This article was downloaded by: *[University at Buffalo (SUNY)]*
On: *9 November 2008*
Access details: *Access Details: [subscription number 784375717]*
Publisher *Informa Healthcare*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK

Nicotine & Tobacco Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713439766

Reduced-nicotine cigarettes increase platelet activation in smokers in vivo: A dilemma in harm reduction

Gaurav Girdhar [a]; Sulan Xu [a]; Danny Bluestein [a]; Jolyon Jesty [b]
[a] Dept. of Biomedical Engineering, Stony Brook University, Stony Brook, NY [b] Division of Hematology, Stony Brook University, Stony Brook, NY

First Published on: 03 November 2008

To cite this Article Girdhar, Gaurav, Xu, Sulan, Bluestein, Danny and Jesty, Jolyon(2008)'Reduced-nicotine cigarettes increase platelet activation in smokers in vivo: A dilemma in harm reduction',Nicotine & Tobacco Research,10:12,

To link to this Article: DOI: 10.1080/14622200802443528

URL: http://dx.doi.org/10.1080/14622200802443528

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Nicotine & Tobacco Research
2008, iFirst Article, 1–8

informa
healthcare

Reduced-nicotine cigarettes increase platelet activation in smokers *in vivo*: A dilemma in harm reduction

Gaurav Girdhar, Sulan Xu, Danny Bluestein, Jolyon Jesty

Received 6 November 2007; accepted 29 March 2008

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

Nicotine is a primary constituent of tobacco and smoke, and its roles in causing addiction and causing disease are commonly conflated. In the present work, we investigated whether nicotine protects smokers' platelets against smoke-induced activation *in vivo*, raising a possible dilemma in harm-reduction strategies. *In vivo* platelet activation state (PAS) was measured by fixing blood at drawing and measuring a standard marker, platelet P-selectin (CD62P). We conducted two studies: (1) 32 smokers smoked three medium-nicotine (0.6 mg nicotine) cigarettes for 1 h. Following this initial conditioning phase, 16 subjects continued with five of the same cigarettes from 1–2.5 h, resulting in a 33% increase in PAS. The other 16 subjects smoked five low/zero-nicotine cigarettes (0.05 mg nicotine), causing a 94% increase in PAS. The increase in PAS caused by nicotine withdrawal in the second group is very significant ($p<.02$). Any compensation in smoke-intake due to nicotine withdrawal in the second group was not measured in this study. (2) To determine whether nicotine modulates platelet activation by secondhand smoke (SHS), 16 nonsmokers were exposed to medium-nicotine smoke and 16 to low/zero-nicotine smoke for 1.5 h on two consecutive days. Exposure to SHS increased PAS by 60% ($p<.01$), but no difference in the medium and zero nicotine groups was observed ($p>.09$). We conclude that in smokers, nicotine modulates platelet activation, and it may significantly moderate the risk of cardiovascular disease caused by non-nicotine smoke components. Conversely, reduced-nicotine cigarettes may increase harm.

Introduction

Nicotine is the addictive component of tobacco smoke, and the means by which tobacco companies exert control over smokers. Despite large reductions over the last 15–20 years, more than 50 million Americans and 1.3 billion adults worldwide smoke cigarettes (Lillard, Plassmann, Kenkel, & Mathios, 2007; White, 2007). It is agreed that tobacco combustion products, among them many complex tars, nitrosamines, oxidants and fine particles, are the major cause of disease, e.g. emphysema, lung cancer, and cardiovascular disease (Ambrose & Barua, 2004; Brody & Spira, 2006). In contrast, pure nicotine largely satisfies the nicotine cravings of people who quit smoking, and the lack of reported adverse reactions strongly suggests that it is essentially harmless as taken. Moreover, it is proven to be effective not only in initially helping smokers to quit smoking, but also in subsequent weaning from nicotine (Etter & Stapleton, 2006). In comparison with anything that accompanies or involves tobacco products, pure nicotine is a very effective means of harm reduction (Joseph & Fu, 2003; McNeill, Foulds, & Bates, 2001; McRobbie & Hajek, 2001).

Increased risk of cardiovascular disease due to cigarette smoke manifests in the form of arterial and venous thrombosis, stroke and myocardial infarction (Ambrose & Barua, 2004; Burns, 2003; Chia & Newby, 2002). The vascular endothelium and blood platelets are major targets, the platelets in particular being critical in both the initiation and propagation of clot formation and the initial inflammatory response (Chung & Lip, 2006; Huo & Ley, 2004; Kereiakes & Michelson, 2006; Malek et al., 2007).

Gaurav Girdhar, Ph.D., Sulan Xu, M.S., Danny Bluestein, Ph.D., Dept. of Biomedical Engineering, Stony Brook University, Stony Brook, NY; Jolyon Jesty, Ph.D., Division of Hematology, Stony Brook University, Stony Brook, NY.

