NEW RESEARCH

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SCIENTIFIC INVESTIGATIONS

The Influence of Intermittent Hypoxemia on Platelet Activation in Obese Patients with Obstructive Sleep Apnea

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Objectives: Literature regarding platelet function in obstructive sleep apnea (OSA) has considerable limitations. Given the central role of platelets in atherothrombosis and the known cardiovascular risk of OSA, we hypothesized that OSA severity is predictive of platelet function, independent of known comorbidities.

Design: Obese subjects, without comorbidities, underwent overnight, in-lab polysomnography. The following morning, 5 biomarkers of platelet activation were measured by whole-blood flow cytometry at baseline and in response to agonists (no stimulation, stimulation with 5 μM ADP agonist, and stimulation with 20 μM ADP agonist): platelet surface P-selectin, activated glycoprotein (GP) IIb/IIIa, and GPIb receptor expression, platelet-monocyte aggregation (PMA) and platelet-neutrophil aggregation (PNA).

Results: Of the 77 subjects, 47 were diagnosed with OSA (median apnea-hypopnea index [AHI] of 24.7 ± 28.1/h in subjects with OSA and 3.0 ± 3.9/h in subjects without OSA, $p < 0.001$). The groups were matched for body mass index, with a mean body mass index of 40.3 ± 9.6 kg/m^2 in subjects with OSA and 38.9 ± 6.0 kg/m^2 in subjects without OSA ($p = 0.48$). A comparison of time spent with an oxygen saturation of less than 90% showed that subjects who had 1 minute or more of desaturation time per hour of sleep had lower GPIb fluorescence in circulating platelets, as compared with those subjects who had less than 1 minute of desaturation time per hour of sleep; similar findings were observed following 5 μM and 20 μM of ADP stimulation, as compared with control vehicle, suggesting higher levels of circulating platelet activity. In multivariate analyses, only nocturnal hypoxemia and female sex predicted agonist response. Platelet surface P-selectin, platelet surface-activated GPIIb/IIIa, PMA, and PNA were not significantly correlated with markers of OSA.

Conclusions: In obese patients with OSA, platelet activation is associated with greater levels of oxygen desaturation, compared with matched control subjects. Metrics other than AHI (e.g., hypoxemia) may determine OSA-related thrombotic risk.

Keywords: Platelet activation, sleep apnea, obesity, hypoxemia, GPIb, lung

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Obstructive sleep apnea (OSA) is a common disorder associated with repeated arousals and oxygen desaturations related to upper airway collapse. Conservative prevalence rates have been reported as 2% to 4% in the general population.[1] OSA has been associated with increased risk of cardiovascular sequelae, including atherothrombotic diseases such as stroke and coronary artery disease. Some have proposed that the repeated cycles of intermittent hypoxemia, sympathetic activation, and sleep disruption may lead to vascular abnormalities, including endothelial dysfunction, vascular inflammation, and increased platelet activation and aggregation.[2]

BRIEF SUMMARY

Current Knowledge/Study Rationale: Much of the currently published literature supports an increase in platelet activation in OSA subjects, but is limited by methodological issues, lack of control for major co-morbidities and lack of generalizability.

Study Impact: Our findings suggest that even relatively "healthy obese" subjects with OSA may have platelet activation if OSA is accompanied by desaturation as measured by GPIb intensity. In addition, our findings suggest that different indices of desaturation may be important in predicting platelet acitivation and thus atherothrombotic complications in those with OSA.

A commentary on this article appears in this issue on page 179.

Platelet activation plays a vital role in atherothrombosis but has been inadequately characterized in those with OSA.[3] For example, a very small early study by Bokinsky et al. used an indwelling catheter to obtain blood samples hourly for flow cytometric analysis of platelet surface P-selectin during sleep and upon awakening in a total of 11 controls and subjects with OSA.[4] The authors found an increase in platelet activation using this marker in patients with OSA who responded to continuous positive airway pressure therapy. However, artifact is suggested by the very high proportion (~20%-55%) of platelets that were P-selectin positive in this study[4]— as compared with the 5% to 7% rate reported to occur in patients who have recently experi-

