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Neutrophil Release of Myeloperoxidase in Systemic Vasculature of Obese Women May Put Them at Risk for Preeclampsia

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Abstract

Obesity is a risk factor for preeclampsia, but the reason for this risk is unknown. Neutrophils infiltrate into systemic blood vessels of both obese and preeclamptic women. Neutrophils are a major source of myeloperoxidase (MPO), which is associated with hypertension. We tested the hypothesis that systemic vasculature of both obese and preeclamptic women will have a significant presence of MPO as a result of neutrophil infiltration. We found that immunohistochemical staining of MPO was significantly greater in subcutaneous fat blood vessels of obese women than overweight women, which was significantly greater than normal weight women. Expression of MPO was significantly greater in maternal blood vessels of preeclamptic women than normal pregnant or normal nonpregnant women. In general, when vessels of overweight or normal pregnant women were stained it was primarily for leukocytes in the lumen and not infiltrated into the vessel. In contrast, in obese and preeclamptic women staining was present for leukocytes in the lumen, flattened, and adhered to the endothelium and infiltrated into the vessel wall. There was also extensive diffuse staining for MPO in vessels of obese and preeclamptic women. In conclusion, both obese and preeclamptic women have increased presence of MPO in systemic vasculature as a result of neutrophil infiltration. We speculate that obese women may be at risk of preeclampsia because their vasculature is already prone to hypertension.

Keywords

preeclampsia, obesity, myeloperoxidase, neutrophils, hypertension

Introduction

Obesity is a risk factor for preeclampsia,[1-8] but the mechanisms involved are not known. The risk of preeclampsia rises strikingly with an increase in prepregnancy body mass index (BMI, kg/m^2). Compared to women with a BMI of 21, the risk of preeclampsia doubles at a BMI of 26, triples at a BMI of 30, and increases further with severe obesity.[1,6,8] Gaining weight between pregnancies also increases the risk of developing preeclampsia in the next pregnancy.[9] Even for women of normal weight, gaining weight between pregnancies increases their risk of preeclampsia, whereas losing weight decreases their risk.[9] Women with the lowest BMI are relatively protected against preeclampsia,[10] whereas those with the highest BMI are at increased risk of severe preeclampsia.[8,11]

Myeloperoxidase (MPO) is a lysosomal enzyme involved with host defense present in azurophilic granules of neutrophils. In the presence of halogens, specifically chloride anion, MPO produces hypochlorous acid from hydrogen peroxide during the neutrophil's respiratory burst as they infiltrate tissue. Hypochlorous acid is a potent cytotoxic oxidant, which is used by neutrophils to kill bacteria, however neutrophil release of MPO can also affect the integrity of the blood vessel wall. In addition, MPO reduces the bioavailability of the endogenous vasodilator nitric oxide,[12-14] and so can lead to hypertension. In fact serum levels of MPO, which is secreted by neutrophils, are inversely related to endothelium-dependent dilation, and impaired endothelium-dependent dilation is related to higher neutrophil counts in the circulation.[15] Elevated levels of MPO are positively associated with blood pressure and this association is strongest in patients with oxidative stress.[16] Preeclampsia is associated with oxidative stress,[17-19] and increased levels of MPO are present in the placenta and maternal circulation,[20] so MPO is a candidate for causing hypertension.

Neutrophils are a major source of MPO in the circulation, and normal pregnancy is characterized by leukocytosis caused by proliferation of neutrophils in the second and third trimesters. The number of neutrophils increases 2.5-fold by 30 weeks of gestation in normal pregnancy[21] and the number

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Table 1. Demographic Data for Nonpregnant Patients.[a]

	Normal Weight (N = 5)	Overweight (N = 5)	Obese (N = 5)
Age, year	41.0 ± 10.3	37.8 ± 8.5	35.8 ± 6.3
BMI, kg/m^2	22.9 ± 1.74	27.4 ± 1.26[b]	33.7 ± 5.00[c,d]
Systolic blood pressure, mm Hg	111.0 ± 8.7	130.0 ± 16.0	115.0 ± 15.8
Diastolic blood pressure, mm Hg	60.4 ± 6.23	83.0 ± 14.6[b]	69.0 ± 10.7
Race			
White	2	3	2
Black	2	2	3
Other	1		

