Niacin action in the atherogenic mixed dyslipidemia of metabolic syndrome: Insights from metabolic biomarker profiling and network analysis

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KEYWORDS: ER niacin; Mixed dyslipidemia; Metabolic syndrome; Triglyceride; LDL; HDL; Inflammation; Insulin resistance; Interleukin-6; Apolipoprotein CIII

BACKGROUND: Niacin as an adjunct to statin treatment to reduce cardiovascular risk is questioned.

OBJECTIVE: To evaluate interrelationships between the effects of niacin on mixed dyslipidemia and a spectrum of metabolic and inflammatory biomarkers.

METHODS: Obese, nondiabetic, hypertriglyceridemic males (n = 19) with low high-density lipoprotein–cholesterol levels received extended-release nicotinic acid for 8 weeks. Multiple biomarkers were measured using enzyme-linked immunosorbent assay, enzymatic/absorptiometric, or multiplex biochip assays. Treatment effects were determined for each variable and a differential correlation network created on the basis of univariate correlations between baseline and response to niacin treatment for all pairs of variables.

RESULTS: Extended-release niacin treatment favoured normalization of plasma lipid and apolipoprotein profile. Plasma markers of inflammation, hepatic function, cellular adhesion and proliferation, and macrophage phenotype were attenuated; however, insulin resistance increased. Differential network analysis revealed that changes in triglycerides and high-density lipoprotein–cholesterol were closely linked; equally, niacin mediated reductions in total cholesterol, apolipoprotein B, low-density lipoprotein–cholesterol and lipoprotein(a) clustered together, as did homeostatic model assessment of insulin resistance, insulin, and interleukin-6 levels. Two clusters of inflammatory markers were identified, involving (1) intercellular adhesion molecule 1 and high-sensitive C-reactive protein and (2) soluble tumor necrosis factor receptors; and novel clusters involving matrix metallopeptidase 9 and apolipoprotein E, and...
**Introduction**

Niacin, or nicotinic acid, (vitamin B3) exerts a broad spectrum of lipid-regulating action on both cholesterol and triglyceride metabolism at pharmacological doses.\(^1\)\(^-\)\(^3\) Indeed, the pharmacotherapeutic profile of niacin is relevant to specific patient populations featuring an atherogenic mixed dyslipidemia, including the metabolic syndrome (MetS) and type 2 diabetes.\(^4\)\(^-\)\(^6\) Thus, in a dose-dependent manner, niacin reduces circulating levels of atherogenic apolipoprotein B (apoB)–containing lipoproteins (triglyceride-rich very low–density lipoproteins [VLDLs] and their remnants, low-density lipoproteins [LDLs]), and lipoprotein(a) [Lp(a)] and raises those of potentially atheroprotective apolipoprotein AI (apoAI)–containing high-density lipoprotein (HDL) particles, across a range of dyslipidemic phenotypes.\(^1\)\(^-\)\(^4\) Niacin action involves marked reduction in VLDL-triglyceride production rate, with enhanced clearance of apoB-containing lipoproteins and consequently reduction in circulating triglyceride levels; by contrast, HDL apoAI production and fractional catabolic rate are increased, with net elevation in circulating apoAI concentrations.\(^5\)\(^-\)\(^11\) Niacin equally appears to enhance HDL biogenesis by upregulating adenosine triphosphate binding cassette subfamily A member 1-mediated cellular cholesterol efflux.\(^12\) Concomitantly, however, niacin impairs glucose homeostasis by inducing reduction in hepatic insulin sensitivity, which is intimately associated with increase in insulinemia and glycermia.\(^6\)\(^,\)\(^7\) The mechanisms of niacin action, which are implicated in these effects, are incompletely understood and may involve inhibition of the hormone-sensitive lipase in adipose tissue, or inhibition of the esterification of diacglycerol to triacylglycerol by diacylglycerol O-acyltransferase 2, or perturbation of insulin signalling, or induction of endoplasmic reticulum stress, or a combination of these.\(^6\)\(^-\)\(^15\)

Whether the lipid-modulating effects of niacin may translate into a reduction in cardiovascular risk in mixed dyslipidemic patients displaying the high triglyceride/low HDL-cholesterol (HDL-C) phenotype remains indeterminate.\(^4\) Importantly, this metabolic phenotype is intimately associated with insulin resistance as manifested in prediabetic and diabetic states, both featuring elevated cardiovascular risk.\(^16\) Indeed, a meta-analysis of the effect of nicotinic acid alone or in combination with a statin on cardiovascular events revealed an overall positive effect representing a decrease in cardiovascular outcomes of 25%; furthermore, most niacin-treated patients showed regression of coronary and carotid atherosclerosis.\(^1\)\(^,\)\(^4\)\(^,\)\(^17\)

