses (Table 4). A standard model including age, sex, peak flow velocity increase, and baseline diameter revealed a significant negative association between MPO and FMD in subjects with abnormal glucose metabolism ($\beta$, −20.9 [95% confidence interval {CI}, −41.7 to −0.2] -$\mu$m change in FMD per 1-SD increment of MPO), but not in the normal glucose metabolism group ($P$ = .83). Myeloperoxidase and NMD were not significantly associated ($P$ = .11), and this association was not modified by glucose metabolism status ($P_{interaction}$ = .27). In the group with abnormal glucose metabolism, the association between MPO and FMD remained significant after adjustment for endothelium-independent vasodilation by including NMD in the model ($\beta$, −31.1 [95% CI, −50.0 to −12.3]) (Table 4, model 1). Further adjustment for mean arterial pressure (Table 4, model 2) did not alter this association. In addition, none of the other variables tested (CRP, ascorbic acid intake, Framingham risk score, waist circumference, prior CVD, or microalbuminuria) attenuated the association between MPO and FMD in individuals with abnormal glucose metabolism.

### 3.4. Accessory analyses

Because CRP and ascorbic acid intake were both independent correlates of MPO, both variables might, either directly or through their association with MPO, be associated with FMD. To gain more insight in these associations, we explored additional linear regression models that were stratified for glucose metabolism status and adjusted for age, sex, baseline diameter, peak flow velocity increase, mean arterial blood pressure, and NMD. In these models, MPO and CRP were negatively and ascorbic acid intake was positively associated with FMD in subjects with abnormal glucose metabolism, but not in individuals with normal glucose metabolism (Fig. 2, open bars). These associations were slightly attenuated after further mutual adjustment for the other variables of interest (MPO, CRP, and ascorbic acid intake). However, the negative association between MPO and FMD as well as the positive association between ascorbic acid intake and FMD in subjects with abnormal glucose metabolism remained significant (Fig. 2, solid bars). In contrast, mutual adjustment rendered the association between CRP and FMD nonsignificant.

## 4. Discussion

Myeloperoxidase plasma concentrations were elevated and FMD was decreased in subjects with an abnormal

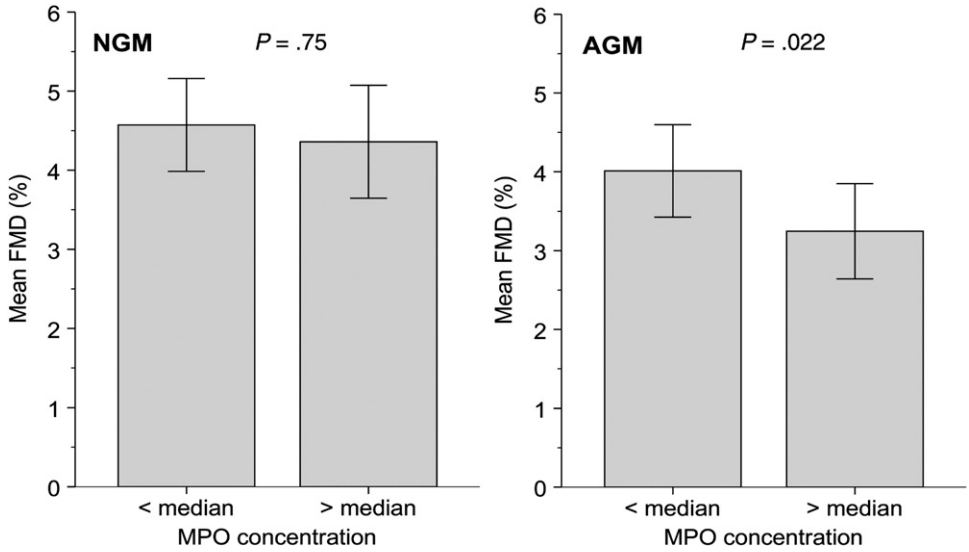


Fig. 1. Flow-mediated dilation of the brachial artery according to low (less than the median [55.3 μg/L] of the entire cohort) or high (greater than the median) concentration of MPO in subjects with normal glucose metabolism (NGM) or abnormal glucose metabolism (AGM), that is, impaired glucose metabolism and type 2 diabetes mellitus diabetes. Flow-mediated dilation data are expressed as percentage change in brachial diameter and presented as means with 95% CI. Significance of differences was tested by Mann-Whitney analysis.

glucose metabolism. Myeloperoxidase was significantly inversely associated with endothelium-dependent vasodilation in individuals with abnormal glucose metabolism, but not in those with normal glucose metabolism.