Correspondence: Danny Bluestein, Ph.D., Dept. of Biomedical Engineering, Health Sciences Center T18-030, Stony Brook University, Stony Brook, NY 11794-8151, USA. Tel: +1 (631) 444-2156; Fax: +1 (613) 444-6646; E-mail: danny.bluestein@sunysb.edu

ISSN 1462-2203 print/ISSN 1469-994X online © 2008 Society for Research on Nicotine and Tobacco

DOI: 10.1080/14622200802443528

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

However, the mechanism of the smoke effect is unclear, and particularly the role of nicotine. To assess the effects on platelets and discriminate clearly between smoke and nicotine, we recently studied the differential effects of extracts of whole smoke, nicotine-free smoke, and pure nicotine on the function of purified platelets *in vitro* (Ramachandran, Rubenstein, Bluestein, & Jesty, 2004; Rubenstein, Jesty, & Bluestein, 2004), and showed that even at a concentration of only 50 nmol/L, which is less than the plasma concentration typically attained in smokers, nicotine markedly reduced the activation of platelets caused by smoke.

Extending our *in vitro* observations to the situation *in vivo*, and considering baseline platelet activation state (PAS) as an important marker of cardiovascular risk, we investigated the effect of acute nicotine withdrawal on the platelets of smokers who were still smoking. In addition to deliberate self-exposure to smoke at high concentrations in the case of smokers, secondhand smoke (SHS), although enormously reduced in recent years in western countries through public policy and societal change, exposes others. We therefore additionally compared, in nonsmokers, the platelet-activating effects of SHS containing or lacking nicotine.

Methods

Human subjects

The studies and consent procedures—smoking, smoke exposure, and phlebotomy—were approved by the Stony Brook Institutional Review Board. Healthy non-pregnant adult smokers (32) and nonsmokers (32) were recruited locally and informed consent was obtained. All were between 20 and 40 years old. Subjects had not taken aspirin for 10 days, and denied any history of lung or cardiovascular disease. The smoker subjects smoked 20–25 cigarettes/day. Nonsmoker subjects reported no exposure to secondhand smoke for 10 days before the study. All experiments started between 9:30am and 11am, and subjects had not fasted. One smoker and two nonsmokers withdrew from the study before completion. The final subject numbers by sex were: (1) Smoking: medium nicotine, 8M+8F; zero-nicotine, 8M+7F. (2) SHS: medium nicotine, 7M+7F; zero nicotine, 8M+8F. Both studies were single-blinded, i.e., the nicotine content of the cigarettes was known to the personnel conducting the study but not the participants; indeed the participants were not aware that nicotine content varied in the study.

Cigarettes

Quest cigarettes are a product of Liggett Vector Brands Inc. (Mebane, NC). Quest 1 cigarettes deliver 0.6 mg nicotine by FTC method, and Quest 3 cigarettes deliver 0.05 mg. Both deliver 10 mg tar by the FTC method, which is in the medium range. In this report we use the terms "medium nicotine" and "zero nicotine" to denote the two types.

Blood drawing; fixing; platelet-rich plasma samples (PRP)

Blood was drawn from the antecubital vein into four 2.7-ml Vacutainer tubes containing: (A and C) 0.3 ml acid citrate dextrose (ACD); (B) 0.3 ml ACD+0.4 ml added 4% paraformaldehyde (PFA); and (D) 0.3 ml EDTA. Sample A, containing any vascular debris from the needle, was discarded. Sample B was centrifuged at $400 \times g$ for 3 min to make fixed platelet-rich plasma (PRP). Sample C was centrifuged to make PRP, which was treated with 1/9 vol 1 mmol/L thrombin-receptor-activating peptide (abbrev. TRAP; Sigma-Aldrich, St Louis, MO) at 37°C for 3 min. It was then fixed by adding 1/7 vol 4% PFA. These samples were used for cytometry quality-control purposes. Sample D was centrifuged at $1200 \times g$ for 15 min to make platelet-poor plasma, which was frozen at –20°C and later used for nicotine analysis.

Flow cytometry; platelet activation state (PAS)

As described above, blood was drawn directly into paraformaldehyde in the collection tube B. Immediate fixation, followed by cytometric measurement of an activation-dependent platelet-surface marker, is both quantitative and a highly sensitive measure of platelet activation (Hagberg & Lyberg, 2000; Michelson, 2006). After removal of the red cells, platelet surface expression of P-selectin was determined by flow cytometry, using phycoerythrein-labeled antibody against P-selectin (CD62P), obtained from BD Biosciences. Activation-dependent transfer of P-selectin from the α granules to the platelet surface is widely accepted as a primary marker of platelet activation (Kappelmayer, Nagy, Miszti-Blasius, Hevessy, & Setiadi, 2004), particularly in the study of cardiovascular disease (Chung & Lip, 2006; Kereiakes & Michelson, 2006; Malek et al., 2007), and a direct causative relationship between increase in P-selectin expression on platelets and cardiovascular disease has been demonstrated (Burger & Wagner, 2003; Rajagopalan et al., 2007). We define PAS as the ratio of gated positive particles to total particles, expressed as a percentage. Our gate settings remained unchanged throughout the study. With these settings, unactivated platelets from nonsmokers have PAS values of 0.5 to 5% (median$\approx$1.5%), and TRAP-activated platelets are in the range 65–96%. A typical set of cytometry measurements for one high-responding subject before and after smoking five zero-nicotine cigarettes, and upon maximal platelet activation with TRAP, is illustrated in Figure 1.