Abbreviations: BMI, body mass index; SD, standard deviation.
[a]Values are mean ± SD.
[b]$P < .05$ compared to normal weight.
[c]$P < .001$ compared to normal weight.
[d]$P < .01$ compared to overweight.

increases further in preeclampsia.[22] For neutrophils to manifest their inflammatory effects, they need to infiltrate tissue. In 2004, we reported the first direct evidence of extensive neutrophil infiltration into the maternal systemic microcirculation in women with preeclampsia[23] and, although all classes of leukocytes are activated,[24,25] vascular infiltration was restricted to neutrophils.[26,27] Neutrophil infiltration was associated with a significant increase in inflammatory markers in the mother's blood vessels, for example, interleukin 8, cyclooxygenase 2 (COX-2), nuclear factor-κ B, and thromboxane synthase,[23,28,29] which could have been caused by neutrophil release of MPO.

Obesity and preeclampsia share many common features, such as inflammation, hypertension, and oxidative stress,[30] which may be related to vascular infiltration of neutrophils and their release of MPO. If so, this might help explain why obesity is a risk factor for preeclampsia. In this study, we used immunohistochemical staining to evaluate the vascular expression of MPO in obese and preeclamptic women. We hypothesized that systemic vasculature of both obese and preeclamptic women will have a significant presence of MPO as a result of neutrophil infiltration.

Materials and Methods

Study Participants

Subcutaneous fat was obtained at abdominal surgery from nonpregnant and pregnant women at MCV Hospitals, Virginia Commonwealth University Medical Center. Subcutaneous fat blood vessels were studied because they are representative of systemic blood vessels and they contribute to total peripheral vascular resistance. Fat samples (approximately 2 cm × 2 cm × 2 cm) were collected at the time of abdominal surgery at the incision site from nonpregnant women undergoing removal of fibroids or tissue biopsies, and from pregnant women undergoing cesarean section. Cesarean sections for normal pregnant women were performed because of previous cesarean section or secondary to latent herpes simplex virus or fetal malposition. Nonpregnant patients were categorized by weight into the following groups: normal weight (BMI 18.5-24.9), overweight (BMI 25-29.9), and obese (BMI ≥ 30). Preeclampsia was defined as sustained blood pressure of ≥140/90 mm Hg with readings at least 6 hours apart and proteinuria (≥300 mg/24 h or ≥ 1+ urine dipstick). Blood pressure was recorded for each patient at preoperation admittance by surgery registered nurses. Exclusion criteria for nonpregnant patients included infection, active sexually transmitted diseases (STDs), diabetes, and inflammatory disease and for pregnant patients included chorioamnionitis, maternal infection, active STDs, diabetes, and smoking because these conditions could impact vascular inflammation. Pregnant patients were matched for BMI and were not in labor. Informed consent was obtained prior to surgery. The Office of Research Subjects Protection of Virginia Commonwealth University approved this study.

Immunohistochemistry

Tissues were formalin-fixed, paraffin embedded and cut into 8 μm sections as previously described.[23,28,31] Heat citrate buffer antigen retrieval was used. Tissues were immunostained with a prediluted polyclonal rabbit immunoglobulin (Ig) G antibody specific for MPO using SuperPicTure Kit with diaminobenzidine, which results in a brown stain (Life Technologies, Grand Island, New York). A mouse IgM antihuman monoclonal antibody specific for CD66b (1:50; BD BioSciences, San Diego, California) was used to identify neutrophils. Negative controls were stained with a rabbit IgG negative control prediluted in phosphate-buffered saline (Life Technologies). Slides were counterstained with hematoxylin. The staining protocol was the same for all samples with regard to processing, incubation times, and temperature.

Data Analysis

Slides were analyzed with an Olympus BH2 microscope attached to a digital camera (Olympus QColor5) using image analysis software (cellSens, Olympus America, Center Valley, Pennsylvania). Vessels between 10 μm and 200 μm were analyzed as representative of resistance-sized blood vessels. Each slide was analyzed blindly, meaning that the observer was unaware of the patient group. For each patient, an average of 36

Table 2. Demographic Data for Pregnant Patients.