enced a myocardial infarction.[5] More recent studies by Shimizu et al. have used larger cohorts (94 subjects with OSA and 31 control subjects) and obtained samples by fresh venipuncture in the morning to avoid possible activation due to an indwelling catheter.[6] These researchers measured platelet surface-activated glycoprotein (GP) IIb/IIIa receptor and P-selectin to determine platelet activation. The levels of surface-activated GPIIb/IIIa (identified by monoclonal antibody PAC-1) were markedly elevated, i.e., again, on average higher than those in patients who had had a recent myocardial infarction, and certain subjects with OSA had almost 95% of platelets positive for this marker. This was an unexpected finding given that patients with a recent myocardial infarction had approximately 38% of platelets positive for activated GPIIb/IIIa.[5] Additionally, this and other such studies[6-9] have included predominantly men, have not always controlled for age and body mass index (BMI), and have included subjects with preexisting atherosclerotic disease, leaving unclear whether the increased platelet activation is due to confounding differences in patient groups or truly due to OSA. Additionally, these and other studies investigating the role of OSA in platelet function have recruited patients referred to sleep clinics, who are much more likely to have daytime sleepiness (and are generally sicker), as compared with a community cohort, thus introducing a bias. Therefore, much of the published literature, though largely uniform in demonstrating that OSA is associated with increased platelet activation, is limited by serious methodological issues, lack of control for major comorbidities, and probable lack of generalizability.

To understand the independent role of OSA on platelet function, we investigated the role of OSA in a cohort of healthy obese subjects (obese subjects with and without OSA but with no other known cardiovascular risk factors). We hypothesized that OSA severity would be predictive of platelet activation, reactivity, and aggregability in these obese subjects.

RESEARCH DESIGN AND METHODS

Study Design

Obese individuals were recruited from the community from October 2005 to September 2008. Following an outpatient screening visit to determine eligibility, subjects underwent a standard in-laboratory overnight polysomnogram. Subjects remained fasting from 22:00 until the study was completed the next morning and refrained from any vigorous physical activity. Blood collection for platelet testing was performed on the morning following the polysomnogram, approximately 2 hours after awakening. Flow cytometry samples were labeled for immunophenotypic analysis within 30 minutes of blood draw and were stabilized by fixation. Samples were analyzed on the flow cytometer within the next 24 to 48 hours, a time frame that does not result in artifactual platelet activation.[10]

Study Population

Obese individuals with a BMI $\geq$ 30 kg/m^2, aged 18 to 70 years, were enrolled if they were nonsmokers and free of cardiac, pulmonary, endocrine, or sleep disorders (other than hypertension in 3 patients and the presence of OSA). All subjects underwent thorough history and physical examination by a licensed physician and laboratory testing for fasting blood glucose concentration, fasting lipid panel, complete blood count, and thyroid stimulating hormone level. Subjects with abnormal results on physical exams or laboratory values were excluded. Prior to the study night, all subjects were asked to keep a 2-week sleep diary, and those with partial sleep deprivation or erratic sleep schedules were excluded. Subjects were also excluded if they were taking medications that could affect cardiovascular function or sleep, such as oral contraceptive agents, hormone replacement therapy, sedatives, steroids, antiplatelet drugs (including nonsteroidal antiinflammatory drugs), - antihypertensive medications, and lipid-lowering agents. Thus, we excluded any subjects with known preexisting or unstable vascular disease, inflammatory diseases, or infections that could affect platelet function. All subjects provided written informed consent. The study was approved by the Institutional Review Board and General Center for Clinical Research of Brigham and Women's Hospital.

Laboratory Measurements

Blood samples were obtained using standard sterile technique from the antecubital vein. Plasma glucose concentration, total serum cholesterol level, and triglyceride levels were measured using the Synchoron CX analyzer (Beckman Systems, Fullerton, CA). High-density lipoprotein serum cholesterol levels were measured directly (Sigma, St. Louis, MO), and low-density lipoprotein levels were calculated. The glycated hemoglobin (HbA_{1c}) concentration was measured in whole blood with ion-exchange high-performance liquid chromatography. Complete blood counts (CBC), thyroid stimulating hormone levels, and fasting glucose concentrations were measured in a core laboratory using standard laboratory techniques.