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

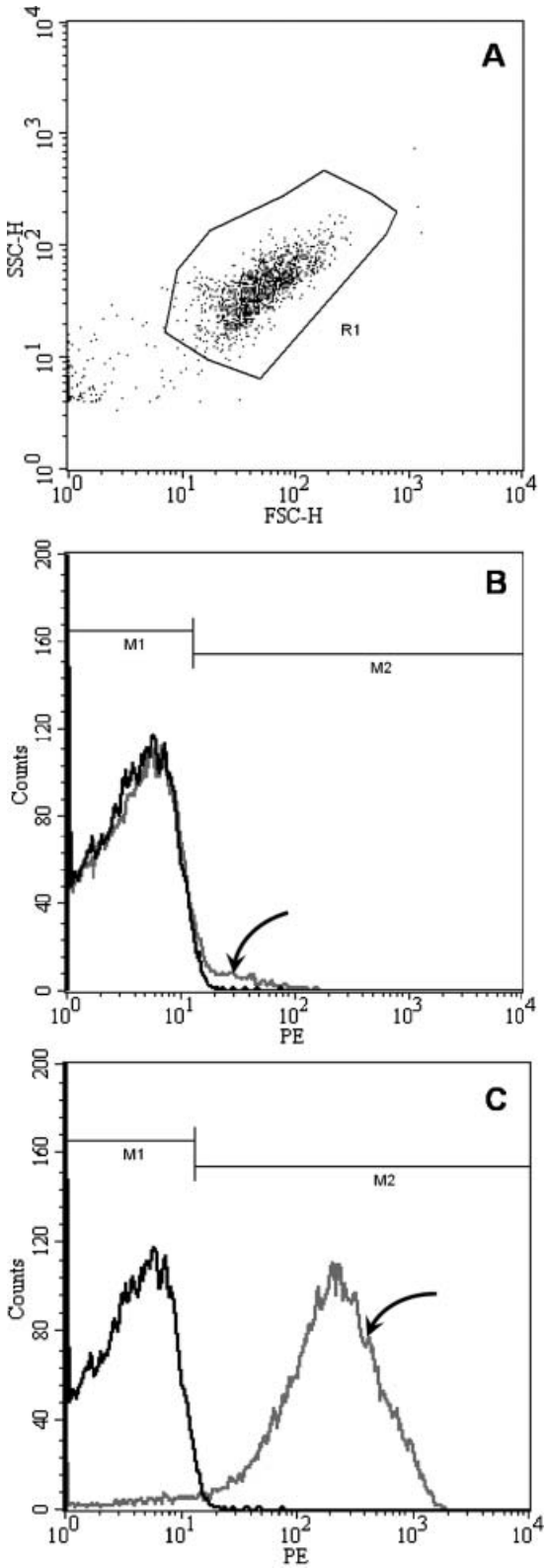

Figure 1. Determination of platelet activation state (PAS) by flow cytometry: an example of a high-responding smoker. Platelets were labeled with PE-conjugated anti-P-selectin antibody and analyzed by cytometry. The subject smoked medium-nicotine cigarettes for 1 h, at which time the first blood sample was drawn. (A) Gating, which was held constant through all subjects and samples in the entire study, is indicated by the region R1. These settings exclude microparticles $<1\,\mu m$. (B) In a plot of fluorescence intensity vs. gated events, the platelet state at that point is shown by the black line (no arrow), with PAS calculated as M2/(M1+M2)=1.1%. After the subject smoked five zero-nicotine cigarettes in 1.5 h, PAS increased to 5.9% (grey line, arrow). (C) In the positive control of TRAP-activated platelets from this subject, PAS increased to 95.9% (grey line, arrow).

Fluorescence intensity was determined for 25,000 events (panel A, region R1). The areas under the black and gray lines (panel B) represent the total fluorescence intensity of the platelets before and after switching to zero-nicotine cigarettes. The number of P-selectin positive platelets (PAS) was determined by the M2 statistic. For the particular individual shown, PAS increased from 1.1% to 5.9% after smoking five zero-nicotine cigarettes. Panel C shows the near-maximal activation of this individual's platelets by TRAP.

Readers unfamiliar with platelets should note that base platelet activation state naturally varies very widely among individuals; the variability in the results presented in this study derives almost entirely from variation in the platelet state, and not the cytometric assay.

Setting

(1) In the smoker study, with 3–5 subjects per session in a well-ventilated room, individual *subjects* were assigned randomly to the zero and medium nicotine groups.
(2) In the SHS exposure study, in a poorly ventilated room, alternating *groups* (two per week) were exposed to smoke containing zero or medium nicotine. The protocols for the respective studies—1 (with smokers) and 2 (with nonsmokers exposed to SHS)—are described in following sections.