	Normal Nonpregnant (N = 5)	Normal Pregnant (N = 5)	Preeclamptic (N = 5)
Maternal age, year	39.6 ± 4.7[a]	24.8 ± 5.4	26.0 ± 4.6
Prepregnancy BMI, kg/m^2	26.4 ± 2.7	29.4 ± 4.5	29.9 ± 5.5
Systolic blood pressure, mm Hg	128.2 ± 16.8	120.8 ± 13.8	163.4 ± 9.0[a]
Diastolic blood pressure, mm Hg	80.8 ± 20.5	64.0 ± 6.1	103.0 ± 15.5[b]
Proteinuria, mg/24 h	NA	ND	232.0 ± 96.9 (N = 2)
Protein urinary dipstick	NA	ND	3.0 ± 1.0 (N = 3)
Parity	NA	1.8 ± 0.8	0.4 ± 0.5
Gestational age, week	NA	39.8 ± 1.0	35.6 ± 3.0[b]
Infant birth weight, g	NA	3618 ± 461	1730 ± 1129[b]
Race			
White	3	2	1
Black	2	2	4
Hispanic		1	

Abbreviations: BMI, body mass index; NA, not applicable; ND, not determined; SD, standard deviation.
[a]Values are mean ± SD.
[a]$P < .001$ compared to other groups.
[b]$P < .01$ compared to normal pregnant.