Polysomnography

Recorded polysomnographic signals included electroencephalogram (C4-A1, C3-A2, O2-A1, and O1-A2), left and right electrooculogram, submental and bilateral tibial electromyogram, electrocardiogram with surface electrodes, airflow, chest and abdominal excursion (piezo bands), oxyhemoglobin saturation (SaO_2), and body position. All data were collected and stored on Nihon Kohden, a digital polysomnography system (Nihon Kohden; Foothill Ranch, CA). Polysomnograms were scored by a blinded, experienced sleep technician and staged according to standard criteria.[11] Arousals and respiratory events were scored according to published guidelines.[12,13] An apnea was scored if airflow was absent for 10 seconds, and a hypopnea was scored if there was at least a 50% reduction in airflow for 10 seconds or a discernable decrement in airflow for 10 seconds in association with a either an oxyhemoglobin desaturation of at least 3% or an arousal. A respiratory-disturbance index (RDI) was then calculated based on the number of apneas and hypopneas per hour of sleep. The time spent with an SaO_2 below 90% was represented as minutes of desaturation time < 90% divided by the hours of total sleep time (TST) (T < 90%). The SaO_2 nadir represented the lowest oxygen saturation during sleep, and the oxygen desaturation index (ODI) was based on the number of desaturations (decrease of at least 4% from baseline) per hour of sleep. The TST, arousal index (AI), and sleep efficiency (SE) were determined after manual scoring of the polysomnographic record.

Measurement of Platelet Activation

Five biomarkers of platelet activation were measured by whole-blood flow cytometry in 3 states (no stimulation, stimulation with 5 μM ADP, and stimulation with 20 μM ADP): platelet-surface P-selectin, platelet surface-activated GPIIb/IIIa, platelet-surface GPIb, platelet-monocyte aggregates, and platelet-neutrophil aggregates. To minimize platelet activation during blood collection, blood samples were obtained with a 21-gauge needle into a sodium citrate blue-top tube. The initial 4 mL of blood drawn was not used for platelet testing, and the tube was handled with care to avoid inadvertent activation of platelets. Preparation of whole-blood samples for flow-cytometry analysis has been previously described in detail.[14,15] In brief, samples for flow cytometry were prepared by a highly trained investigator within 30 minutes of blood's being drawn. For platelet-activation studies, whole-blood samples were labeled with phycoerythrin (PE)-labeled P-selectin-specific monoclonal antibody (CD62P, Santa Cruz Biotech, Santa Cruz, CA), fluorescein isothiocyanate (FITC)-labeled monoclonal antibody specific for activated GPIIb/IIIa (PAC-1, Becton Dickinson [BD], Franklin Lakes, NJ), PE-Cy5-labeled GPIb-specific monoclonal antibody (CD42b, BD-Pharmingen) and exposed to 1 of 3 activation conditions for 15 minutes: (1) buffer, (2) 0.5 μM ADP, (3) or 20 μM ADP. Following this exposure, platelet samples were fixed with HEPES-saline-1% ultrapure formaldehyde (Polysciences, Inc., Warrington, PA). Nonspecific PE-antibody binding was determined using mouse $IgG1_{2a}$-PE (Santa Cruz Biotech) in place of the P-selectin-specific antibody. Nonspecific FITC fluorescence was determined by adding 2.5 μg/mL eptifibatide (a small-molecule GPIIb/IIIa antagonist, Millennium Pharmaceuticals, Cambridge, MA) to block site-specific PAC-1–FITC binding. For platelet-monocyte and platelet-neutrophil aggregation studies, whole-blood samples were labeled with anti-CD14 (PE-Cy5, Beckman Coulter, Inc., Brea, CA) to identify monocytes (bright) and neutrophils (dim) and a PE-labeled GPIX-specific monoclonal antibody (CD42a, BD-Pharmingen) to identify bound platelets and then were exposed to 1 of 3 activation conditions for 15 minutes: (1) buffer, (2) 0.5 μM ADP, or (3) 20 μM ADP. Following this exposure, samples were fixed with Becton Dickinson FACS lysing solution, a single-step fix-lyse reagent. Nonspecific PE fluorescence was determined by using MIgG1κ-PE (BD-Pharmingen) in place of the anti-GPIX antibody. Samples were analyzed in a FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) within 24 to 48 hours with 488-nm excitation and appropriate color-compensation settings. The flow cytometer was calibrated daily with Spherotech (Lake Forest, IL) calibration particles to adjust PMT voltages, which ensured consistent fluorescence values during the course of the study.