Glucose metabolism parameters were associated with MPO concentrations. Furthermore, CRP, a marker of inflammation, was an independent correlate of MPO. These findings are in agreement with previous observations of increased inflammation in impaired glucose metabolism and type 2 diabetes mellitus [26], and elevated levels of the MPO cosubstrate hydrogen peroxide at high glucose concentrations [13]. Hence, MPO, an enzyme that converts hydrogen peroxide to even more reactive compounds, may be particularly detrimental to the vascular endothelium in the abnormal glucose metabolism group.

The role of vitamin consumption in preventing CVD and influencing plasma MPO concentrations has only partially been elucidated. Ascorbic acid can inhibit and reverse hypochlorous acid–induced chlorination of LDL in vitro [27] and protect against NO scavenging [28]. In the present study, a significant inverse independent association was found between MPO and intake of ascorbic acid. This association was not observed with both other vitamins.

Myeloperoxidase was inversely associated with FMD, independent of NMD, but only in subjects with abnormal glucose metabolism. Flow-mediated dilation reflects "total" vascular function, whereas NMD is thought to reflect the proportion of vascular function that does not depend on endothelium-derived vasodilators. None of the traditional risk factors, including microalbuminuria and prior CVD,

Table 4
Multivariable regression models for the relation between MPO and FMD (dependent variable) according to glucose tolerance status

| Model | Normal glucose metabolism | | Abnormal glucose metabolism | |
|---|---|---|---|---|
| | β (95% CI) | P value | β (95% CI) | P value |
| Standard model | 2.0 (−16.0 to 20.0) | .83 | −20.9 (−41.7 to −0.2) | .048 |
| Model 1: standard model + NMD | 0.2 (−16.0 to 16.5) | .98 | −31.1 (−50.0 to −12.3) | .001 |
| Model 2: model 1 + mean arterial pressure | 0.8 (−15.4 to 17.0) | .92 | −28.8 (−47.9 to −9.7) | .003 |
| Model 2 + CRP[a] | 1.8 (−15.0 to 18.6) | .83 | −25.4 (−45.2 to −5.6) | .012 |
| Model 2 + ascorbic acid intake[a] | 0.6 (−16.1 to 17.3) | .95 | −25.6 (−44.5 to −6.6) | .008 |
| Model 2 + Framingham risk score | 1.0 (−15.2 to 17.2) | .91 | −25.5 (−44.2 to −6.7) | .008 |
| Model 2 + waist circumference | 0.2 (−15.9 to 16.3) | .98 | −26.8 (−46.0 to −7.6) | .006 |
| Model 2 + prior CVD | 0.9 (−15.5 to 17.2) | .92 | −28.9 (−48.1 to −9.6) | .003 |
| Model 2 + microalbuminuria | 1.0 (−15.2 to 17.2) | .90 | −28.0 (−47.0 to −8.9) | .004 |

Regression coefficients are expressed as absolute change in diameter (in micrometers) per 1-SD (19.3 μg/L) increment of MPO. Standard model: MPO + age, sex, peak flow velocity increase, and baseline diameter.

[a] Skewed variables were $\log_e$ transformed before linear regression analyses.