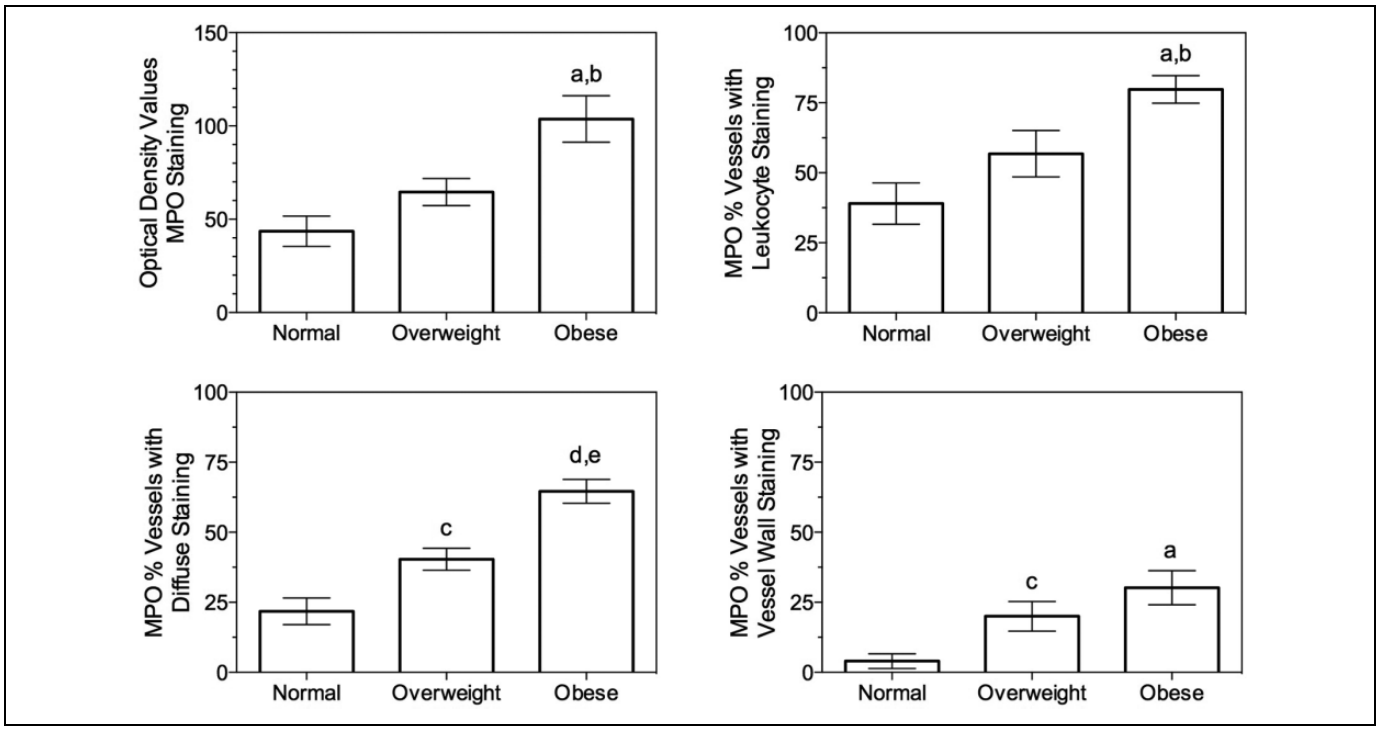

Figure 1. Optical density of myeloperoxidase (MPO) staining and percentage of vessels with leukocyte staining, diffuse staining, and vessel wall staining for normal weight, overweight, and obese nonpregnant women. In all cases, staining for MPO increased according to weight categories with the greatest amount of staining present for obese women. a, $P < .01$ compared to normal. b, $P < .05$ compared to overweight. c, $P < .05$ compared to normal. d, $P < .001$ compared normal. e, $P < .01$ compared to overweight.

vessels was evaluated. Vessels were evaluated for percentage of vessels with leukocyte staining, diffuse staining, and vessel wall staining, as well as optical density. Optical density was determined with the closed polygon tool of the analysis software (cellSens, Olympus). The mean gray intensity value measurement was used and adjusted so 0 = white and 255 = black. A consistent length of 20 µm was used for each vessel so that a vessel with only 1 leukocyte would have a lower density than a vessel with many leukocytes and/or diffuse staining. Circular, circumscribed staining identified leukocytes. Diffuse staining represented larger, noncircumscribed areas of staining.

Statistical Analysis

Patient demographic data, density of staining, and percentage of vessels stained were analyzed by one-way analysis of variance with Newman-Keuls post hoc test. Bar graph data are reported as mean ± standard error. A probability level of