Data acquisition and analysis were performed with BD CellQuest Pro 2 Software (Becton Dickinson Biosciences). Platelets were identified by their characteristic light scatter and positive labeling with platelet-specific anti-GPIb monoclonal antibody,and these platelets were then assessed for percentage of platelets positive for PAC-1 binding and P-selectin and also assessed for mean fluorescence of PAC-1, P-selectin, and GPIb (indicating average receptor density). Monocytes and neutrophils were identified by their characteristic light scatter and CD14 fluorescence. Platelet-monocyte aggregates (PMA) and platelet-neutrophil aggregates (PNA) were defined by the percentage of monocytes or neutrophils that were positive for platelet-specific marker GPIX.

Statistical Analysis

Patient characteristics are represented as means and statistical differences and were calculated using t tests for 2-group comparison. Variables with significant departure from the normal distribution were presented as median and interquartile range and analyzed with nonparametric tests: Mann-Whitney for 2-group comparison and Kruskal-Wallis for comparison between larger groups. The Pearson χ^2 test was used for comparison among categorical variables. The biomarkers of platelet activation were assessed in relation to T < 90% status (dichotomized as explained below), ODI (dichotomized into groups above and below the median value of 4.5 events/h), OSA status, TST, AI, and SE. The T < 90% value was dichotomized based on a clinically significant time of 1 or more minutes per hour of sleep. A multiple linear-regression analysis was performed to determine if relevant OSA and sleep parameters in all subjects were associated with platelet activation, reactivity, and aggregability independently of the BMI, age, sex, and logAHI. To avoid model overfitting, we used forward stepwise regression to identify potential significant predictors ($p < 0.10$) for retention in the model. Only a portion of our subjects with OSA had significant desaturations; thus, in posthoc analysis, subjects were also separated into 3 groups: (1) those with no OSA, (2) those with OSA but minimal desaturation (T < 90% was < 1 min/h of sleep), and (3) those with OSA and desaturation (T < 90% was ≥ 1min/h of sleep). All reported p values are 2 sided, and $p < 0.05$ was considered to be significant.

RESULTS

Subject Characteristics

Of the 341 potential subjects who underwent screening, 77 met inclusion and exclusion criteria and were enrolled. Characteristics of the 77 subjects studied are shown in **Table 1A**. The groups were well matched for BMI, as intended, and had similar body-fat percentages. Differences in age and sex among the groups with and without OSA were statistically adjusted, as shown below. Based on an AHI of at least 10 events per hour to define OSA, 47 of the 77 studied subjects had OSA (see **Table 1B**). The median AHI in subjects with OSA was 24.7 ± 28.1 events/hour and 3.0 ± 3.9 events/hour in subjects without OSA ($p < 0.001$). Additionally, there were the expected differences between the 2 groups in the SaO_2 nadir, T < 90%, ODI, and AI. TST and SE were similar in both groups.

Platelet Activation

The relationships between platelet activation and OSA status, T < 90%, ODI, TST, AI, and SE were examined. Those subjects with desaturations above the cutoff of more than 1 minute of desaturation time showed a significant decrease in GPIb fluorescence intensity in circulating platelets and after mild and strong stimulation with ADP (with 0.5 μM and 20 μM of ADP) (**Figure 1**), indicating circulating platelet activation

Table 1A—Subject characteristics, blood pressure, and results of laboratory studies

	Non-OSA n = 30	OSA n = 47	p value
Characteristics			
Age, y	31.8 ± 11.8	43.5 ± 9.3	< 0.001
Female, %	70	47	0.046
BMI, kg/m^2	38.9 ± 6.0	40.3 ± 9.6	0.483
Body fat, %	40.3 ± 10.2	40.8 ± 10.9	0.855
Waist:hip ratio	0.85 ± 0.10	0.95 ± 0.09	< 0.001
Blood pressure, mm Hg			
Systolic	127.7 ± 13.1	130.0 ± 10.6	0.404
Diastolic	73.5 ± 7.0	78.6 ± 10.0	0.018
Values from laboratory studies			
WBC, K/μL	6.9 ± 1.6	7.2 ± 1.8	0.506
Hematocrit, K/μL	39.9 ± 3.5	40.3 ± 7.5	0.714
Platelet count, K/μL	293.6 ± 59.5	318.4 ± 85.2	0.168
Total cholesterol, mg/dL	185.9 ± 34.0	190.3 ± 33.5	0.579
LDL, mg/dL	109.0 ± 29.5	112.0 ± 27.2	0.652
HDL, mg/dL	56.4 ± 15.5	48.3 ± 12.8	0.016
Fasting glucose, mg/dL	79.8 ± 11.3	86.1 ± 14.6	0.048
HgA_{1c}, %	5.4 ± 0.4	5.6 ± 0.4	0.022
TSH, mIU/L	1.9 ± 0.8	1.9 ± 0.9	0.890