Smoker study: Nicotine withdrawal

(1) Baseline or conditioning phase: 32 smokers, in groups of 3–5, each smoked three medium-nicotine Quest 1 cigarettes over 1 h in a well-ventilated room, followed by blood drawing.
(2) Experimental phase: smoking continued at the same rate, when five cigarettes smoked were either medium nicotine (16 subjects; Quest 1) or zero nicotine (16 subjects; Quest 3), over the next 1.5 h. This was immediately followed by a second blood drawing. The cigarette type in the second phase was randomized across all subjects, and within daily groups.

This study design and rationale for the baseline or conditioning phase for both groups is explained as follows. Nicotine is oxidized in the liver to cotinine,

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

and its half-life in the plasma is 1½–2h (Benowitz, Kuyt, & Jacob, 1982). Balancing the ability to confine our subjects against this relatively rapid clearance rate, we thought it should be possible to detect a change in platelet activation state in smokers within 1.5 h after nicotine withdrawal, while they continued to smoke otherwise-identical nicotine-free cigarettes at the same rate. To partly stabilize smoke exposure before the study started, all subjects smoked three medium-nicotine cigarettes over 1 h before the first blood sample was taken. Half the group was then switched to five zero-nicotine cigarettes, while the control group (medium nicotine) continued smoking the five medium-nicotine ones. Smoking continued for 1.5 h.

Nonsmokers: Secondhand smoke (SHS) exposure (zero or medium nicotine) study

Nonsmokers (32) were exposed to high concentrations of either medium-nicotine (16) or zero-nicotine (16) SHS (cigarette type alternating between groups) for 1.5 h on each of two successive days; the SHS produced by a smoking machine (Borgwaldt, Hamburg, Germany) at the rate of 10 cigarettes/h, with a puff frequency of $2\,min^{-1}$ and a puff volume of 35 mL, in a room of $19\,m^3$ ventilated at an airflow of approx. $1.5\,m^3$/min. Although quantitative air smoke measurements were not possible, the equilibrium level of respirable suspended particulates (or RSP) in the room may be estimated as between 200–$300\,\mu g/m^3$, which is close to the levels found in a smoky bar or lounge (Repace & Lowrey, 1980). The PAS measurements therefore represent the extent of platelet activation upon repeated exposure to such environments. On Day One, blood samples were drawn before exposure (A), immediately after 1.5 h exposure (B), and 4 h after exposure (C). The regimen was repeated on Day Two on the same individuals, producing samples D–F.

Statistical analyses

The hypotheses being tested were: (1) PAS increases with time both during smoking (study 1) and on exposure to secondhand smoke (study 2), and (2) most importantly this change in PAS correlates with the nicotine content of the cigarettes. For both studies, statistical analyses were performed with a repeated measures two-way analysis of variance (ANOVA) test, using a statistical software package (SPSS Inc., Chicago, IL). A significance level of $p=.05$ was adopted for all tests. The analyses were performed with repeated measures on PAS with time for each subject (i.e., T=0, 1.5, 5.5, 24, 25.5, 29.5 h for the nonsmoker study; and T=1, 2.5 h for the smoker study) and between two independent groups based on the nicotine content of cigarettes (zero nicotine and medium nicotine). Post-hoc Bonferroni *t* test was conducted to identify differences in PAS at individual time points for the nonsmoker study. Post-hoc paired *t* test was conducted to confirm the difference in Δ(PAS) between the zero nicotine and medium nicotine smoker study groups.

Nicotine analysis

Selected frozen plasma samples (samples D, in EDTA) were analyzed for nicotine by Labstat International ULC (Kitchener, Ontario, Canada), with a detection limit ~20 nmol/L. In the smoker study, plasma samples were taken before and after the 1.5-h smoking period from two randomly-selected subjects in each group (zero and medium nicotine). Similarly in the SHS study, plasma samples were taken before and after the 1.5-h initial smoke exposure from three randomly-selected subjects in each group (zero and medium nicotine). Please note that samples from all subjects in both studies were not analyzed due to financial constraints.

Results

In subsequent sections, we present the results of changes in PAS and its correlation with nicotine content of the cigarettes for the smoker study followed by identical measurements in the nonsmoker study.

Change in plasma nicotine in smokers

We verified qualitatively that cigarette nicotine content rapidly affects plasma nicotine levels by selecting two subjects randomly from each group for plasma nicotine analysis (Figure 2A). In the two control subjects (medium nicotine), nicotine levels rose by 40%; this increase reflected the relatively high smoking rate chosen for these experiments (3.2 cigarettes/h), and a correspondingly high overall smoke exposure. Two subjects who were switched to zero-nicotine cigarettes showed a reduction in plasma nicotine of 64%. Although we were unable to analyze plasma nicotine in all subjects, these data suffice to show that the Quest 3 (“zero nicotine”) cigarettes cause acute reduction in plasma nicotine levels in smokers.

