The *in vitro* effects of niacin on platelet biomarkers in human volunteers

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**Summary**

Niacin is a natural pyridine derivative, proven to favorably modulate the blood lipid profile by increasing levels of high-density lipoprotein (HDL) cholesterol, and by reducing total cholesterol, low-density lipoprotein (LDL) cholesterol, triglycerides, and Lp(a) lipoprotein concentrations. Considering that platelet activity is important in predicting vascular outcomes, and that HDL heavily constitutes platelet cellular membranes, we sought to evaluate the effect of niacin on human platelet activity indices. The blood obtained from 30 aspirin-naïve volunteers was preincubated with escalating concentrations of niacin in vitro. Platelet tests included whole blood and plasma aggregometry, rapid cartridge-based analyser, expression of major surface receptors by flow cytometry, and plasma prostaglandins by ELISA. Preincubation of blood with niacin at 0.3, 1.0 and 3.0 mM resulted in significant inhibition of maximal adenosine diphosphate (ADP)- (p=0.03), and collagen-induced platelet aggregation (p=0.01), and reduced activity by VerifyNow™ (p=0.007) bedside analyser. Surface platelet PAR-1 (MoAb WEDE-15; p=0.04), and vitronectin (CD51/CD61; p=0.02) receptors were up-regulated. Niacin was associated with a two- to three-fold increase of thromboxane B2, prostaglandins D2, and E2. Formation of platelet-monoocyte microparticles (CD14+/CD151), and expression of PECAM-1 (CD31), thrombospondin (CD36), GPIIb/IIIa (CD41a) antigen, and activity with MoAb PAC-1, GPIb (CD42b), P-selectin (CD62p), LAMP-3 (CD63), LAMP-1 (CD107a), CD40-ligand (CD154), GP37 (CD165), were not affected by niacin, suggesting no effect on prostacyclin release. In conclusion, niacin in vitro affects platelet activity by mildly inhibiting aggregation, and stimulating significant prostaglandin release, with mostly intact major platelet receptor expression. The effect of niacin is unique, differs from other known antiplatelet agents, and suggests potential opportunities for therapeutic combination, particularly in patients with low levels of HDL-C. These preliminary data, while intriguing, require confirmation in subjects receiving orally dosed extended-release niacin in order to determine whether these findings are clinically relevant.

**Keywords**

Niacin, lipids, platelets, *in vitro*

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**Introduction**

Current concept of atherothrombosis link abnormalities in the oxidative state of the vascular wall with interactions in the immune system and platelets, leading to a cycle of localised inflammatory, growth, release, and thrombotic responses that result in the maturity and rupture of atherosclerotic lesions (1). Oxidative modification of lipids, particularly oxidised low-density lipoprotein (LDL) cholesterol, is thought to be an important step in the pathogenesis of atherosclerosis (2). It has been documented that lipoproteins modulate the activity of platelets, while elevated levels of LDL increase the sensitivity of platelets to several agonists via an apoB-100-receptor-mediated mechanism, although the platelet plasma membrane receptor activity remains uncharacterised. It is also thought that LDL transfers lipids to the platelet membrane, causing excessive activation (3). By contrast, high-density lipoprotein (HDL) cholesterol, which is also heavily present in the platelet membrane (4), improves membrane fluidity and permeability causing platelet inhibition, and may favourably affect vascular clinical outcomes (5), and even reduce mortality (6).

Niacin, also known for decades as vitamin B3 or nicotinic acid, is an organic naturally occurring compound with the formula C6H5NCO2H being a pyridine derivative (7). Extensive literature has unequivocally shown that niacin favourably modulates the blood lipid profile via reducing concentrations of total cholesterol, LDL-cholesterol, triglycerides, and Lp(a) lipoproteins, and by increasing HDL-cholesterol (8). Niacin is also an established medication when used as either monotherapy, or in combination with statins in patients with low levels of HDL and/or elevated triglycerides.

However, the effects of the agent on the platelet activity are not well defined, or even entirely unknown. Considering the fact that HDL constitutes an integral component of the platelet cellular membranes, HDL augmentation by niacin may represent an alter-
native and attractive (yet unidentified) mechanism of platelet inhibition, and may potentially distinguish therapeutically niacin from other agents affecting platelets activity biomarkers, independent of HDL-induced effects. Therefore, as a first step, we sought to test a hypothesis that niacin in vitro may cause changes in the platelet activity biomarkers.