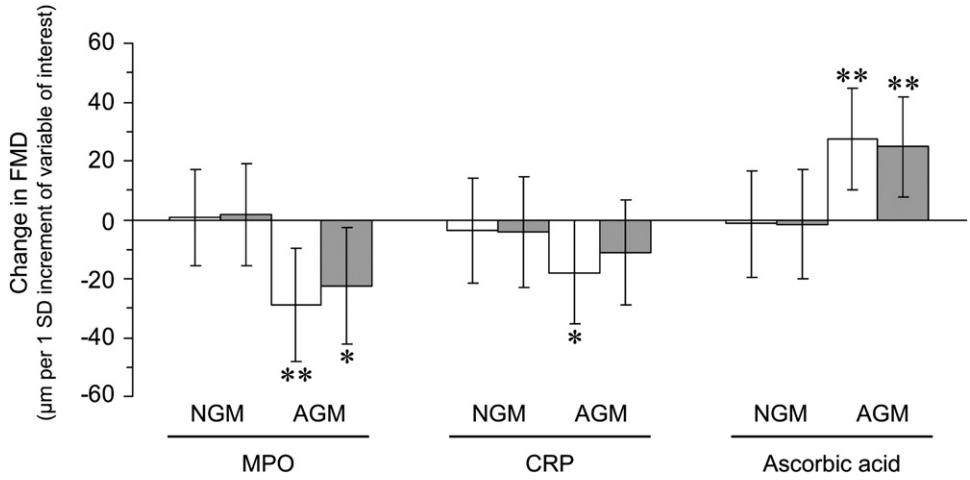


Fig. 2. Changes in absolute FMD (95% CI) per SD increment in plasma concentrations of MPO, CRP, and ascorbic acid intake (the latter two after $\log_e$ transformation) in subjects with normal glucose metabolism (NGM) and abnormal glucose metabolism (AGM). Open bars represent the association of the variable of interest (MPO, CRP, or ascorbic acid consumption) with FMD, adjusted for age, sex, baseline diameter, pulse wave velocity increase, mean arterial blood pressure, and NMD. Solid bars represent the association of the variable of interest (MPO, CRP, or ascorbic acid) with FMD after additional adjustment for both other variables of interest. $^{*}P < .05$ and $^{**}P < .01$.

altered the strength of the relation between MPO and FMD. Taken together, these results strongly suggest that MPO is an independent correlate of endothelium-dependent vascular function in persons with abnormal glucose metabolism.

A positive association between ascorbic acid and FMD was anticipated because it has been shown that ascorbic acid protects constituents of the artery wall from oxidation by HOCl [29]. In addition, improvement of FMD after both acute and chronic ascorbic acid administration has been reported in patients with coronary artery disease [30] and chronic heart failure [31] and in insulin resistant subjects and smokers [32], although such an improvement was not found in healthy subjects. In line with these reports, we found a positive association between ascorbic acid intake and endothelium-dependent vascular function, but only in individuals with abnormal glucose metabolism. The negative association between ascorbic acid intake and MPO concentration in the present study suggests that lowering of MPO may be one of the routes by which ascorbic acid reduces oxidative stress and improves vascular function in subjects with abnormal glucose metabolism. On the other hand, ascorbic acid intake and MPO were both independent correlates of FMD and remained so after mutual adjustment, indicating that ascorbic acid may improve vascular function by both MPO-dependent and MPO-independent mechanisms.

C-reactive protein is known to stimulate polymorphonuclear cells to release MPO [33]. In the present study, CRP was, like ascorbic acid intake, an independent correlate of plasma MPO concentration and was associated with FMD in individuals with abnormal glucose metabolism. However, the association between CRP and FMD was weaker and less robust in comparison with the association between ascorbic acid intake and FMD, and lost significance after mutual adjustment. This is in agreement with previous studies in which associations between CRP and FMD were either nonsignificant [34] or modest and rendered nonsignificant after adjustment for traditional risk factors [12]. Both inflammation and oxidative stress may be involved in impairment of endothelium-dependent vascular function. The fact that MPO is a marker of both inflammation and oxidative stress, whereas CRP only reflects inflammation, may therefore explain the stronger and more robust association of MPO with FMD. Local release and retention of MPO in the vasculature, as contrasted to CRP that is mainly of hepatic origin, may also contribute to a stronger association of MPO with FMD.

Our study had some limitations. First, the study population was limited to elderly subjects; and because atherosclerosis progresses with age, the results may be different in other age groups. A second limitation is that vitamin intake was assessed by questionnaire, and it is conceivable that measurement of ascorbic acid in plasma would have revealed a more pronounced association with MPO. Third, because MPO may be released from activated leukocytes in serum and heparin plasma during sample handling, MPO values differ considerably between serum, heparin plasma, and EDTA plasma [35,36]. EDTA plasma was our choice because it may best reflect circulating concentrations of MPO. It should be noted, however, that measurement of MPO in serum or heparin plasma or even whole blood would have provided other, but potentially equally valuable, information because it probably better reflects the total reservoir of MPO. Major strengths of our study include the large number of subjects and the availability of a wide array of clinical and biochemical variables to control for potential confounding.