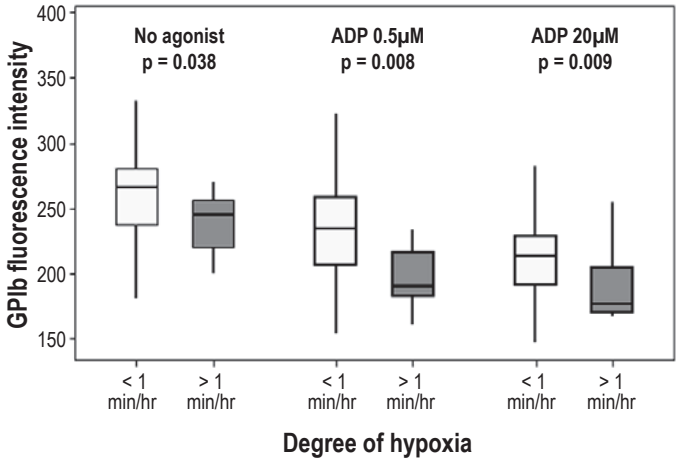
OSA, obstructive sleep apnea; BMI, body mass index; WBC, white blood cell count; LDL, low-density lipoprotein fraction; HDL, high-density lipoprotein fraction; HgA_{1c}, glycated hemoglobin A_{1c} fraction.

Table 1B—Sleep characteristics

Sleep Characteristic	Non-OSA n = 30	OSA n = 47	p value
AHI, events/h	3.0 ± 3.9	24.7 ± 28.1	< 0.001
SaO_2 nadir, %	91.3 ± 2.8	81.2 ± 9.0	< 0.001
T < 90%, min/h of sleep	0 ± 0	0.36 ± 3.5	< 0.001
ODI, events/h	0.9 ± 1.7	14.4 ± 31.7	< 0.001
TST, min	360 ± 69	342 ± 74	0.295
AI, arousals/h	16.0 ± 7.3	37.3 ± 27.3	< 0.001
SE, %	88.9 ± 15.7	84.5 ± 21.1	0.037

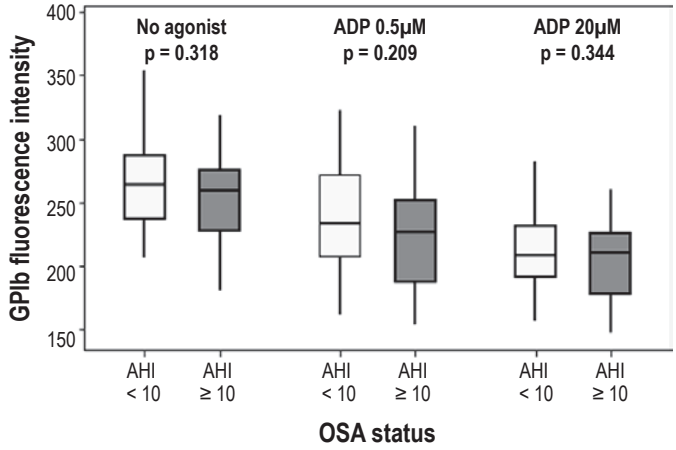
OSA, obstructive sleep apnea; AHI, apnea-hypopnea index; T < 90%, the time spent with an SaO_2 below 90% divided by the hours of total sleep time (TST); ODI, oxygen desaturation index; AI, arousal index; SE, sleep efficiency.

Figure 1—A greater degree of hypoxia is predictive of platelet activation



Platelet surface glycoprotein Ib (GPIb) fluorescence intensity (expressed as arbitrary units) is decreased in subjects with greater desaturation (expressed as minutes of time spent with an SaO_2 < 90%, per hour of sleep). Data between the first and third quartiles are shown as a box, which is bisected by the median value; whiskers are used to represent the upper and lower limits. p values were determined using the Mann-Whitney test.