Methods

Subjects

Thirty volunteers donated blood for the study. All of them underwent pre-employment drug screening. Apparently healthy men and women ≥21 years of age independently from the risk factors defined according to the criteria of the American Heart Association: family history of coronary disease, sedentary life-style, diabetes mellitus, hypertension, obesity, hypercholesterolaemia, postmenopausal or postoophorectomy women, current or recent smokers were included. Exclusion criteria were a history of bleeding diathesis, stroke, major surgery, or significant trauma in the past six months; blood pressure >200/110 mmHg; receiving aspirin, any medications containing aspirin, or any antiplatelet therapy including GPIIb/IIIa inhibitors or thienopyridines within the past two weeks; history of platelet count <100 × 10^9/l; or blood dyscrasia. No subjects were treated with statins. Informed consent was obtained from all participating subjects. Central IRB approval was obtained to conduct the platelet studies (The Quorum IRB, Seattle, WA, USA; Protocol 278154/08).

Samples

Blood samples were obtained for platelet aggregation, rapid analysers, flow cytometry, and ELISA studies. All subjects underwent blood sampling after at least 30 minutes (min) of rest and 2 hours (h) of fasting. Eight tubes (4.5 ml and containing 3.2% sodium citrate) of blood for a total of 36 ml of whole blood-citrate mixture were collected from each participant between 8 a.m. and 10 a.m. Blood sampling after at least 30 minutes (min) of rest and 2 hours (h) of fasting. Eight tubes (4.5 ml and containing 3.2% sodium citrate) of blood for a total of 36 ml of whole blood-citrate mixture were collected from each participant between 8 a.m. and 10 a.m. to avoid diurnal variations. All specimens were obtained by venipuncture after a 4.5 ml discard sample. Considering that niacin is easily soluble in saline, fresh solutions of the niacin powder were prepared ex tempore on the morning during which platelet studies were performed. Whole blood-citrate mixture was incubated for 60 min at 37°C to achieve final concentrations of 0.3, 1.0 and 3.0 mM of niacin, and one portion of blood-citrate mixture was collected for each sample with a Coulter Counter ZM (Coulter Co., Hialeah, FL, USA). Platelet numbers were adjusted to 3.50 × 10^8/ml with homologous platelet-poor plasma (PPP). Platelet aggregation was induced by 5 μM ADP and 5 μg/ml collagen obtained from Chronolog Corporation (Havertown, PA, USA). Aggregation studies were performed using a four-channel Chronolog Lumi-Aggregometer (model 560–Ca). Aggregation was expressed as the maximum percentage of light transmittance change (% max) from baseline at the end of the recording time, using PPP as a reference. Aggregation curves were recorded for 6 min and analysed according to internationally established standards (10). Maximal and final platelet aggregation have been assessed and reported.

Platelet studies

Aggregometry

A. Optical, platelet-rich plasma

The whole blood-citrate mixture was centrifuged at 1,200 g for 5 min in order to obtain platelet-rich plasma, which was kept at room temperature for use within 1 h. Platelet counts were determined for each sample with a Coulter Counter ZM (Coulter Co., Hialeah, FL, USA). Platelet numbers were adjusted to 3.50 × 10^8/ml with homologous platelet-poor plasma (PPP). Platelet aggregation was induced by 5 μM ADP and 5 μg/ml collagen obtained from Chronolog Corporation (Havertown, PA, USA). Aggregation studies were performed using a four-channel Chronolog Lumi-Aggregometer (model 560–Ca). Aggregation was expressed as the maximum percentage of light transmittance change (% max) from baseline at the end of the recording time, using PPP as a reference. Aggregation curves were recorded for 6 min and analysed according to internationally established standards (10). Maximal and final platelet aggregation have been assessed and reported.

B. Impedance, whole blood

The whole blood-citrate mixture was diluted 1:1 with 0.5 ml phosphate-buffered saline and gently swirled. The sample was allowed to warm to 37°C for 5 min then transferred to the assay well. The electrode was then placed in the cuvette, and platelet aggregation was stimulated with 1 μg/ml collagen, and measured in ohms. The change in electrical impedance was recorded as previously described utilizing a Chronolog whole blood aggregometer (11).