In summary, in individuals with abnormal glucose metabolism, MPO was elevated and independently inversely associated with endothelium-dependent vasodilation.

## References


[1] Zhang R, Brennan ML, Fu X, Aviles RJ, Pearce GL, Penn MS, et al. Association between myeloperoxidase levels and risk of coronary artery disease. JAMA 2001;286:2136-42.

[2] Brennan ML, Penn MS, Van Lente F, Nambi V, Shishehbor MH, Aviles RJ, et al. Prognostic value of myeloperoxidase in patients with chest pain. N Engl J Med 2003;349:1595-604.

[3] Baldus S, Rudolph V, Roiss M, Ito WD, Rudolph TK, Eiserich JP, et al. Heparins increase endothelial nitric oxide bioavailability by liberating vessel-immobilized myeloperoxidase. Circulation 2006;113:1871-8.

[4] Mocatta TJ, Pilbrow AP, Cameron VA, Senthilmohan R, Frampton CM, Richards AM, et al. Plasma concentrations of myeloperoxidase predict mortality after myocardial infarction. J Am Coll Cardiol 2007; 49:1993-2000.

[5] Brevetti G, Schiano V, Laurenzano E, Giugliano G, Petretta M, Scopacasa F, et al. Myeloperoxidase, but not C-reactive protein, predicts cardiovascular risk in peripheral arterial disease. Eur Heart J 2008;29:224-30.

[6] Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005; 77:598-625.

[7] Miller RA, Britigan BE. Role of oxidants in microbial pathophysiology. Clin Microbiol Rev 1997;10:1-18.

[8] Wang Z, Nicholls SJ, Rodriguez ER, Kummu O, Horkko S, Barnard J, et al. Protein carbamylation links inflammation, smoking, uremia and atherogenesis. Nat Med 2007;13:1176-84.

[9] Schindhelm RK, van der Zwan LP, Teerlink T, Scheffer PG. Myeloperoxidase: a useful biomarker for cardiovascular disease risk stratification? Clin Chem 2009;55:1462-70.

[10] Shao B, Oda MN, Oram JF, Heinecke JW. Myeloperoxidase: an inflammatory enzyme for generating dysfunctional high density lipoprotein. Curr Opin Cardiol 2006;21:322-8.

[11] Abu-Soud HM, Hazen SL. Nitric oxide is a physiological substrate for mammalian peroxidases. J Biol Chem 2000;275:37524-32.

[12] Vita JA, Brennan ML, Gokce N, Mann SA, Goormastic M, Shishehbor MH, et al. Serum myeloperoxidase levels independently predict endothelial dysfunction in humans. Circulation 2004;110:1134-9.

[13] Zhang C, Yang J, Jennings LK. Leukocyte-derived myeloperoxidase amplifies high-glucose–induced endothelial dysfunction through interaction with high-glucose–stimulated, vascular non–leukocyte-derived reactive oxygen species. Diabetes 2004;53:2950-9.

[14] Guzik TJ, Mussa S, Gastaldi D, Sadowski J, Ratnatunga C, Pillai R, et al. Mechanisms of increased vascular superoxide production in human diabetes mellitus: role of NAD(P)H oxidase and endothelial nitric oxide synthase. Circulation 2002;105:1656-62.

[15] Mooy JM, Grootenhuis PA, De Vries H, Valkenburg HA, Bouter LM, Kostense PJ, et al. Prevalence and determinants of glucose intolerance in a Dutch Caucasian population. The Hoorn Study. Diabetes Care 1995;18:1270-3.

[16] Spijkerman AM, Adriaanse MC, Dekker JM, Nijpels G, Stehouwer CD, Bouter LM, et al. Diabetic patients detected by population-based stepwise screening already have a diabetic cardiovascular risk profile. Diabetes Care 2002;25:1784-9.

[17] Liu PY, Liu YW, Lin LJ, Chen JH, Liao JK. Evidence for statin pleiotropy in humans: differential effects of statins and ezetimibe on rho-associated coiled-coil containing protein kinase activity, endothelial function, and inflammation. Circulation 2009;119:131-8.

[18] Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 1998;15:539-53.

[19] Schindhelm RK, Alssema M, Diamant M, Teerlink T, Dekker JM, Kok A, et al. Comparison of two consecutive fat-rich and carbohydrate-rich meals on postprandial myeloperoxidase response in women with and without type 2 diabetes mellitus. Metabolism 2008;57:262-7.