Change in PAS in smokers

Figure 2B shows the corresponding changes in the platelets for all subjects in the two groups. The average increase in PAS (Δ[PAS]) in the medium-nicotine group was 33%, confirming our suspicion that the specified smoking rate was higher than

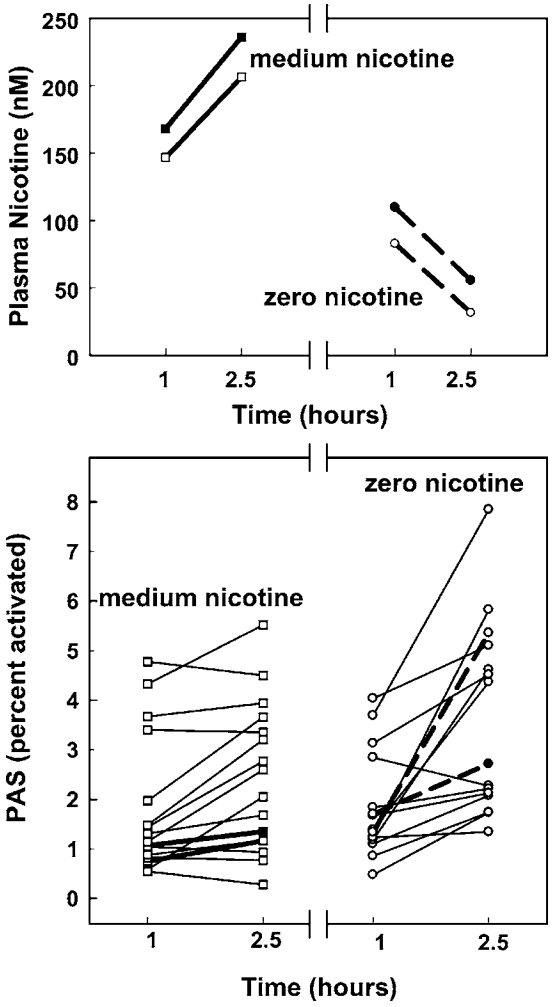

Figure 2. Plasma nicotine levels and platelet activation state in smokers. All subjects smoked three medium-nicotine cigarettes from 0 to 1 h. From 1 to 2.5 h, half the subjects continued to smoke five medium-nicotine cigarettes, and the others smoked five zero-nicotine cigarettes. (A) Plasma nicotine, measured in two randomly selected subjects in each group (top panel). (B) Platelet activation state (P-selectin expression) for all subjects individually (bottom panel). The bold lines in Figure 2B (solid and dashed) show the platelet-activation data for the four individuals who were selected for nicotine analysis, shown in Figure 2A.

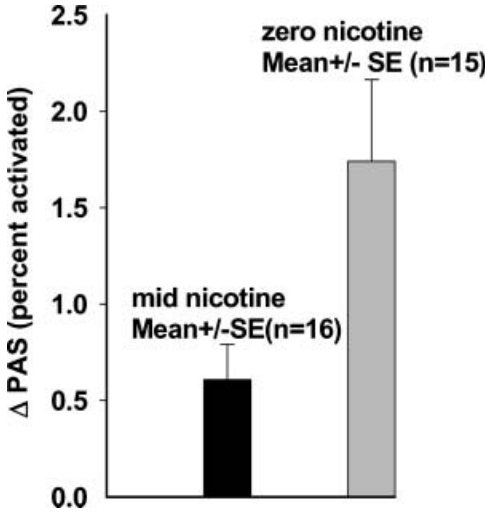

Figure 3. Statistical analysis of platelet activation in smokers of medium- and zero-nicotine cigarettes. The mean arithmetic change in PAS from 1 to 2.5 h within individuals for the two groups (zero and medium nicotine) is shown.

"normal" (cf. nicotine levels, above). In marked contrast, in the zero-nicotine group, PAS increased nearly three times more, by 94%. The mean arithmetic change of platelet activation within individuals in each group is plotted (Figure 3) and computed as $\Delta(\text{PAS}) = 0.61 \pm 0.18$ (SEM) and 1.74 ± 0.42 percentage points for the medium- and zero-nicotine groups respectively.

Statistical analyses show this increase in PAS to be significantly different from the baseline in both groups ($p < .01$). More importantly and central to the hypothesis that nicotine modulates platelet activation, the interaction between multiple measures of PAS with time, and nicotine content of cigarettes (two groups) was highly significant ($p < .02$). We further confirmed the results obtained with repeated measures two-way ANOVA for significant difference of Δ(PAS) between the two groups, with a post-hoc paired t test ($p < .02$; between zero and medium nicotine groups). We may therefore conclude that cigarette nicotine modulates platelet activation *in vivo* in smokers.

Change in plasma nicotine in nonsmokers exposed to SHS

Nicotine levels in plasma samples from three randomly selected subjects in each group were not within the detection range of the analysis procedure ($\leqslant 20$ nmol/L).