VerifyNow-Aspirin Assay

The device (Accumetrics, San Diego, CA, USA), is a cartridge-based rapid analyser using arachidonic acid as agonist is an optical detection system, which measures platelet induced aggregation as an increase in light transmittance. When the activated platelets are exposed to the fibrinogen-coated micro particles, agglutination occurs in proportion to the number of available platelet receptors. The whole blood citrate mixture is being added to the cartridge, and agglutination between platelets and coated beads is being recorded (12), and the results are reported as Aspirin Reactive Units (ARU). The data mirror turbidometric platelet aggregation induced by arachidonic acid and reflect the degree of platelet activity achieved after conventional aspirin.

Whole blood flow cytometry

The surface expression of platelet receptors was determined by flow cytometry using the following monoclonal antibodies: CD 41 antigen (GP IIb/IIIa), CD 42b (GP Ib), CD 62p (P-selectin), PAC-1 (GP IIb/IIIa activity), CD 31 (platelet/endothelial cell adhesion molecule [PECAM]-1), CD 51/CD 61 (vitronectin receptor), CD 63 (LIMP or LAMP-3), CD 107a (LAMP-1), CD 154 (CD40-ligand), CD 165 (GP37) (PharMingen, San Diego, CA, USA); CD36...
(thrombospondin, GPIV), WEDE15, and SPAN12 (Beckman Coulter, Brea, CA, USA). Formation of platelet-leukocyte aggregates was assessed by dual labelling with pan-platelet marker (CD151), and then with CD14, the macrophage receptor for endotoxin lipopolysaccharides. The blood-citrate mixture (50 μl) was diluted with 450 μl Tris-buffered saline (TBS) (10 mM Tris, 0.15 M sodium chloride) and mixed by inverting an Eppendorf tube gently two times. The appropriate primary antibody was then added (5 μl) and incubated at 37°C for 30 min, and then a secondary antibody was applied if needed. After incubation, 400 μl of 2% buffered paraformaldehyde was added for fixation. The samples were analysed on a Becton Dickinson FACScan flow cytometer (San Diego, CA, USA) measuring fluorescent light scatter as previously described (13, 14). All parameters were collected using four-decade logarithmic amplification. The data were collected in list mode files and then analysed. P-selectin was expressed as % positive cells as previously described (15). Other antigens were expressed as log mean fluorescence intensity.

Prostaglandins

The blood-citrate mixture was centrifuged at 3000 g for 15 minutes. The resulting platelet-poor plasma was frozen at –80 °C and batched for further ELISA assays. Platelet-derived thromboxane (TXB2), final breakdown prostacyclin metabolite (6-keto PGF1alpha), prostaglandin E (PGE), and prostaglandin D (PGD) were measured by enzyme-linked immunosorbent assays (ELISA) (R&D Systems, Minneapolis, MN, USA). Each sample was measured in triplicate, and the expected overall intra-assay coefficient of variation was in the range between 2.7% ± 0.4% and 8.1% ± 1.6%, with a plasma recovery rate between 84.2% and 98.6%.

Table 1: Demographics and clinical characteristics.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N=30</th>
</tr>
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<tbody>
<tr>
<td>Age, years</td>
<td>46.4 ± 6</td>
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<tr>
<td>Male</td>
<td>18 (60%)</td>
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<tr>
<td>Ethnic origin</td>
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<tr>
<td>Caucasian</td>
<td>21 (73%)</td>
</tr>
<tr>
<td>African-American</td>
<td>3 (10%)</td>
</tr>
<tr>
<td>Asian</td>
<td>6 (17%)</td>
</tr>
<tr>
<td>Risk factors</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Sedentary life style</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Metabolic syndrome</td>
<td>8 (26%)</td>
</tr>
<tr>
<td>Obesity</td>
<td>12 (40%)</td>
</tr>
<tr>
<td>Current or past smoker</td>
<td>10 (30%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>10 (30%)</td>
</tr>
</tbody>
</table>

Statistical analysis

Analysis of variance was used to define significance of differences in biomarkers between baseline and post-niacin incubation. The Mann-Whitney U test was used to analyse non-parametric data. The differences among flow cytometry histograms were assessed by Smirnov-Kholmogorov test incorporated in the CellQuest™ software (Becton Dickinson). Normally distributed data were expressed as mean ± standard error (M ± SE), and skewed data as median (range). All p-values were two sided. Linear regression analysis was performed using the SPSS v9.0 program (SPSS Inc., Chicago, IL, USA). The chosen power of 90%, and a two-sided α-level of 0.025 has been applied for the multiple planned comparisons according to Bonferroni correction method.