The use of niacin as an adjunct to background statin treatment to reduce cardiovascular risk has however been brought into question in recent placebo-controlled randomized trials in patients with atherosclerotic vascular disease (Atherothrombosis intervention in MetS with low HDL/high triglycerides: Impact on Global Health Outcomes [AIM-HIGH]; Heart Protection Study 2–Treatment of HDL to reduce the Incidence of Vascular Events [HPS2-THRIVE]), in which ER niacin in combination with intensive statin therapy (plus ezetimibe as required to attain predetermined levels of LDL-cholesterol [LDL-C] or total cholesterol) failed to significantly reduce the risk of major cardiovascular events.\(^18\)\(^,\)\(^19\) To what degree these findings reflect either the low baseline levels of LDL-C (<75 mg/dL) at entry in both trials, or the low on-treatment reduction (6%–7%; placebo-corrected) in LDL-C levels, or the relative paucity of inclusion of subjects presenting a mixed dyslipidemia, or the potential stabilizing effect on lipid-rich plaque of the extensive use of intensive statin therapy (frequently in combination with ezetimibe) in trial participants prior to inclusion, or the association of laropiprant with niacin (HPS2-THRIVE), or a combination of the above, is unresolved.\(^3\) Equally, the possibility that treatment-emergent adverse effects, such as deterioration in glycemic status, increase in new-onset diabetes, deterioration in glycemic control in participants with diabetes at baseline, or a catecholamine surge associated with a switch from fatty acids to glucose in myocardial energy supply in response to evening dosing (and typically involving triggering of a counter-regulatory hormonal response to increase hepatic glucose production), alone or in combination, may have contributed to attenuate potential niacin-derived benefit on cardiovascular outcomes in the AIM-HIGH and HPS2-THRIVE trials cannot be excluded.\(^4\)\(^,\)\(^6\)\(^,\)\(^18\)\(^-\)\(^20\)

The potential for cardiovascular benefit from niacin therapy in subjects displaying the mixed dyslipidemic phenotype may extend beyond its lipid-modulating actions to include its well-documented nonlipid effects.\(^1\)\(^,\)\(^21\)\(^-\)\(^26\) Interestingly, the putative nonlipid-mediated effects of niacin include reduction of vascular inflammation, attenuation of oxidative stress, protection against endothelial dysfunction, antithrombotic actions, and promotion of endothelial progenitor cell–mediated endothelial repair.\(^1\)\(^,\)\(^21\)\(^-\)\(^27\) Moreover, recent evidence has demonstrated a key role for HDL in endothelial-protective functions; in
addition, the impaired endothelial-protective actions of HDL in type 2 diabetes are markedly improved by niacin treatment. The putative cardioprotective benefits of niacin may therefore result from integrated actions on both atherogenic as well as nonatherogenic lipids and lipoproteins, but equally from its nonlipid, off-target actions.

It is of immediate relevance that nicotinic acid can acutely or chronically modulate gene expression and cellular signaling mechanisms in multiple cell types and tissues both by direct and indirect mechanisms. The direct mechanisms are mediated in part by hydroxycarboxylic acid receptor-2 (HCAR2; previously termed GPR109A), a G-protein-coupled receptor. This specific receptor for niacin is expressed abundantly in adipose tissue and transiently inhibits hormone-sensitive lipase to attenuate free fatty acid release. In addition, non-HCAR2-mediated in vivo effects may be mediated either by off-target mechanisms or by changes in levels of plasma free fatty acids, lipids, or hormones.

To evaluate the mechanisms underlying the effects of niacin on hypertriglyceridemia and glucose/insulin homeostasis, a placebo-controlled, randomized, cross-over clinical trial of 8 weeks involving niacin monotherapy was undertaken in subjects with atherogenic mixed dyslipidemia and the risk factor cluster of MetS by the Niacin Study Group. Initial findings documenting reduction in VLDL-triglyceride production, attenuation of insulin sensitivity, and induction of hepatic insulin resistance in this nondiabetic cohort were reported earlier. Furthermore, and to obtain an integrated view of niacin action as it might relate to cardiovascular risk, we concomitantly evaluated on-target action on the plasma lipid, lipoprotein, and apolipoprotein profile together with a targeted biomarker approach to probe nonlipid effects using multiplex arrays. This approach allowed differential network analysis at high stringency, thereby probing effects on insulin resistance, inflammation, hepatic and renal function, cellular adhesion, growth factors, coagulation, and monocyte-derived macrophage phenotype.

Research protocol and methods

Subjects

Twenty healthy, dyslipidemic, nondiabetic, nonhypertensive male volunteers who were nonsmokers and presented the risk factor cluster of MetS (as defined by the criteria of the International Diabetes Federation Task Force Consensus Group) were recruited at 2 Research Centers in Human Nutrition in France: (1) Hôtel Dieu University Hospital, Nantes, and (2) Edouard Herriot University Hospital, Lyon. This clinical trial was registered as NCT01216956 at clinicaltrials.gov. Exclusion criteria included triglycerides >400 mg/dL, hypertension, hyperglycemia (>110 mg/dL), any secondary dyslipidemia, treatment with a lipid-lowering agent in the 4 weeks preceding the study, any chronic medical treatment with an agent affecting lipid metabolism, allergy to aspirin, and excess consumption of alcohol (>20 g/d). All subjects gave written informed consent to participate in the study. The experimental protocol was approved by the Scientific Ethics Committee of the Nantes region (Pays de Loire N°2) and AFSSAPS (French Safety Agency for Health Products) and complied with both the French “Huriet-Serusclat” law and the Second Declaration of Helsinki.