Change in PAS in nonsmokers exposed to SHS

Following the smoker phase of the study, we asked whether nicotine might similarly modulate platelet activation in the case of secondhand smoke (SHS) exposure, in which the smoke and nicotine doses are very much lower than in smokers. (Note: in times past SHS has also been called *environmental tobacco smoke*, and exposure to SHS has sometimes been called *passive smoking*.) We extended the study to two days to discern any early effects of repeated exposure. On each day 16 subjects were exposed for 1.5 h to smoke from medium-nicotine cigarettes, and 16 to zero-nicotine smoke. For depiction and analysis, the PAS of the zero-time sample (A) for each individual was subtracted from the remaining data (B–F) (Figure 4).

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

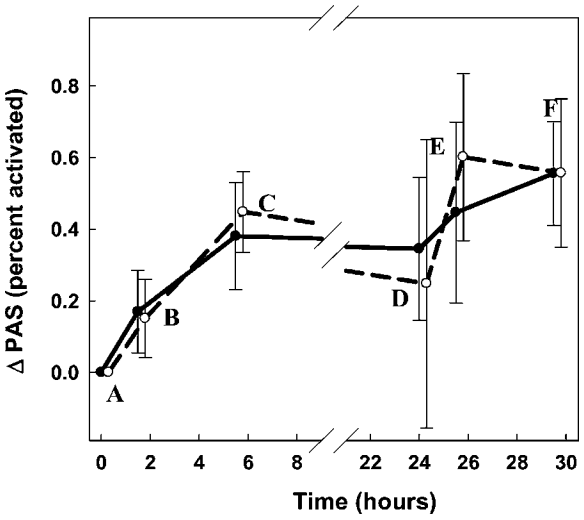

Figure 4. Platelet activation in nonsmokers exposed to secondhand smoke. The average change in PAS over time for nonsmokers exposed to either medium-nicotine cigarette SHS (dashed line), or zero-nicotine SHS (solid line) is shown ± SEM. For each subject the PAS value at 0 time (A) was subtracted from subsequent values (B-F). No statistical difference in extent of platelet activation was found between the medium and zero nicotine groups ($p>.09$). However, platelet activation was highly significant in both groups at 5.5 h (C) and 29.5 h (F) relative to A ($p<.01$).

Statistical analyses with repeated measures two-way ANOVA show that (1) In marked contrast to the protective effect of nicotine in smokers, any nicotine in SHS is irrelevant to platelet activation state across the entire 30-h experimental course. This is confirmed by the lack of statistical significance for the interaction between repeated measures of PAS, and the nicotine content of the cigarettes ($p>.09$). (2) However, the increase in PAS (at time points C and F) was found to be significantly different from the baseline (time point A) in both groups ($p<.01$).

A post-hoc Bonferroni's t test was conducted to determine statistical significance for differences in PAS at individual time points, and the following was found: (1) Over the initial 1.5 h, SHS exposure causes platelet activation, but the change is quite small and, because of the variability, of doubtful significance ($p<.01$). (2) Activation continues unchecked over the next 4 h *after* exposure (A vs. C; $p<.01$). (3) Despite no overnight exposure, this increased activation state substantially persists for 20 h (C vs. D; $p>.08$). (4) A second 1.5-h exposure on Day Two further raises the platelet activation state, although the increase is less than that caused by the Day One exposure. In summary, over the 30-h course, entailing just two 1.5-h exposures to SHS, PAS rose 60%, and this increase is extremely significant (A vs. F; $p<.01$).

Discussion

Nonsmoker study

The observation that nicotine is irrelevant in SHS exposure may appear to be a negative result, but it allows the important conclusions, (1) that it is solely the non-nicotine components of SHS that are activating platelets, and (2) that platelets activated by SHS cannot quickly recover from this insult. We also re-emphasize that the focus here is the baseline platelet state, and not measures of platelet responsiveness to physiological agonists that have been reported in previous studies of SHS exposure (Schmid et al., 1996, Burghuber, Punzengruber, Sinzinger, Haber, & Silberbauer, 1986). In these studies, the inhaled smoke is the agonist, and it is a very substantial one. Our findings confirm the persistence in platelet activation upon repeated SHS exposures to nonsmokers (Schmid et al., 1996). In this previous study, the baseline platelet activation in nonsmokers, after repeated 1 h SHS exposures over 5 days, approached levels found in active smokers. It is also worthwhile to note that platelet activation was indeed the first mechanistic evidence cited for cardiovascular disease risk due to SHS exposures (Barnoya & Glantz, 2005).

Further, the fact that nicotine in cigarette smoke may not be the cause of atherogenesis is additionally supported by a study by Glantz and colleagues (Sun et al., 2001) where rabbits were exposed to SHS (with or without nicotine). They reported that SHS exposure, presumably due to combustion products in the smoke and independent of nicotine, increased arterial lesion lipids (or development of atheromas) in rabbits. Our nonsmoker study therefore confirms previous findings that repeated exposure to SHS leads to persistent and significant platelet activation, and the latter may not be influenced by extremely low levels of nicotine found in SHS.