Results

Demographic data and risk factors are presented in Table 1. The study population was unremarkable for the Baltimore metropolitan area. Participants were predominantly Caucasian middle-aged men. Some of the volunteers had a family history of vascular disease, obesity, and attained to sedentary life style, while smoking, disorders of lipid metabolism, and history of hypertension were less frequent.

Platelet data

The combined results of the platelet studies are summarised in Table 2. Preincubation with niacin (1.0–3.0 mM) resulted in a significant inhibition of ADP-(p=0.03), and collagen-induced (p=0.01) platelet aggregation, and reduced activity by VerifyNow™ (p=0.007) bedside analyser. Surface platelet PAR-1 (MoAb WEDE-15; p=0.04), and vitronectin (CD51/CD61; p=0.02) receptors were up-regulated. Niacin cause dose-dependent two- to three-fold increase of thromboxane B2, prostaglandins D2, and E2. Formation of platelet-monocyte microparticles (CD14+CD151), and expression of PECAM-1 (CD31), thrombospondin (CD36), P-selectin (CD62p), LAMP-3 (CD63), LAMP-1 (CD107a), CD40-ligand (CD 154), GP37 (CD165), GP IIb/IIIa (CD41a) antigen, and activity, GPIb (CD42b), so as prostacyclin release were not affected by niacin. Individual data for collagen-induced aggregation with corresponding odds ratios (OR), and confidence intervals (CI) are presented in Figure 1.

Discussion

The data from the present study suggest that niacin in a therapeutic range exhibit a direct inhibitory effect on human platelet activity.
The antiplatelet profile of this agent, at least in vitro, is quite different from the established platelet inhibitors such as aspirin, dipyridamole, or glycoprotein IIb/IIIa inhibitors. In fact, niacin, targets specific combination of platelet indices which is not similar to the in vitro antiplatelet properties of the indirect platelet inhibitors done at the same laboratory with selective serotonin reuptake inhibitors (16), acid ester of probucol (AGI-1067) (17), astaxanthine (18), fibrin-derived peptide FX-06 (19) or angiotensin receptor blockers (20). Interestingly, the lowest used concentration of niacin was not sufficient to cause moderate inhibition of platelet aggregability; therefore, dose-dependency pattern suggests that there may be need for higher levels to achieve the sustained antiplatelet potency with this agent. Considering that niacin is a potent modulator of platelet membrane content, it would be reasonable to expect not only inhibition of pure functional activity, but similar down regulation of platelet receptors. However, surface receptor expression in these normal volunteers was predominantly intact, with the exception of mild up-regulation of G-coupled PAR-1, and vitronectin receptors. It is possible that the in vitro setting may not be suitable for adequate assessment of receptor expression after niacin, or the exposure time was not sufficient to impact such changes.

The clinical benefits of nicotinic acid have been well established for decades. The encouraging results obtained with niacin, especially with the extended-release formulation which both decreases LDL, triglycerides, and Lp(a) lipoprotein concentrations while raising HDL-C, represents an important therapeutic approach in patients with atherosclerosis who exhibit mixed dyslipidaemia. On the other hand, platelet activity is heavily dependent on cell fluidity and microvascular permeability, which in part are