Experimental protocol

The details of the experimental design were reported earlier and involved an 8-week crossover, single-blind, randomized study with an 8-week active treatment period preceded by a washout period of 3 weeks (Fig. S1, Supplementary Materials). For the original metabolic study, sample size determination was based on the ability of ER niacin to increase HDL-C significantly by 20% with a variation coefficient of 15% and α and β error values of 0.05 and 0.01, respectively. With respect to the presented biomarker study, the null hypothesis indicated that effects of ER niacin, which can be expected to be ≥15% for all major lipid classes and all biomarkers, can be detected with P < 0.05 for variables with coefficients of variation (CVs) ≤ 20% in a group of at least 15 subjects. Importantly, inter- and intra-CV values were less than 10% for all biomarker assays (see below). Given that no significant changes were observed in any of the basal anthropometric and metabolic parameters in the placebo arms, we analyzed data from the active arms, that is, at baseline and on treatment. Subjects received 2 g/d of Niaspan (Abbott Laboratories, Rungis, France) in the evening in addition to 300 mg/d of aspirin. The dose of nicotinic acid (as Niaspan) was initiated at a starting dose of 0.5 g/d for the first week (Fig. S1, J0) and progressively titrated upward from 0.5 g/wk to 2 g/d at the start of week 4 (Fig. S1, Supplementary Materials). Fasting blood samples were withdrawn at baseline (J0) and at completion of the active niacin and placebo arms at 8 weeks (W8) in the Clinical Unit from the cubital vein into precooled, EDTA-containing Vacutainer tubes (final concentration: 1 mg/mL) at baseline and at day 53 of each 2-month period after the final intake of Niaspan at 8 PM on the preceding day. Plasmas were rapidly separated by centrifugation and frozen at −80°C until analysis.

Anthropometric measurements, analyses of plasma lipids, lipoproteins, apolipoproteins, and biomarker profiling were performed at baseline and after 8 weeks of niacin/aspirin treatment. On the basis of concomitant elevation of biomarkers of liver disease and systemic inflammation, 1 patient was excluded from the cohort; experimental findings are therefore presented for n = 19 patients. For comparative purposes, plasma samples from age-matched, healthy, normolipidemic, normotensive, nondiabetic, normoglycemic nonobese, male control subjects (n = 20) who were nonsmokers were retrieved from the biobank at National Institute for Health and Medical Research Unit 939 and analyzed with the same procedures as patient samples.
Analytical methods

Plasma lipid (triglycerides, total cholesterol, HDL-C, and LDL-C) and apolipoprotein (apoAI and apoB) analyses were performed using standard methods on a KoneLab 20 Autoanalyzer (Thermo Fisher, Illkirch, France) with DiaSys Diagnostic System reagents and corresponding reference standards (Tours, France) as described earlier. Apo-CIIII (BioScience Innovations, Interchim, Montlucon, France), apolipoprotein E (apoE; DiaSys Poles, Condum, France), neopterin (Brahms France, Clichy, France), and gamma-glutamyltransferase (GGT) and total bilirubin (Thermo Fisher, Illkirch, France) were quantified by enzyme-linked immunoassays.

Biomarker profiling

The following multiplex (biochip) arrays (Randox Laboratories, Crumlin, N. Ireland) were used to evaluate the biomarker profile in plasma samples:

1. High Sensitivity Cytokine Array I: interleukin (IL)-2, IL-4, IL-6, IL-8, IL-10, vascular endothelial growth factor (VEGF), interferon gamma (IFNG), tumour necrosis factor alpha, IL-1A, IL-1B, monocyte chemoattractant protein-1 (MCP1), and epidermal growth factor (EGF).
2. Adhesion Protein Array: vascular cell adhesion molecule-1 (VCAM1), intercellular adhesion molecule 1 (ICAM1), and E, L, and P selectins
3. Cardiac + Array: creatine kinase MB type (CKMB), myoglobin (MYO), glycogen phosphorylase BB (GPBB), heart-type fatty-acid binding protein (H-FABP), carbonic anhydrase III (CAIII), and troponin I, cardiac (CTNI)
4. Cytokine Array IV: soluble interleukin-2 alpha, soluble interleukin-6 receptor, soluble tumor necrosis factor receptor 1 (sTNFRI), sTNFRII, and matrix metallopeptidase 9 (MMP-9)
5. Cytokine Array V: IL-3, IL-7, IL-12p70, IL-13, and IL-23
6. Cerebral II Array: C-reactive protein (CRP), D-dimer (D-DMER), neuron-specific enolase (NSE), neutrophil gelatinase-associated lipocalin (NGAL), sTNFRI, and thrombomodulin (TM)
7. MetS Array I: C-peptide (CPEP), ferritin (FERR), IL-6, resistin, insulin, tumour necrosis factor alpha, IL-1A, leptin, and phosphoribosylanthranilate isomerase 1
8. MetS Array II: Adiponectin, Cystatin C, high-sensitive C-reactive protein

Intra- and inter-assay CVs for all of these biomarkers were in the range from 4% to 15%. Biomarkers whose values were below the limit of detection at both baseline and on-treatment time points were excluded. Additional details of this biochip array technology, of the sensitivities of these assays, and of the lower limit of the sensitivity of the respective biomarkers are included in the manufacturer’s instructions for use and in the Supplementary Materials.