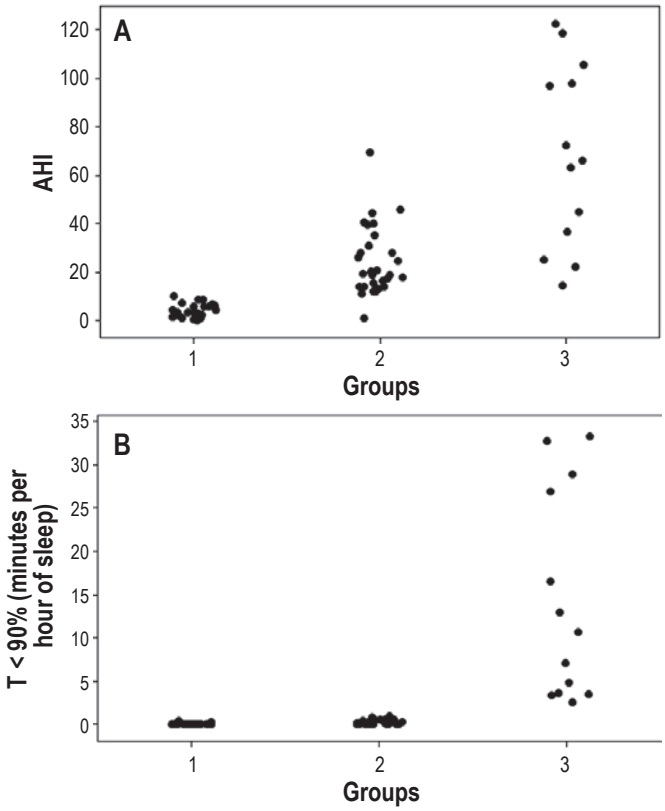
Figure 2—Obstructive sleep apnea (OSA) status does not predict platelet activation



Subjects with an apnea-hypopnea index (AHI) < 10 events/h (n = 30) had glycoprotein (GP)Ib fluorescence intensity (expressed as arbitrary units) values similar to those of subjects with an AHI > 10 events/h. Data between the first and third quartiles are shown as a box, which is bisected by the median value; whiskers are used to represent the upper and lower limits. p values were determined using the Mann-Whitney test.

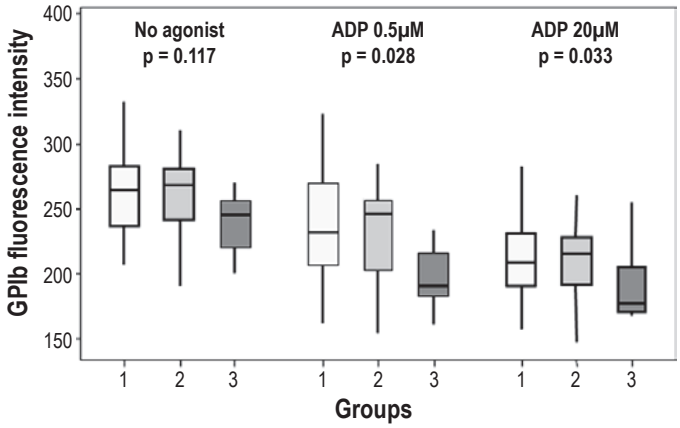
and increased platelet reactivity. Multiple linear regression analysis revealed that T < 90% and female sex were the only significant independent predictors of GPIb fluorescence intensity in a model that included T < 90% status, OSA status, age, sex, and BMI. Although OSA status (AHI ≥ 10 events/h) did not predict platelet activation, as determined by GPIb fluorescence intensity (**Figure 2**), most subjects with longer desaturation times also had more severe OSA (**Figures 3A** and **3B**). However, the relationship between AHI and T < 90% was not linear. Thirteen subjects with OSA and desaturations (T < 90% was ≥ 1 min/h of sleep, Group 3) had more activated platelets than did the 29 subjects with OSA and minimal desaturations (T < 90% was < 1 min/h of sleep, Group 2) or the 27 subjects without OSA (Group 1, **Figure 4**). A greater ODI was not statistically significantly predictive of GPIb levels. There were no changes in other markers of platelet activation (platelet surface-activated GPIIb/IIIa, platelet surface P-selectin, PMA, and PNA) in relationship to OSA status, T < 90%, ODI, TST, AI, or SE.

Figure 3—Shown below are the apnea-hypopnea index (AHI) and desaturation times of groups 1-3



Group 1: no obstructive sleep apnea (OSA) (n = 27), Group 2: OSA, but minimal desaturation (time spent at an SaO_2 of < 90% [T < 90%] was < 1 min/h of sleep, n = 29), and Group 3: OSA and desaturation (T < 90% was ≥ 1min/h of sleep, n = 13).