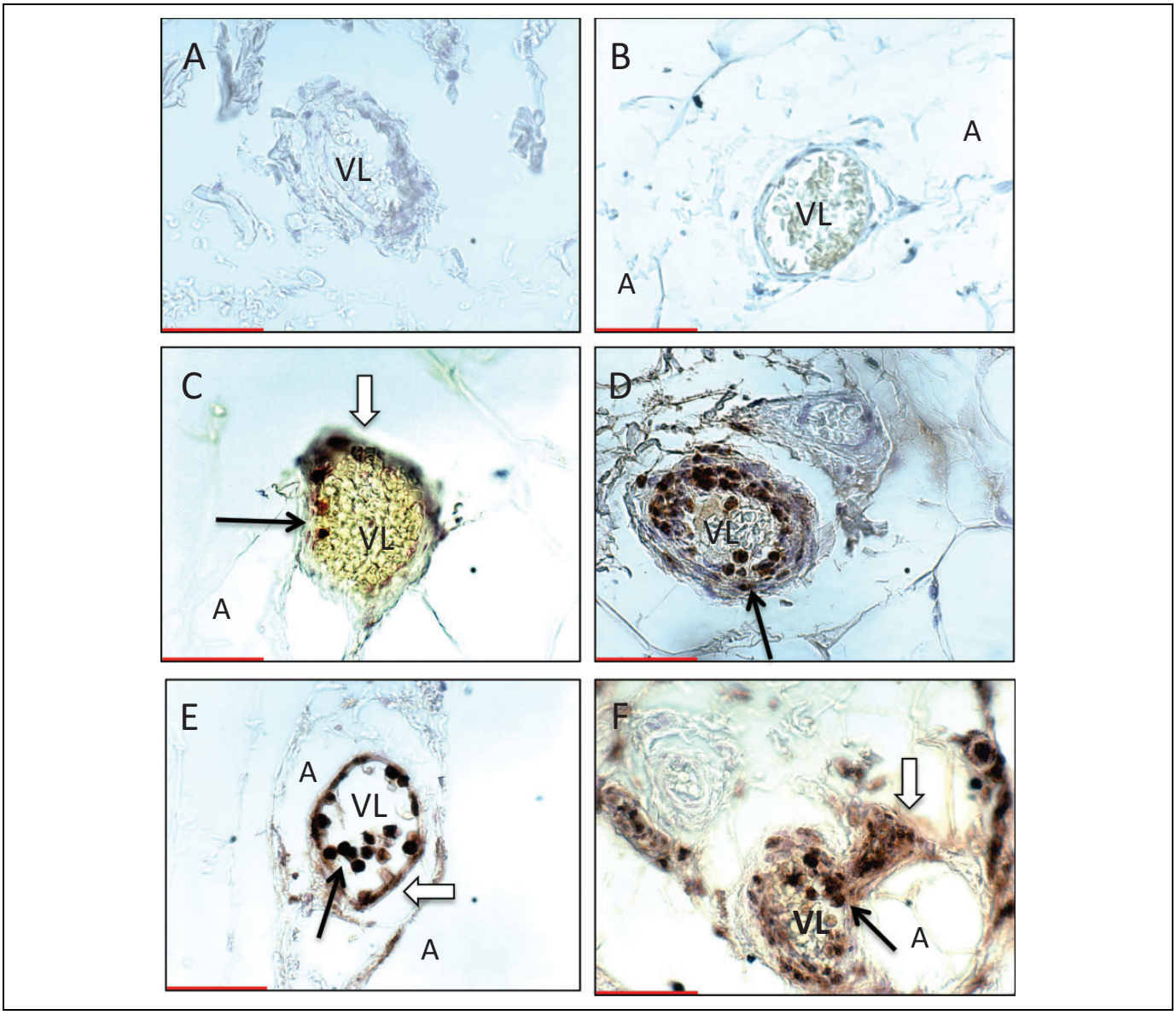

Figure 2. Representative examples of MPO staining in subcutaneous vessels of normal weight, overweight, and obese women. Expression of MPO was significantly greater in the systemic vasculature of obese women (Panels D-F) than in overweight women (Panel C), which was significantly greater than that of normal weight women (Panel B). While overweight women showed MPO staining, it was not as intense and diffuse as it was for the obese women. Leukocytes stained for MPO and were present in the lumen, flattened and adhered to the endothelium, and infiltrated into the vessel wall in overweight and obese women (black arrows). There was considerable diffuse vascular staining for MPO in overweight and obese women (white arrows). Panel A, negative control for an obese woman. Red scale bar, 50 μm. All pictures were taken with a 40× lens. A indicates adipocyte; VL, vessel lumen; MPO, myeloperoxidase.

<.05 was considered significant. A statistical software application was used (Prism 4.0 for Macintosh, GraphPad Software, Inc, San Diego, California).

Results

Demographic data for nonpregnant and pregnant patients are given in Tables 1 and 2, respectively. Summarized results for optical density, percentage of vessels with leukocyte staining, percentage of vessels with diffuse staining, and percentage of vessels with staining in the vessel wall for nonpregnant patients are shown in Figure 1. In all cases, there was a progressive increase in staining from normal weight to overweight to obese. In all cases, obese patients had significantly more staining than normal weight or overweight patients, except for vessel wall staining for which the number of vessels stained in the obese group was not significantly greater than the overweight group. Figure 2 shows representative pictures of vascular staining of MPO for each group. There was little or no staining for normal weight patients (Panel B), more staining for overweight patients (Panel C), and considerable staining for obese patients (Panels D-F). In obese patients, staining was present in