Smoker study

The irrelevancy of nicotine in SHS contrasts with the major modulatory effect of nicotine that we see in smokers, where both smoke and nicotine doses—and measured plasma nicotine levels—are very much higher. The simplest explanation is that platelets are activated by non-nicotine smoke components in both cases, but only in smokers is the nicotine level high enough to moderate this activation. In simple terms of nicotine binding to a receptor or other effector molecule, a binding constant, K_d, of 20–50 nmol/L could explain such a result.

It should be noted that we did not measure expired carbon-monoxide (CO) levels in the two groups of smokers to account for any compensation that might have occurred as a consequence of

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

nicotine withdrawal. This is a limitation of our smoker study but nevertheless confirms the risk of nicotine withdrawal as a cessation alternative for smokers. Moreover the fact that non-nicotine combustion products of cigarette smoke increase platelet activation is confirmed and also well supported by the SHS (nonsmoker) study findings.

Potentially reduced exposure products (PREPs) and policy considerations

The soundness of "potential reduced-exposure products" (PREPs) (Strasser, Lerman, Sanborn, Pickworth, & Feldman, 2007) that involve tobacco and/or smoke—and many have recently been proposed and manufactured—is not so clear. Apart from being a proposed means of harm reduction (Henningfield et al., 1998; Zacny & Stitzer, 1988), regulation of cigarette nicotine is currently the subject of much political discussion. The goal is to reduce the addictiveness of tobacco products, but it rests on the untested assumption that nicotine reduction is actually beneficial. Vector Tobacco developed a genetically modified nicotine-deficient tobacco for the production of cigarettes with medium, low, and near-zero nicotine levels (trade-name Quest 1, 2, and 3; in our studies we tested Quest 1 or medium-nicotine and Quest 3 or zero/low-nicotine cigarettes), and these are generally classified as PREPs. It was not known until our study whether such PREPs can cause harm.

Pronouncements concerning the negative consequences of cigarette nicotine content, and current political efforts to regulate cigarettes nicotine content, rest on the obvious fact that it is nicotine that causes tobacco dependency. The argument is unassailable when addressing smoking prevention. However, in existing smokers who are unable or unwilling to quit, the key question is harm reduction, and the logic is not so simple. There are two dilemmas that need thinking about. (1) At the levels attained in smokers ($\leqslant 200$ nmol/L), nicotine *per se* is not notably harmful, and this is borne out by the safe record of pure nicotine as a tobacco substitute. Thus, while nicotine is the root addiction that drives smoking, it is non-nicotine smoke constituents that cause the vast majority of smoking-related disease. (2) Nevertheless, the converse of protection by nicotine leads to the second dilemma: for a significant portion of the world's population that consists of smokers, reductions in cigarette nicotine content will cause substantially more platelet activation, and may increase the risk of cardiovascular disease, rather than reduce it. More awkward still, this study suggests that nicotine might exert a statistically significantly protective effect on smokers against part of the cardiovascular risk caused by smoke. We emphasize that platelets are not solely useful experimental markers; their activation is central in thrombogenesis and cardiovascular disease.

This study was small, and focused solely on platelet activation, but it raises concerns about whether nicotine reduction should have any place in discussions about harm reduction in smokers. More studies of *in vivo* responses to nicotine are clearly needed.

Acknowledgments

This study was supported in part by a grant from the Flight Attendants Medical Research Institute to Danny Bluestein. The authors have no competing interests.

References

Ambrose, J. A., & Barua, R. S. (2004). The pathophysiology of cigarette smoking and cardiovascular disease: An update. *Journal of the American College of Cardiology*, *43*, 1731–1737.

Barnoya, J., & Glantz, S. A. (2005). Cardiovascular effects of secondhand smoke: Nearly as large as smoking. *Circulation*, *111*, 2684–2698.

Benowitz, N. L., Kuyt, F., & Jacob, P., III. (1982). Circadian blood nicotine concentrations during cigarette smoking. *Clinical Pharmacology & Therapeutics*, *32*, 758–764.

Brody, J. S., & Spira, A. (2006). State of the art. Chronic obstructive pulmonary disease, inflammation, and lung cancer. *Proceedings of the American Thoracic Society*, *3*, 535–537.

Burger, P. C., & Wagner, D. D. (2003). Platelet P-selectin facilitates atherosclerotic lesion development. *Blood*, *101*, 2661–2666.

Burghuber, O. C., Punzengruber, C., Sinzinger, H., Haber, P., & Silberbauer, K. (1986). Platelet sensitivity to prostacyclin in smokers and non-smokers. *Chest*, *90*, 34–38.

Burns, D. M. (2003). Epidemiology of smoking-induced cardiovascular disease. *Progress in Cardiovascular Diseases*, *46*, 11–29.