Statistical analyses

Data are presented as mean ± standard error of the mean. Data were analyzed by either the nonparametric Wilcoxon test or Student’s t-test (as indicated in tables) using GraphPad (GraphPad Software Inc, La Jolla, CA). First, the differences between baseline and on-treatment samples were calculated. The normality for the distribution of differences was tested by calculating skewness (S) and kurtosis (K) as well as their standard errors (SE₅ and SE₆). Normality was tested using the test statistics abs(S/SES) < 1.96 and abs((K-3)/SEK) < 1.96. Data were compared between baseline and on-treatment samples using a paired t-test for normally distributed differences or by Wilcoxon signed rank sum test for non-normally distributed differences as indicated in Table 1. P-values were adjusted, based on q-value analysis (R-package QVALUE). Briefly, variables were selected to be included in the analysis based on a priori knowledge about their function, and therefore statistical tests cannot be assumed to be independent. Traditional adjustment for multiple tests may therefore be too conservative regarding false discovery rate. Instead, the q-values, which control the false discovery rate, were calculated. In Table 1, q-values ≤ 0.05 were considered significant.

Differential correlation networks

Differential correlation networks were constructed from the correlation matrix of the differences between baseline and niacin treatment. Pairwise correlations between all 49 variables were initially calculated. Significant correlations/edges between biomarkers/nodes were included in the model if (1) at least one of the biomarkers was significantly changed on niacin treatment (see Table 1) and (2) the P-value for the correlation was lower than the prespecified value (either 0.001 or 0.01).

To further strengthen the stringency of the differential network, we used a “leave-one-out approach.” The network was reconstructed 19 times with 1 observation (ie, data from 1 individual) omitted at each iteration. Only edges that were consistently reconstructed in the networks were included in the final network. Additional details of differential correlation network construction are provided in the Supplementary Materials. In addition, the correlation heatmap was constructed using correlations between all biomarkers but equally included those which did not change on niacin treatment.

Results

Patient characteristics at baseline

All subjects (n = 19) concomitantly presented abdominal obesity, an atherogenic mixed dyslipidemia and insulin resistance (mean age: 47 ± 13 years; body mass index: 32 ± 2.4 kg/m²; waist circumference: 106 ± 5.2 cm;
Table 1  Impact of ER niacin treatment in metabolic syndrome: Effect on biomarkers of lipids and lipoproteins, insulin resistance, inflammation, hepatic function, adhesion molecules, macrophages, growth factors, and coagulation after 8 wk (W8) of ER niacin treatment (see Fig. S1, Supplementary Materials)