<table>
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<tr>
<th>Biomarker</th>
<th>Baseline</th>
<th>Niacin (mM)</th>
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<tbody>
<tr>
<td></td>
<td>0.3</td>
<td>1.0</td>
</tr>
<tr>
<td><strong>Aggregometry</strong></td>
<td></td>
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</tr>
<tr>
<td>Maximal ADP 5 μM (%)</td>
<td>70 ± 8</td>
<td>70 ± 8</td>
</tr>
<tr>
<td>Final ADP 5 μM (%)</td>
<td>48 ± 9</td>
<td>50 ± 10</td>
</tr>
<tr>
<td>Maximal Collagen 5 μg/ml (%)</td>
<td>89 ± 9</td>
<td>83 ± 8</td>
</tr>
<tr>
<td>Final Collagen 5 μg/ml (%)</td>
<td>68 ± 8</td>
<td>60 ± 9</td>
</tr>
<tr>
<td>Whole Blood 1 μg/ml (ohms)</td>
<td>16 ± 2</td>
<td>13 ± 1</td>
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<tr>
<td><strong>Rapid Bedside Analyzer</strong></td>
<td></td>
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<tr>
<td>VerifyNow (ARU)</td>
<td>590 ± 69</td>
<td>560 ± 64</td>
</tr>
<tr>
<td><strong>Flow cytometry</strong></td>
<td></td>
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<tr>
<td>CD31 (MFI)</td>
<td>73 ± 8</td>
<td>72 ± 8</td>
</tr>
<tr>
<td>CD36 (MFI)</td>
<td>12.7 ± 1.2</td>
<td>12.3 ± 1.9</td>
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<tr>
<td>CD41a antigen (MFI)</td>
<td>478 ± 51</td>
<td>490 ± 52</td>
</tr>
<tr>
<td>CD41a activity (MFI)</td>
<td>9.3 ± 1.3</td>
<td>11.6 ± 1.5</td>
</tr>
<tr>
<td>CD42b (MFI)</td>
<td>309 ± 45</td>
<td>310 ± 31</td>
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<tr>
<td>CD51/61 (MFI)</td>
<td>12.8 ± 1.7</td>
<td>14.8 ± 2.3</td>
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<tr>
<td>CD62p (% positive cells)</td>
<td>11.6 ± 1.3</td>
<td>10.4 ± 1.1</td>
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<tr>
<td>CD63 (MFI)</td>
<td>8.1 ± 0.8</td>
<td>8.5 ± 0.9</td>
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<tr>
<td>CD107a (MFI)</td>
<td>5.8 ± 0.5</td>
<td>5.9 ± 0.6</td>
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<tr>
<td>CD151 + CD14 (MFI)</td>
<td>172 ± 21</td>
<td>177 ± 22</td>
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<tr>
<td>CD154 (MFI)</td>
<td>6.5 ± 1.1</td>
<td>7.1 ± 1.8</td>
</tr>
<tr>
<td>CD165 (MFI)</td>
<td>34 ± 5</td>
<td>34 ± 5</td>
</tr>
<tr>
<td>PAR-1 intact (MFI)</td>
<td>50 ± 5</td>
<td>53 ± 5</td>
</tr>
<tr>
<td>PAR-1 activated (MFI)</td>
<td>36 ± 4</td>
<td>37 ± 4</td>
</tr>
<tr>
<td><strong>ELISA</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thromboxane (pg/ml)</td>
<td>379 ± 39</td>
<td>739 ± 76*</td>
</tr>
<tr>
<td>Prostacyclin (pg/ml)</td>
<td>160 ± 18</td>
<td>139 ± 15</td>
</tr>
<tr>
<td>Prostaglandin D2, (pg/ml)</td>
<td>730 ± 81</td>
<td>942 ± 99*</td>
</tr>
<tr>
<td>Prostaglandin E2 (pg/ml)</td>
<td>399 ± 41</td>
<td>711 ± 74*</td>
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The samples were not pooled, measured individually, and the baseline represents vehicle alone.
directly related to the lipid content of the platelet membranes. Therefore, role of LDL, HDL, and LDL/HDL interplay on platelet indices is critical for better explanation of niacin benefit as an adjunct in patients with vascular disease.

The potential link between lipid biomarkers and platelet activity is not new, but is still a matter of considerable controversy. Some reports suggest that HDL per se exhibited no direct effect on platelet aggregation (21), and may inhibit platelets via stimulation of endothelial release of nitric oxide (NO). Similarly, HDL had no effect on NO-synthase protein expression, although pretreatment of platelets with HDL blocked the stimulation of NO synthase activity (and thus platelet activity) by oxidised-LDL (22). These findings indicate an interaction between LDL and HDL in terms of their effects on platelet activity. In contrast, apolipoprotein E-containing HDL strongly inhibits platelet aggregation by ADP, epinephrine, and collagen, and subsequently stimulates NO synthase activity (23). These results directly implicate the L-arginine NO signal transduction pathway, which may act as an intermediate step in HDL-induced platelet inhibition. Importantly, apoE-containing HDL inhibition of platelet aggregation probably does not go through a platelet cholesterol depletion mechanism (24, 25). It seems that while HDL in general may not inhibit platelet responsiveness to exogenous agonists, proteins specific to subclasses of HDL, such as apolipoprotein may be implicated in inhibition pathways.