Chia, S., & Newby, D. E. (2002). Atherosclerosis, cigarette smoking, and endogenous fibrinolysis: Is there a direct link? *Current Atherosclerosis Reports*, *4*, 143–148.

Chung, I., & Lip, G. Y. (2006). Platelets and heart failure. *European Heart Journal*, *27*, 2623–2631.

Etter, J. F., & Stapleton, J. A. (2006). Nicotine replacement therapy for long-term smoking cessation: A meta-analysis. *Tobacco Control*, *15*, 280–285.

Hagberg, I. A., & Lyberg, T. (2000). Blood platelet activation evaluated by flow cytometry: Optimised methods for clinical studies. *Platelets*, *11*, 137–150.

Henningfield, J. E., Benowitz, N. L., Slade, J., Houston, T. P., Davis, R. M., & Deitchman, S. D. (1998). Reducing the addictiveness of cigarettes. Council on Scientific Affairs, American Medical Association. *Tobacco Control*, *7*, 281–293.

Huo, Y., & Ley, K. F. (2004). Role of platelets in the development of atherosclerosis. *Trends in Cardiovascular Medicine*, *14*, 18–22.

Joseph, A. M., & Fu, S. S. (2003). Safety issues in pharmacotherapy for smoking in patients with cardiovascular disease. *Progress in Cardiovascular Diseases*, *45*, 429–441.

Kappelmayer, J., Nagy, B., Jr., Miszti-Blasius, K., Hevessy, Z., & Setiadi, H. (2004). The emerging value of P-selectin as a disease marker. *Clinical Chemistry & Laboratory Medicine*, *42*, 475–486.

Kereiakes, D. J., & Michelson, A. D. (2006). Platelet activation and progression to complications. *Reviews in Cardiovascular Medicine*, *7*, 75–81.

Lillard, D. R., Plassmann, V., Kenkel, D., & Mathios, A. (2007). Who kicks the habit and how they do it: Socioeconomic differences across methods of quitting smoking in the USA. *Social Science & Medicine*, *64*, 2504–2519.

Malek, L. A., Spiewak, M., Filipiak, K. J., Grabowski, M., Szpotanska, M., & Rosiak, M., et al. (2007). Persistent platelet

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008

activation is related to very early cardiovascular events in patients with acute coronary syndromes. *Kardiologia Polska*, *65*, 40–45.

McNeill, A., Foulds, J., & Bates, C. (2001). Regulation of nicotine replacement therapies (NRT): A critique of current practice. *Addiction*, *96*, 1757–1768.

McRobbie, H., & Hajek, P. (2001). Nicotine replacement therapy in patients with cardiovascular disease: Guidelines for health professionals. *Addiction*, *96*, 1547–1551.

Michelson, A. D. (2006). Evaluation of platelet function by flow cytometry. *Pathophysiology of Haemostasis & Thrombosis*, *35*, 67–82.

Rajagopalan, S., Mckay, I., Ford, I., Bachoo, P., Greaves, M., & Brittenden, J. (2007). Platelet activation increases with the severity of peripheral arterial disease: Implications for clinical management. *Journal of Vascular Surgery*, *46*, 485–490.

Ramachandran, J., Rubenstein, D., Bluestein, D., & Jesty, J. (2004). Activation of platelets exposed to shear stress in the presence of smoke extracts of low-nicotine and zero-nicotine cigarettes: The protective effect of nicotine. *Nicotine & Tobacco Research*, *6*, 835–841.

Repace, J. L., & Lowrey, A. H. (1980). Indoor air pollution, tobacco smoke, and public health. *Science*, *208*, 464–472.

Rubenstein, D., Jesty, J., & Bluestein, D. (2004). Differences between mainstream and sidestream cigarette smoke extracts and nicotine in the activation of platelets under static and flow conditions. *Circulation*, *109*, 78–83.

Schmid, P., Karanikas, G., Kritz, H., Pirich, C., Stamatopoulos, Y., & Peskar, B. A., et al. (1996). Passive smoking and platelet thromboxane. *Thrombosis Research*, *81*, 451–460.

Strasser, A. A., Lerman, C., Sanborn, P. M., Pickworth, W. B., & Feldman, E. A. (2007). New lower nicotine cigarettes can produce compensatory smoking and increased carbon monoxide exposure. *Drug & Alcohol Dependence*, *86*, 294–300.

Sun, Y. P., Zhu, B. Q., Browne, A. E., Sievers, R. E., Bekker, J. M., & Chatterjee, K., et al. (2001). Nicotine does not influence arterial lipid deposits in rabbits exposed to second-hand smoke. *Circulation*, *104*, 810–814.

White, W. B. (2007). Smoking-related morbidity and mortality in the cardiovascular setting. *Preventive Cardiology*, *10*, 1–4.

Zacny, J. P., & Stitzer, M. L. (1988). Cigarette brand-switching: Effects on smoke exposure and smoking behavior. *Journal of Pharmacology & Experimental Therapeutics*, *246*, 619–627.

Downloaded By: [University at Buffalo (SUNY)] At: 15:27 9 November 2008