[20] Grooteman MP, Gritters M, Wauters IM, Schalkwijk CG, Stam F, Twisk J, et al. Patient characteristics rather than the type of dialyser predict the variability of endothelial derived surface molecules in chronic haemodialysis patients. Nephrol Dial Transplant 2005;20: 2751-8.

[21] Van der Zwan LP, Teerlink T, Dekker JM, Henry RM, Stehouwer CD, Jakobs C, et al. Circulating oxidized LDL: determinants and association with brachial flow-mediated dilation. J Lipid Res 2009;50:342-9.

[22] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force. J Am Coll Cardiol 2002;39:257-65.

[23] Henry RM, Ferreira I, Kostense PJ, Dekker JM, Nijpels G, Heine RJ, et al. Type 2 diabetes is associated with impaired endothelium-dependent, flow-mediated dilation, but impaired glucose metabolism is not. The Hoorn Study. Atherosclerosis 2004;174:49-56.

[24] Wilson PW, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. Circulation 1998;97:1837-47.

[25] Ocke MC, Bueno-de-Mesquita HB, Pols MA, Smit HA, van Staveren WA, Kromhout D. The Dutch EPIC food frequency questionnaire. II. Relative validity and reproducibility for nutrients. Int J Epidemiol 1997;26(Suppl 1):S49-S58.

[26] Muntner P, He J, Chen J, Fonseca V, Whelton PK. Prevalence of non-traditional cardiovascular disease risk factors among persons with impaired fasting glucose, impaired glucose tolerance, diabetes, and the metabolic syndrome: analysis of the Third National Health and Nutrition Examination Survey (NHANES III). Ann Epidemiol 2004; 14:686-95.

[27] Carr AC, McCall MR, Frei B. Oxidation of LDL by myeloperoxidase and reactive nitrogen species: reaction pathways and antioxidant protection. Arterioscler Thromb Vasc Biol 2000;20:1716-23.

[28] Heller R, Werner-Felmayer G, Werner ER. Antioxidants and endothelial nitric oxide synthesis. Eur J Clin Pharmacol 2005;62:21-8.

[29] Jenner AM, Ruiz JE, Dunster C, Halliwell B, Mann GE, Siow RC. Vitamin C protects against hypochlorous acid–induced glutathione depletion and DNA base and protein damage in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 2002;22:574-80.

[30] Gokce N, Keaney Jr JF, Frei B, Holbrook M, Olesiak M, Zachariah BJ, et al. Long-term ascorbic acid administration reverses endothelial vasomotor dysfunction in patients with coronary artery disease. Circulation 1999;99:3234-40.

[31] Ellis GR, Anderson RA, Lang D, Blackman DJ, Morris RH, Morris-Thurgood J, et al. Neutrophil superoxide anion–generating capacity, endothelial function and oxidative stress in chronic heart failure: effects of short- and long-term vitamin C therapy. J Am Coll Cardiol 2000;36:1474-82.

[32] Hirai N, Kawano H, Hirashima O, Motoyama T, Moriyama Y, Sakamoto T, et al. Insulin resistance and endothelial dysfunction in smokers: effects of vitamin C. Am J Physiol Heart Circ Physiol 2000; 279:H1172-8.

[33] Singh U, Devaraj S, Jialal I. C-reactive protein stimulates myeloperoxidase release from polymorphonuclear cells and monocytes: implications for acute coronary syndromes. Clin Chem 2009; 55:361-4.

[34] Lind L, Siegbahn A, Hulthe J, Elmgren A. C-reactive protein and e-selectin levels are related to vasodilation in resistance, but not conductance arteries in the elderly: the prospective investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Atherosclerosis 2008; 199:129-37.

[35] Chang PY, Wu TL, Hung CC, Tsao KC, Sun CF, Wu LL, et al. Development of an ELISA for myeloperoxidase on microplate: normal reference values and effect of temperature on specimen preparation. Clin Chim Acta 2006;373:158-63.

[36] Scheffer PG, van der Zwan LP, Schindhelm RK, Vermue HP, Teerlink T. Myeloperoxidase concentrations in EDTA-plasma of healthy subjects are discordant with concentrations in heparin-plasma and serum. Clin Biochem 2009;42:1490-2.