Results from the earlier studies, however, demonstrate slightly inconsistent findings suggesting that HDL had the capacity to inhibit LDL binding, and this inhibition was independent of the apoE content of the HDL (26). Interestingly, various subclasses of HDL demonstrate marked differences in their interactions with platelets. HDL3, for example, activates the platelet Na+/H+ exchange with acidification by sodium propionate and thrombin (27). This activity is unaffected by indomethacin, thereby excluding the possibility that cyclooxygenase activity is responsible for this effect, but the presence of GPIIb/IIIa platelet receptor is required. HDL3 has also been implicated in the generation of phosphatidic acid in platelets via phospholipase D activity, and GPIIb/IIIa receptor presence is mandatory as well (28). Also, it has been shown that pretreatment of platelets with HDL in vitro lowered the ionised calcium release (29).

Chemically modified forms of lipoproteins, like those that have been oxidised, are of particular importance when discussing lipoprotein-platelet interactions in atherothrombosis. Altered forms of HDL and LDL are heavily present, and may be important in the pathogenesis of atherosclerotic plaques and thrombosis. It has been demonstrated that oxidised HDL and oxidised LDL both exhibit exaggerated interactions with platelets as compared to their native forms (30). In its native state, LDL increase platelet aggregability, decrease membrane fluidity, increase thromboxane B2 production, and enhance the mobilisation of arachidonate from phospholipids; oxidised LDL can actually induce aggregation on its own and enhances all the above effects. Interestingly, while native HDL inhibit platelet activity and increase membrane fluidity, oxidised HDL actually promote platelet aggregation. In short, membrane fluidity changes that determine the responses of platelets to different lipoprotein signals are the most critical in assessing integrated response.

In terms of direct effects of niacin on platelet activity, the literature is scant. There are some data related to the effects of nicotinic acid on platelets. Importantly, Niaspan® has been associated with small, but statistically significant, dose-related reductions in platelet count with a mean decrease of 11% after 2,000 mg niacin dose (31), which strongly suggests a mild direct antiplatelet effect. Such observations are in agreement with our data. Importantly, we applied niacin concentrations in the range known not to cause any cellular toxicity, and mimicking relevant clinical scenarios with re-

Figure 1: Dots represent individual measures of collagen-induced aggregation in 30 blood samples from healthy volunteers. Samples were pretreated with the vehicle, and escalating concentrations of niacin (0.3; 1.0 and 3.0 mM). Corresponding statistics apply when compared with niacin levels against the vehicle.
gard to optimal safety profile (9). However, there is no definite evidence that the most optimal and clinically relevant concentrations of niacin were used in the index study. Efficacy of these doses of Niaspan® are currently being tested in two large outcome-driven studies, namely, NIH funded AIM-HIGH (NCT00120289), and the University of Oxford HPS2-THRIVE trial (NCT00461630). Nagakawa et al. demonstrated that niacin inhibited ADP-, collagen-, and adrenaline-induced platelet aggregation in vitro (32), which is in full agreement with our index data. Moreover, in patients with cardiovascular disease, the same investigators found that Niaspan® treatment for eight days produced long-lasting (4–8 weeks) inhibition of platelet aggregation. These data led the authors to conclude that extended-release niacin not only decreases platelet activity by indirect action via fluctuating lipoprotein levels (32), but also by inhibiting platelets themselves, possibly by modulating thromboxane synthesis (33), which is likewise concordant with our data. These data were consistent independently of the platelet activity biomarker measured, with the exception of the final extend of ADP-induced platelet aggregation which was not significantly inhibited even for the highest niacin concentration.