Figure 4—Subjects with obstructive sleep apnea (OSA) with desaturation had greater platelet reactivity



Group 1 represents no OSA (n = 27), Group 2 represents OSA with minimal desaturation (time spent with an SaO_2 < 90%—represented as minutes of desaturation time < 90% divided by the hours of total sleep time [T < 90%]— was less than 1 min/h of sleep, n = 29), and Group 3 represents OSA and significant desaturation (T < 90% was ≥ 1 min/h of sleep, n = 13). Platelet-surface glycoprotein (GP)Ib fluorescence intensity (expressed as arbitrary units) was not decreased significantly in circulating platelets (no agonist) but was decreased upon stimulation with 0.5 μM ADP and 20 μM ADP, indicating increased platelet reactivity in Group 3. Data between the first and third quartiles are shown as a box, which is bisected by the median value; whiskers are used to represent the upper and lower limits. p values were determined using the Kruskall-Wallis test.

DISCUSSION

The principal finding of our study was platelet activation, as measured by a decrease in platelet surface GPIb receptor fluorescence intensity, in association with greater desaturations in subjects with OSA. This decrease in fluorescence intensity indicates a decrease in the surface receptor density for GPIb. GPIb, a platelet surface receptor for vWF, has, to our knowledge, not been previously studied in the context of OSA.[16] GPIb plays a key role in primary hemostasis and arterial thrombosis and is downregulated upon activation of platelets.[16-19] Michelson et al.[18] have demonstrated that platelet-surface GPIb is internalized upon platelet activation and remains altered for much longer than other markers of platelet activation. Thus, platelet-surface GPIb levels are ideally suited for measurement of platelet activation during the day from a stimulus that occurs during the night, such as sleep disordered breathing.[20] In addition, there is an activation-dependent irreversible proteolysis of the α chain of GPIb by neutrophil cathepsin G,[21] tumor necrosis factor-α–converting enzyme (TACE, ADAM17),[22] and other proteases. Importantly, the binding site of the monoclonal antibody that we used to detect platelet-surface GPIb in this study is in the distal proteolytic fragment of GPIbα. In this study, the activation-dependent reduction in platelet-surface GPIb receptor therefore detected the activation-dependent decrease in platelet-surface GPIb irrespective of whether the mechanism was (a) activation-dependent relocation of GPIb into the interior of the platelet (b) activation-dependent proteolysis of GPIb, or (c) a combination thereof.

Our results suggest that the presence of oxyhemoglobin desaturation in a cohort of otherwise healthy obese subjects with OSA is associated with platelet activation and are consistent with prior evidence.[23-25] Oxyhemoglobin desaturation in such individuals may be important independently, or it may be a marker of other processes that coexist with worsening hypoxemia. In fact, the severity of desaturation in subjects with OSA has been positively correlated with catecholamine excess.[26-28] Additionally, activation of the sympathetic nervous system and increases in shear stress due to surges in blood pressure are known to activate platelets and may also increase as hypoxemia worsens in OSA.[29-31] A desaturation-based definition of hypopnea (and thus AHI) was previously found to be more predictive of cardiovascular outcomes than is one based on awakenings.[32] Thus, based on prior literature and our new findings, the degree of hypoxemia in OSA may be viewed as a possible marker for adverse atherothrombotic consequences of OSA, even in an otherwise healthy obese patient. Of notable interest, desaturation time, but not ODI, was predictive of platelet activation. ODI and T < 90 both reflect desaturation but may differentially reflect important physiological variations in subjects with OSA,

such as differences in lung volumes, length and frequency of desaturation cycles, and duration of apneas. Thus, further characterization of desaturation patterns is necessary, since desaturation indices may allow us to predict platelet activation and other sequelae of OSA. Our data further suggest different sensitivities of various platelet markers for use in the daytime assessment of platelet reactivity following overnight exposure to apnea. These findings have implications for subsequent studies because some platelet biomarkers would be predicted to be more sensitive than others, depending on the time of day of the assessment and the proximity of the measurement to antecedent sleep apnea.