<table>
<thead>
<tr>
<th>Biological pathway</th>
<th>Biomarker</th>
<th>Method</th>
<th>Before</th>
<th>After</th>
<th>% Change</th>
<th>Q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipids and lipoproteins</td>
<td>Triglycerides</td>
<td>PA</td>
<td>210 ± 15.3</td>
<td>145 ± 14.9</td>
<td>-31</td>
<td>0.003†</td>
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<td></td>
<td>NEFA</td>
<td>ELISA</td>
<td>0.43 ± 0.04</td>
<td>0.48 ± 0.07</td>
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<td>0.19</td>
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<tr>
<td></td>
<td>Total cholesterol</td>
<td>PA</td>
<td>201 ± 8.1</td>
<td>172 ± 7.0</td>
<td>-14</td>
<td>0.001†</td>
</tr>
<tr>
<td></td>
<td>LDL-C</td>
<td>PA</td>
<td>125 ± 6.7</td>
<td>103 ± 6.2</td>
<td>-17</td>
<td>0.001†</td>
</tr>
<tr>
<td></td>
<td>ApoB</td>
<td>PA</td>
<td>120 ± 5.2</td>
<td>95 ± 4.5</td>
<td>-21</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td></td>
<td>HDL-C</td>
<td>PA</td>
<td>41.7 ± 8.5</td>
<td>47.2 ± 8.1</td>
<td>13.2</td>
<td>&lt;0.01†</td>
</tr>
<tr>
<td></td>
<td>HDL-C/ApoAI ratio</td>
<td>Calculated</td>
<td>0.32 ± 0.00</td>
<td>0.37 ± 0.01</td>
<td>18</td>
<td>&lt;0.001†</td>
</tr>
<tr>
<td>Insulin resistance</td>
<td>Adiponectin</td>
<td>ARRAY</td>
<td>2.3 ± 1.5</td>
<td>3.9 ± 1.7</td>
<td>72</td>
<td>0.002**</td>
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<td>HOMA-IR</td>
<td>Calculated</td>
<td>5.80 ± 0.91</td>
<td>7.67 ± 1.01</td>
<td>32</td>
<td>0.015†</td>
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<td>Insulin</td>
<td>ARRAY</td>
<td>13.0 ± 1.8</td>
<td>16.0 ± 1.7</td>
<td>22</td>
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<td>C-peptide</td>
<td>ARRAY</td>
<td>1.36 ± 0.15</td>
<td>1.64 ± 0.16</td>
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<td>0.07</td>
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<td>Leptin</td>
<td>ARRAY</td>
<td>0.61 ± 0.08</td>
<td>0.73 ± 0.09</td>
<td>20</td>
<td>0.09*</td>
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<tr>
<td></td>
<td>Glucose</td>
<td>PA</td>
<td>98 ± 2.2</td>
<td>104 ± 2.3</td>
<td>6</td>
<td>0.006†</td>
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<td>Resistin</td>
<td>ARRAY</td>
<td>3.42 ± 0.20</td>
<td>3.24 ± 0.19</td>
<td>-5</td>
<td>0.20</td>
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<td>Inflammation</td>
<td>IL-1α</td>
<td>ARRAY</td>
<td>7.2 ± 1.8</td>
<td>8.2 ± 2.6</td>
<td>14</td>
<td>0.20*</td>
</tr>
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<td>IL-6</td>
<td>ARRAY</td>
<td>1.6 ± 0.26</td>
<td>1.8 ± 0.19</td>
<td>11</td>
<td>0.16*</td>
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<td>SIL6R</td>
<td>ARRAY</td>
<td>1.5 ± 0.16</td>
<td>1.5 ± 0.18</td>
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<td>TNF-α</td>
<td>ELISA</td>
<td>9.8 ± 0.75</td>
<td>10.0 ± 1.11</td>
<td>2</td>
<td>0.28*</td>
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<tr>
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<td>Lp-PLA2</td>
<td>ELISA</td>
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<td>130 ± 8.8</td>
<td>-8</td>
<td>0.07</td>
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<td>PAI-1</td>
<td>ARRAY</td>
<td>80.0 ± 7.7</td>
<td>70.4 ± 8.2</td>
<td>-12</td>
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<td>TNF-α</td>
<td>ARRAY</td>
<td>15.3 ± 1.5</td>
<td>13.4 ± 1.4</td>
<td>-12</td>
<td>0.16*</td>
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<td>TNFRII</td>
<td>ARRAY</td>
<td>0.54 ± 0.06</td>
<td>0.47 ± 0.04</td>
<td>-12</td>
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<td>TNFRI</td>
<td>ARRAY</td>
<td>0.39 ± 0.03</td>
<td>0.34 ± 0.03</td>
<td>-14</td>
<td>0.017†</td>
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<td></td>
<td>IL-7</td>
<td>ARRAY</td>
<td>12.0 ± 1.03</td>
<td>8.8 ± 0.84</td>
<td>-26</td>
<td>0.005†</td>
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<td>CRP</td>
<td>ARRAY</td>
<td>2.8 ± 0.59</td>
<td>1.8 ± 0.27</td>
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<td>0.034†</td>
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<td>hsCRP</td>
<td>ELISA</td>
<td>2.7 ± 0.55</td>
<td>1.6 ± 0.25</td>
<td>-40</td>
<td>0.017†</td>
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<td>Hepatic function</td>
<td>Homocysteine</td>
<td>ELISA</td>
<td>7.6 ± 0.41</td>
<td>9.6 ± 0.44</td>
<td>26</td>
<td>&lt;0.001†</td>
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<td>FABP</td>
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<td>1.3 ± 0.08</td>
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<td>Bilirubin</td>
<td>PA</td>
<td>7.0 ± 0.73</td>
<td>6.3 ± 0.95</td>
<td>-10</td>
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<td>Ferritin</td>
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<td>145 ± 22.5</td>
<td>125 ± 20.7</td>
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<td>0.16</td>
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<tr>
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<td>GGT</td>
<td>PA</td>
<td>52.5 ± 7.8</td>
<td>36.7 ± 5.2</td>
<td>-30</td>
<td>&lt;0.001†</td>
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<td>Adhesion molecules</td>
<td>VCAM-1</td>
<td>ARRAY</td>
<td>585 ± 36.5</td>
<td>568 ± 42.9</td>
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<td>L-SEL</td>
<td>ARRAY</td>
<td>1096 ± 67.9</td>
<td>993 ± 63.8</td>
<td>-9</td>
<td>0.014†</td>
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<td>ICAM-1</td>
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<td>E-SEL</td>
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<td>19.9 ± 1.52</td>
<td>17.5 ± 1.23</td>
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<td>P-SEL</td>
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<td>196 ± 11.6</td>
<td>160 ± 10.1</td>
<td>-18</td>
<td>&lt;0.001†</td>
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<td>Macrophages</td>
<td>Cystatin C</td>
<td>ARRAY</td>
<td>256.1 ± 122</td>
<td>285.4 ± 148</td>
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<td>0.26*</td>
</tr>
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<td>MCP-1</td>
<td>ARRAY</td>
<td>157 ± 15.6</td>
<td>149 ± 19.6</td>
<td>-5</td>
<td>0.14*</td>
</tr>
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<td>Neopterin</td>
<td>ELISA</td>
<td>5.4 ± 0.24</td>
<td>4.8 ± 0.39</td>
<td>-10</td>
<td>0.10*</td>
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<td>MMP-9</td>
<td>ARRAY</td>
<td>17.6 ± 4.4</td>
<td>13.3 ± 3.1</td>
<td>-25</td>
<td>0.031†</td>
</tr>
<tr>
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<td>NSE</td>
<td>ARRAY</td>
<td>21.3 ± 2.7</td>
<td>14.4 ± 2.0</td>
<td>-32</td>
<td>0.006†</td>
</tr>
<tr>
<td>Growth factors</td>
<td>VEGF</td>
<td>ARRAY</td>
<td>38.2 ± 4.5</td>
<td>35.2 ± 6.2</td>
<td>-8</td>
<td>0.055*</td>
</tr>
<tr>
<td></td>
<td>EGF</td>
<td>ARRAY</td>
<td>55.5 ± 9.6</td>
<td>35.0 ± 8.2</td>
<td>-37</td>
<td>0.002†</td>
</tr>
</tbody>
</table>