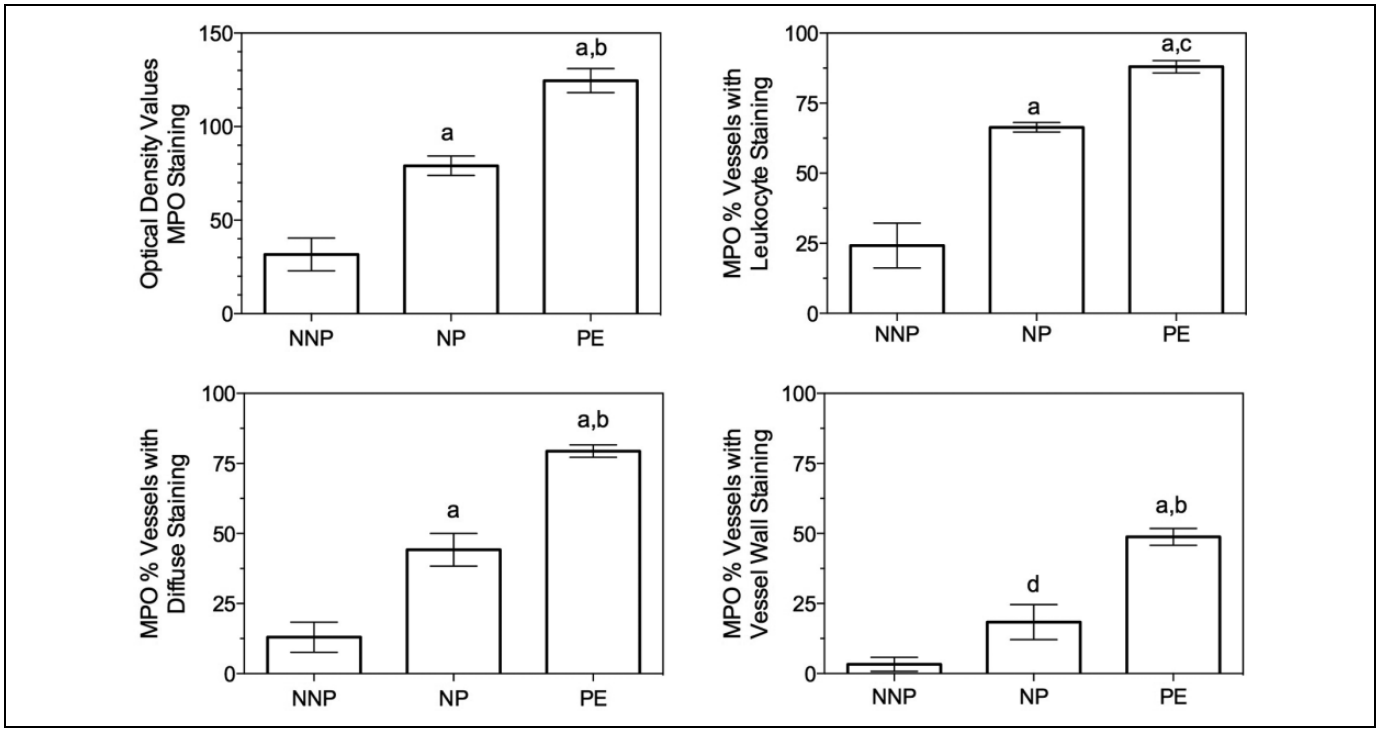

Figure 3. Optical density of myeloperoxidase (MPO) staining and percentage of vessels with leukocyte staining, diffuse staining, and vessel wall staining for normal nonpregnant (NNP), normal pregnant (NP) women, and women with preeclampsia (PE). In all cases, staining for women with PE was significantly greater than for NP women, which was significantly greater than for NNP women. a, $P < .001$ compared to NNP. b, $P < .001$ compared NP. c, $P < .01$ compared NP. d, $P < .05$ compared to NNP.

leukocytes present in the lumen, adhered to the endothelium and infiltrated into the vessel wall. Diffuse staining was also very prominent in obese patients.

Summarized results for pregnant patients are shown in Figure 3. In all cases, staining for patients with preeclampsia was significantly greater than normal pregnant patients, which was significantly greater than normal nonpregnant patients. Figure 4 shows representative pictures of the staining. There was little or no staining in normal nonpregnant patients (Panel B). There was more staining in normal pregnant patients (Panel C), but when there was staining, it was localized to a few leukocytes primarily in the lumen of the vessel. In contrast, there was extensive staining for MPO in vessels of preeclamptic women (Panels D-G). In preeclamptic vessels, many leukocytes stained that were in the lumen, but also ones that were flattened and adhered to the endothelium and infiltrated into the vessel wall. There was considerable diffuse staining in the vessels of women with preeclampsia. Staining of MPO was primarily localized to areas where neutrophils were present (Panels G and H).

Discussion

In this study, we show extensive staining for MPO in systemic blood vessels of obese women and women with preeclampsia. This may help explain why obesity is a risk factor for preeclampsia. Although our obese women were not hypertensive, other than superimposed preeclampsia, women who become preeclamptic do not have elevated blood pressure prior to pregnancy. The reason obese women are at risk for preeclampsia may be because their blood vessels are predisposed to hypertension due to the presence of MPO. The association of MPO with elevated blood pressure is strongest in the presence of oxidative stress,[16] so when an obese women becomes pregnant and is exposed to the additional oxidative stress imposed by placental secretion of lipid peroxides, her blood pressure could worsen leading to preeclampsia.