The present study revealed that niacin in vitro in clinically relevant concentrations causes a significant dose-dependent release of various prostaglandins. Most of the research, however, is focused on the underlying mechanisms, and platelet involvement in the niacin-induced flushing (e.g. [34]). It is well established that niacin induces flushing through dermal Langerhans cells where the activation of G protein–coupled receptor 109A (GPR109A) increases arachidonic acid and prostaglandin release, especially prostaglandin D2 and prostaglandin E2 (34, 35). Both plasma prostaglandins were significantly and dose-dependently increased in our study supporting the proposed mechanism of niacin action. Importantly, aspirin administration can reduce this flush, presumably by decreasing prostaglandin D2, and potentially serotonin release from macrophages (35).

Lastly, our novel finding of PAR-1 platelet receptor up-regulation by niacin may be related to the similar modulation of the GRP109A which is also G-coupled protein. Considering the key role of PAR-1 receptors in the modulation of platelet-thrombin interplay affecting not only primary haemostasis but also coagulation cascade, anti-PAR-1 properties of Niaspan® may be important. In addition, our data support previous observations that niacin increases platelet thromboxane release (36). In contrast to another earlier report (37), we found no evidence that niacin in vitro affects release of prostacyclin. Considering that prostacyclin is exclusively released by vascular endothelium upon stimulation, it is unclear how its plasma levels can be affected in the in vitro setting with no cell contact with the endothelium.

There are obvious limitations of the present study. We conducted the experiments in the in vitro setting, and are not drawing any clinical conclusions based solely on the index data. Although compatible in size with other similar investigations, this study was performed in a small cohort, so the play of chance represents a plausible alternative explanation, and, therefore, should be considered as pure hypothesis generating. Although study participants were aspirin free, other infrequent concomitant medications may have affected the platelet characteristics; however, the use of an autologous baseline sample as an internal reference minimise these confounding effects. In addition, the expression of multiple activation-dependent platelet receptors was studied but their individual roles are yet unknown. Finally, since the effects of niacin on the vascular wall are critical and prominent, the in vitro setting as used in the present study neglects any potential impact of the endothelium, which will ultimately require an ex vivo study design to confirm the current findings. It will be also important in the future to directly compare the antiplatelet potency and durability profile of niacin with those of established antiplatelet agents such as aspirin, dipyridamole, thienopyridines, and GP IIb/IIIa inhibitors. Importantly, it remains unknown how prominently niacin will impact platelet activity in a clinical setting in patients already treated with conventional antiplatelet agents such as clopidogrel or aspirin. It is possible that the small in vitro effect of niacin on aggregation measurements may not translate into the meaningful ex vivo potency when patients are receiving antiplatelet agent(s). Finally, we did not obtain biochemical characteristics from study participants, some of which (especially lipid profile) may be important in the future to correlate with the platelet activity biomarkers. Further ex vivo studies should be focused on the platelet characteristics in patients with diabetes, metabolic syndrome, which may be modulated by oral niacin. Additional platelet aggregation studies induced by TRAP as a universal “global” marker of platelet reactivity are warranted as well.

We conclude that niacin in vitro affects platelet activity by mildly inhibiting aggregation, and stimulating significant prostaglandin release, but does not measurably affect major surface receptor expression. The effect of niacin differs from other known antiplatelet agents, suggesting potentially important opportunities for therapeutic combination. These preliminary data, while intri-

What is known about this topic?

- Niacin, or nicotinic acid is an established modulator or blood lipids by reducing concentrations of total cholesterol, low-density lipoproteins, triglycerides, and Lp(a) lipoproteins; and by increasing high-density lipoproteins.
- Niacin is also an estimated medication when used as either monotherapy, or in combination with statins.
- It is unclear how niacin affects platelet activity.

What does this paper add?

- Niacin in vitro affects platelet activity by mildly inhibiting aggregation, and stimulating significant prostaglandin release, with mostly intact major platelet receptor expression.
- The effect of niacin is unique, differs from other known antiplatelet agents, and suggests potential opportunities for therapeutic combination, particularly in patients with low levels of high-density lipoproteins.
- These preliminary data, while intriguing, require confirmation in subjects receiving orally dosed extended-release niacin in order to determine whether these findings are clinically relevant.
guing, need to be confirmed in subjects receiving orally dosed extended release niacin (Niaspan®) in order to determine whether these findings are clinically relevant.

Acknowledgements
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