(continued on next page)

plasma triglycerides: 217 ± 72 mg/dL; LDL-C: 125 ± 6.7 mg/dL; HDL-C: 33 ± 5.9 mg/dL; Lp(a): 27.2 ± 7.7 mg/dL; apoB: 120 ± 5.2 mg/dL; apoAI: 127 ± 3.7 mg/dL; homeostatic model assessment of insulin resistance [HOMA-IR]: 6.1 ± 4.2); they displayed the risk factor cluster of MetS but had no history of cardiovascular disease. These values are consistent with those published earlier for the entire group (n = 20).
Effects of ER niacin on anthropometric parameters, lipids, lipoproteins, and apolipoproteins

No changes in body mass index or waist circumference were observed in this male cohort following niacin treatment. Niacin treatment significantly attenuated the mixed dyslipidemic profile, including reduction in atherogenic apoB-containing lipoprotein lipids (triglycerides, −31%; LDL-C, −17%; and Lp(a), −21%), and in apolipoproteins B, CIII, and E (range −20% to −25%) (Table 1). Although no significant change in plasma apoAI levels occurred, the HDL cholesterol/apoAI ratio increased significantly reflecting a 13% increment in HDL-C (Table 1).

Effects of ER niacin on nonlipid plasma biomarkers

As indicated in Table 1, niacin exerted significant effects on circulating levels of biomarkers of insulin resistance, inflammation, adhesion molecules, macrophage activation, growth factors, and hepatic function in obese subjects presenting dyslipidemia and MetS.

Integrated response of plasma biomarkers to niacin treatment

Clustering at high stringency (P < .001)

At a significance level of 0.001, 7 distinct biomarker clusters were identified (data not shown). First, decrement in triglyceride levels was strongly negatively correlated with increment in those of HDL-C; furthermore, the HDL-C/apoAI ratio was integral to this cluster. Reduction in total cholesterol and LDL-C was the main drivers of a cluster integrating reductions in apoB and Lp(a); apoAI was significantly associated with this cluster despite lack of response to niacin treatment.

A third cluster indicative of insulin resistance status featured HOMA-IR, insulin, and IL-6 levels. However, fasting glucose levels were independent of this cluster, despite significant elevation on treatment (Table 1). On-treatment reduction in apoE levels did not correlate to changes in any lipid parameter but rather clustered with those of circulating MMP-9, a matrix-remodelling metalloprotease produced by monocyte-derived macrophages and smooth muscle cells.

In a separate cluster, elevation in adiponectin on treatment correlated with a nonsignificant increment in cystatin C. Two distinct clusters of inflammatory markers were identified: one focused on ICAM1 and CRP and the other on TNF receptors.

Remarkably, and despite a 20% decrease on treatment concomitant with those in atherogenic lipoprotein lipids, changes in apo-CIII levels were independent of all other biomarkers. Similarly, circulating levels of the cell adhesion proteins, E-SEL, L-SEL, and P-SEL, fell upon treatment but were independent of other niacin-induced marker modifications.

With respect to biomarkers associated with hepatic function and metabolism, GGT levels decreased independently while those of homocysteine increased on treatment.

The NSE, a potential marker of macrophage activation, was independently reduced. EGF, which together with EGF-like ligands may be implicated in accelerated vascular disease and chronic inflammation, was independently and markedly reduced by niacin treatment. Moreover, levels of IL-7, a cytokine contributing to humoral inflammation, were also independently decreased.