Expression of MPO was increased in women with normal pregnancy as compared to normal nonpregnant women, which would be expected due to the leukocytosis that occurs with normal pregnancy.[21,32] However, despite the increase in MPO expression, blood vessels of normal pregnant women do not show markers of inflammation as do blood vessels of women with preeclampsia.[23,28,29] The reason for this is probably because expression of MPO in normal pregnancy is primarily restricted to leukocytes in the lumen of vessels and not leukocytes that have infiltrated into the vessels. Therefore, even though the leukocytes contain MPO, it is not released to cause vessel damage.

Although both monocytes/macrophages and neutrophils are rich sources of MPO, neutrophils appear to be the main source in blood vessels of obese women and women with preeclampsia because we previously showed that leukocyte

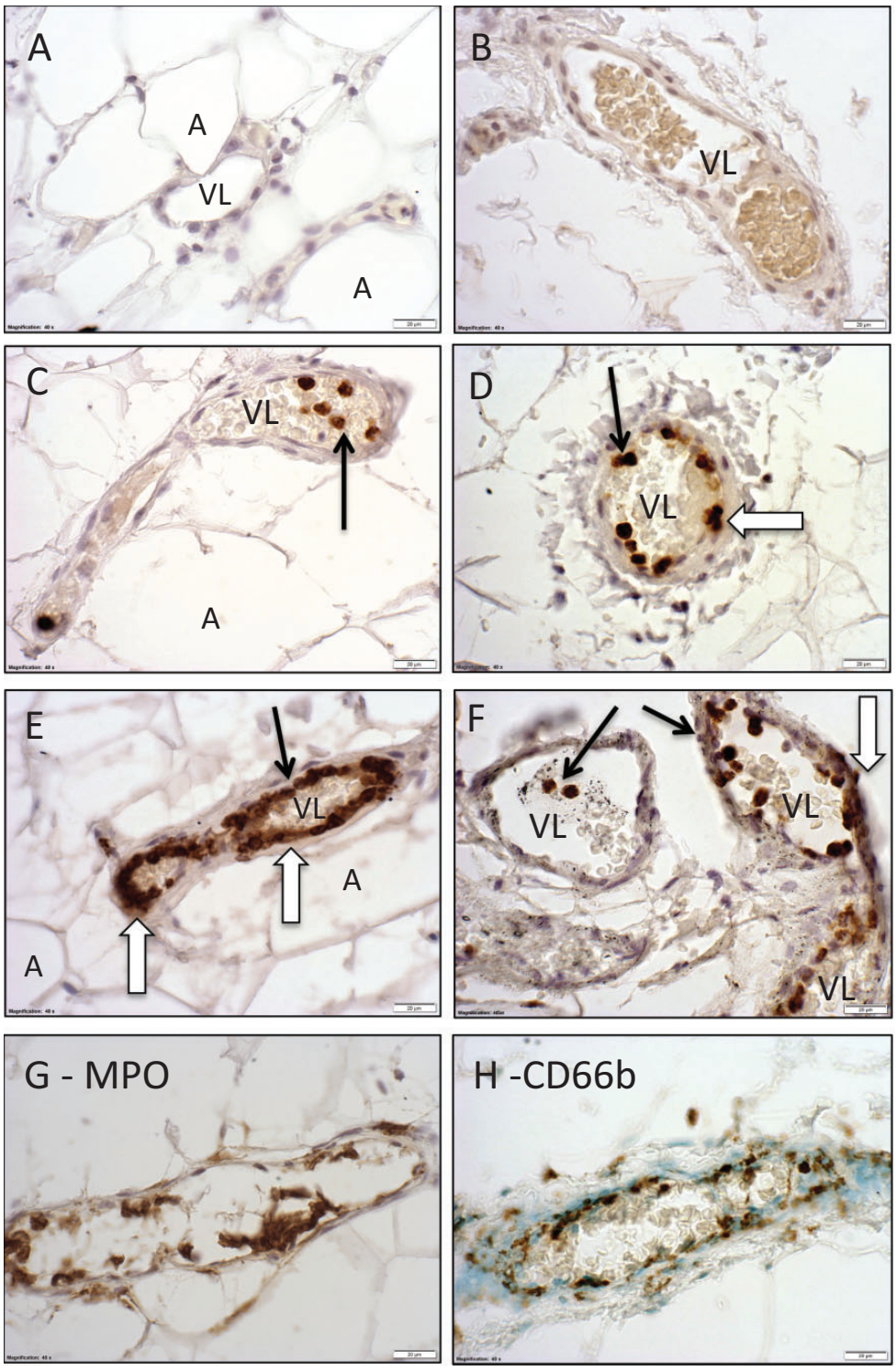

Figure 4. Representative examples of MPO staining in subcutaneous fat vessels of normal nonpregnant women, normal pregnant women, and women with preeclampsia. Expression of MPO was significantly greater in the maternal systemic vasculature in women with preeclampsia (Panels D-G) than in normal pregnant women (Panel C) or normal nonpregnant women (Panel B). While normal pregnant women did show MPO staining, it was not as intense and diffuse as it was for the women with preeclampsia. In general, when vessels of normal pregnant women were stained, it was for leukocytes in the lumen and not infiltrated into the vessel (black arrow, Panel C). In contrast, in women with preeclampsia, staining was present for leukocytes in the lumen, flattened and adhered to the endothelium, and infiltrated into the vessel wall (black arrows, Panels D-F). There was also extensive diffuse staining for MPO in vessels of preeclamptic women (white arrows, Panels D-F). Panels G and H are serial sections of a vessel showing that MPO staining is localized to areas that stain for the neutrophil marker, CD66b. Panel A, negative control for a woman with preeclampsia. White scale bar, 20 μm. All pictures were taken with a 40× lens. A indicates adipocyte; VL, vessel lumen; MPO, myeloperoxidase.

infiltration into subcutaneous and omental fat blood vessels is primarily restricted to neutrophils.[26,27,31] For example, in women with preeclampsia, 80% of vessels stained for neutrophils, whereas only 20% stained for monocytes/macrophages or lymphocytes, and when there was staining there were only 1 or 2 monocytes/macrophages or lymphocytes per vessel.