In our study, platelet activation was also associated with female sex. Although the number of subjects with desaturation was too small to allow secondary analysis by sex, female sex has been associated with increased platelet activity in premenopausal women, compared with age-matched men.[33] Platelet function has also been shown to vary with the menstrual cycle.[34] Further complicating the matter of sex effects on platelet function is that many of our obese premenopausal women did not have regular menses; thus, they were not studied in a specific phase in their menstrual cycle. Additionally, postmenopausal women have been noted to have increased thrombotic events. However, specific studies on platelet activity defined by age and hormone status are lacking.[35] Hormonal therapy appears to be beneficial, with a reduction in platelet activation in the presence of estrogen in vitro; however, in vivo studies have had mixed results.[36] We did not have any subjects taking oral contraceptive agents or hormone replacement therapy. Only 1 of the 4 women present in Group 3 with OSA and significant desaturation was postmenopausal. Sex differences in platelet reactivity in OSA could not be fully addressed in this cohort and will require further study.

The independent impact of obesity on platelet function has not yet been fully explored and, specifically, not explored while controlling for the presence of sleep disordered breathing. Obesity was observed to increase platelet reactivity in a large cohort of more than 2000 participants.[37] However, the obese subjects had significantly more diabetes and hypertension than did the lean subjects, and the cohort was not screened for sleep apnea. A more carefully selected, but smaller, cohort of 100 obese (BMI > 30 kg/m^2) subjects studied by Coban et al. were found to have a higher mean platelet volume, as compared with 100 age- and sex-matched control subjects (BMI < 30 kg/m^2).[38] Though some subjects were smokers, hypertension, diabetes, hyperlipidemia, and other serious systemic diseases (but not sleep apnea) were excluded. Thus, though obesity may influence platelet reactivity, the evidence does not yet support a relationship independent of other cardiovascular risk factors.

Strengths of our study were the exclusion of subjects with many cardiovascular risk factors and preexisting cardiovascular diseases and the inclusion of only obese subjects. Thus, confounding from obesity and preexisting cardiovascular disease was minimized. However, exclusion of patients without these confounders resulted in younger subjects with milder OSA, compared with some previous cohorts. Additionally, by design, our subjects had higher BMIs and were more likely to be women (56.5% overall) than were prior cohorts.

Limitations

Although our study had a number of strengths, including our relatively large sample size for a physiological study, our rigorous exclusion of potential confounding variables and our use of gold-standard techniques (in-laboratory polysomnography and flow cytometry with response to agonists performed in a laboratory with considerable expertise in platelet biology), we acknowledge the following limitations. This is an observational study, and thus, associations of platelet function with various indices of OSA severity do not prove causality. Differences in platelet function may be related to unrecognized differences in subjects who have more severe desaturation, rather than to sleep apnea, per se. Additionally, samples were collected after the subject awakened and was in an upright position. Prior data suggest that the upright position activates platelets, and our study design, which included the collection of platelets after the subject assumed the upright position, could thus bias our findings toward the null hypothesis.[4] However, this procedure was systematic (in both groups) and not altered throughout the study period. Additionally, in prior studies examining the role of OSA in platelet activation, blood was collected from patients shortly after they awakened, whereas we collected blood 2 hours after the subjects awakened. Biomarkers, such as platelet surface P-selectin and platelet surface-activated GPIIb/IIIa, remain activated for a shorter time and return to baseline values faster than does GPIb. Thus, timing of our blood draw (2 hours after awakening rather than immediately after awakening or during sleep) may also explain the lack of platelet activation in subjects with OSA, as assessed by some markers. We also accept that, because we used multiple metrics of platelet activation to provide a thorough assessment of platelet function, we performed multiple comparisons. Thus, any significant results may be purely due to chance alone, although we doubt that all of our findings are spurious. Finally, one could argue that our exclusion of covariates yielded an 'exceptionally healthy' sample of obese subjects, which could lead to a lack of generalizability of our results. For example, perhaps the concurrent presence of OSA, diabetes, and obesity all interactively affect platelet function. Although we accept that our conclusions are limited to the population studied, we believe that isolated variables should be assessed before these complex interactions can be defined in cohorts that would yield more generalizable results.

Clinical Significance

Our findings suggest that even relatively "healthy obese" subjects with OSA may have platelet activation if OSA is accompanied by desaturation. These findings suggest that, when assessing the severity of OSA and the potential for cardiovascular complications developing, desaturation time must be considered in addition to the AHI. The findings of the present study will require confirmation in other patient cohorts. Further studies may help stratify which patients with OSA should be targeted therapeutically with antiplatelet agents for the prevention of subsequent atherothrombotic disease.

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