Clustering at intermediate stringency (P < .01)

Definition of biomarker clustering at the significance level of 0.01 revealed significant relationships between components of the clusters, which were not linked at the higher stringency (Fig. 1, dashed lines). One major cluster involved linkage of the 2 key lipid and apolipoprotein clusters together and in addition, integrated the apoE/MMP-9
Furthermore, IL-6 was critical in linking the insulin resistance cluster to the axis of the principal lipoprotein-lipid and apolipoprotein cluster through total cholesterol, LDL-C, and Lp(a). Similarly increments in adiponectin linked the adiponectin/cystatin-C cluster to that constituted of the main atherogenic lipoprotein/lipids (total cholesterol, LDL-C, Lp(a), and apoB).

The second major cluster linked the 2 inflammatory clusters. The niacin-mediated reduction in Apo-CIII was linked to the entire inflammatory cluster through CRP (Fig. 1).

A correlation heatmap shows the correlation structure among all variables, including those that did not change with treatment (Fig. 2). It should be noted that correlations between biomarkers, which did not change with treatment, likely reflect their internal association, independent of niacin treatment.

Discussion

The present studies are novel in featuring an integrated biomarker approach to analyze the effects of ER niacin in monotherapy on lipid and lipoprotein metabolism, insulin resistance, inflammation, cellular adhesion molecules, macrophage activation, growth factors, coagulation, and hepatic function in an obese patient cohort with MetS. This patient group displayed an atherogenic mixed dyslipidemia involving subnormal levels of HDL-C, hypertriglyceridemia, elevated levels of small dense LDL (M.J. Chapman and A. Kontush, unpublished findings), and insulin resistance (Table 1). It is notable that the Framingham study has confirmed that major elevation in cardiovascular risk is a characteristic of subjects with this phenotype. Overall, our findings indicate that ER niacin treatment substantially attenuated the dyslipidemic profile, induced increment in insulin resistance as estimated by the HOMA-IR index, exerted attenuation of a spectrum of proinflammatory cytokines, cytokine receptors, and biomarkers of systemic inflammation, downregulated circulating levels of both macrophage activation markers and cell adhesion proteins, and reduced levels of the potentially prooxidant enzyme γ-glutamyltransferase. In addition, niacin reduced plasma levels of epidermal growth factor, a
A comprehensive correlation heatmap summarizing the impact of niacin treatment on biomarkers relevant to cardiovascular risk in metabolic syndrome. Plasma biomarkers (denoted in abbreviated form on horizontal and vertical axes) that were modified significantly on niacin treatment at W8 are represented in black, and those which were unchanged in grey (see Table 1); the stringency level is $P < .01$. Colored squares in the heatmap indicate positive (green) or negative (red) correlations. The strength of the correlations was progressively graded, with stronger correlations being denoted in a darker color (see vertical graded color scale at right). After leave-one-out testing of the robustness of the networks, correlations with $P \geq .01$ are denoted with black dots. Parameters are ordered using hierarchical-clustering with distances calculated using the “complete” method. PAI-1, phosphoribosylanthranilate isomerase 1; ApoE, apolipoprotein E; MMP-9, matrix metalloproteinase 9; GGT, gamma-glutamyltransferase; RETN, resistin; FERR, ferritin; IL, interleukin; EGF, epidermal growth factor; LEPT, leptin; VCAM1, vascular cell adhesion molecule 1; LDL-Chol, low-density lipoprotein–cholesterol; ApoAI, apolipoprotein AI; ApoB, apolipoprotein B; Lp(a), lipoprotein(a); HOMA, homeostatic model assessment; INS, insulin; CPEP, C-peptide; TM, thrombomodulin; ApoC-III, apolipoprotein CIII; MCP1, monocyte chemoattractant protein 1; HDL-C, high-density lipoprotein–cholesterol; NSE, neuron-specific enolase; VEGF, vascular endothelial growth factor; TNFR1, soluble tumor necrosis factor receptor 1; TNFRII, soluble tumor necrosis factor receptor II; FABP, fatty-acid binding protein; ICAM-1, intercellular adhesion molecule 1; L-SEL, selectin L; P-SEL, selectin P; E-SEL, selectin E; SIL6R, soluble interleukin 6 receptor; DDMER, D-dimer; CRP, C-reactive protein; hsCRP, high-sensitive C-reactive protein; H-cys, homocysteine; Lp-PLA2, lipoprotein-associated phospholipase A2; NEFA, non-essential fatty acids; tot-TG, total triglycerides; TNFa, tumor necrosis factor alpha.