[26,27] The present study confirms our previous findings of extensive neutrophil infiltration and extends these findings to demonstrate intense diffuse staining of MPO in the vasculature of obese women and women with preeclampsia. Diffuse staining most likely represents MPO secreted by infiltrated neutrophils and is consistent with a previous report that MPO concentrates in the subendothelial matrix of vascular tissues.[33] This is significant because MPO is a potent oxidizing and antimicrobial agent.[34] Myeloperoxidase is released during the degranulation process when neutrophils infiltrate tissues. Hydrogen peroxide is formed by the respiratory burst and combines with chloride to form hypochlorous acid. When released to the outside of the cell, hypochlorous acid can attack normal tissue causing damage.[34] In obese women and women with preeclampsia, neutrophil release of these toxic agents is a likely cause of vascular inflammation[28] and compromised vascular integrity, which in preeclampsia could explain proteinuria and pathologic edema.

Hypertension is common to both obesity and preeclampsia and elevated levels of MPO are associated with increased blood pressure.[16] We have shown that neutrophil infiltration correlates with vascular inflammation and increased blood pressure.[31] There are several mechanisms whereby MPO can increase blood pressure. One is that MPO interacts with the endogenous vasodilator nitric oxide thus reducing its bioavailability.[12-14] Another is that MPO is a potent oxidant[34] and oxidation inhibits prostacyclin synthase,[35,36] thus reducing availability of another vasodilator, prostacyclin. A third mechanism is to stimulate the induction of COX-2 to increase the vasoconstrictor, thromboxane.[37-39] With regard to thromboxane, we recently showed increased expression of thromboxane synthase in systemic blood vessels of women with preeclampsia,[29] so neutrophil release of MPO could be responsible for a local vascular increase in thromboxane.

In summary, both obese women and women with preeclampsia have increased presence of MPO associated with neutrophil infiltration in their systemic blood vessels. Myeloperoxidase was present in neutrophils and diffuse staining for MPO was present in endothelium and vascular smooth muscle. We speculate that the presence of MPO in blood vessels of obese women compromises their vasculature putting them at risk of developing preeclampsia when they become pregnant and are exposed to the additional burden of oxidative stress imposed by placental secretion of lipid peroxides.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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