Two aspects of our findings are of special interest: (1) the relevance of the overall profile of ER niacin action on lipid and nonlipid biomarkers and biologically active factors to the pathophysiology of atherosclerotic vascular disease (ASCVD) and cardiovascular risk, particularly in light
of the ongoing controversy surrounding recent findings on the effect of niacin on cardiovascular outcomes in randomized, placebo-controlled, prospective trials involving combination with statins or statin plus laropiprant, that is, AIM-HIGH and HPS2-THRIVE, respectively, and (ii) the profile of biomarker clusters in relation to the known niacin receptor-dependent (HCAR2) and receptor-independent actions.

With respect to potential cardiovascular benefit, the present study documents a significant degree of normalization of the plasma levels of atherogenic apoB-containing lipoproteins, including Lp(a). Indeed, baseline levels of LDL-C decreased by 17% from a baseline of 125 mg/dL; given that LDL is causal in the pathophysiological pathway of ASCVD, such a reduction—if maintained over a 5 year period—might provide a relative risk reduction of the order of 10% in a composite CV endpoint if transposed to the CTT regression line established on the basis of the statin intervention trials. Moreover, the numerically greater decrease in apoB (−21%) relative to that in LDL-C is suggestive of preferential depletion of atherogenic cholesterol-poor, small dense LDL, the metabolic fingerprint of niacin on LDL metabolism; indeed, our unpublished data (M. J. Chapman and A. Kontush) reveal significant shift in the LDL profile to larger particles on ER niacin. Importantly, niacin-mediated decrease in small LDL levels is correlated with arteriographic and clinical outcome benefit.

Minor elevation in nonatherogenic HDL-C equally occurred, but in the absence of change in apoAI, thereby suggesting a shift in the HDL profile to larger, cholesterol-rich particles. In this context, it is relevant that niacin-induced HDL markedly improved endothelial function and repair in patients with type 2 diabetes and low HDL-C and equally that ER-niacin administered to patients with low HDL-C and coronary artery disease improved endothelial function in the INEF (“Impact of Niacin on Endothelial Function”) study. Furthermore, an inverse relationship was seen between HDL-C and carotid plaque wall area when evaluated by magnetic resonance imaging in patients treated with niacin at 6 months in the Oxford Niaspan Study. In addition, we observed decreases of 20% or more in circulating apolipoprotein CIII (apoC-III) and apoE concentrations, 2 apolipoproteins recently linked to cardiovascular risk in the Bruneck cohort. Furthermore, decrement in these 2 apolipoprotein components of remnant lipoproteins is consistent with the marked reduction (36%) seen in remnant lipoprotein-cholesterol (calculated from Table 1 as total cholesterol–LDL-C–HDL-C). Significantly, these changes were observed in a patient population whose mixed dyslipidemia was matched to the therapeutic action of niacin. Finally, it has become clear that the lipid efficacy of niacin is independent not only of its action at the HCAR2 receptor but also of its ability to suppress, transiently, free fatty acid flux arising through inhibition of HCAR2-mediated adipose tissue lipolysis. The overall evidence then concerning niacin-mediated changes in the lipid profile in mixed dyslipidemia favors an antiatherogenic effect, although the precise molecular mechanisms underlying these effects remain elusive.

Additional metabolic changes were detected under ER niacin treatment, which is relevant to the pathophysiology of atherosclerosis. These included significant reductions in circulating levels of inflammatory markers, cell adhesion proteins, and macrophage activation markers (Table 1). The monocyte-derived macrophage is a key cell in driving both cholesterol accumulation and immune-inflammatory processes within the developing atherosclerotic plaque. Significant reduction in plasma metalloprotease-9 and in NSE concentrations under niacin treatment is indicative on the one hand of attenuated remodelling of extracellular matrix, potentially favoring plaque stability, and on the other, of reduction in macrophage activation. Attenuation of monocyte-macrophage activation is also consistent with reduction in L-selectin expression, a key monocyte-macrophage adhesion protein, which binds to a mucin-like glycoprotein on endothelial cells, and with reduced diapedesis of monocytes into the arterial intima as a consequence of reduction in E-selectin expression by endothelial cells. Levels of the cell surface glycoprotein, ICAM-1, were equally reduced, a signal suggesting integrated down-regulation of immune-mediated and inflammatory processes and which may enhance L-selectin–mediated reduction in trans-endothelial migration of monocytes and other leucocytes. In addition, fall in P-selectin levels may be indicative of a lower propensity for platelets to interact with the arterial endothelium, which itself expresses P-selectin as a receptor for monocytes and neutrophils. These preceding interpretations are of course subject to the limitation that the circulating pool of selectins and ICAM-1 represents the sum of the liberation of these proteins from a wide range of cell types. Finally, reduction in circulating EGF, one of the multiple ligands of the EGF receptor expressed on vascular smooth muscle cells, endothelial cells, and monocyte-macrophages, is suggestive of an additional dimension of niacin-mediated downregulation of vascular inflammation.

Oxidative stress, involving oxidative intracellular signaling, is an integral feature of accelerated atherogenesis in prediabetic and diabetic subjects; indeed, oxidative biomarkers can be considered to integrate risk factor action at the arterial wall. GGT is one such biomarker, possessing negative prognostic power for ischemic heart disease. This enzyme has been localized to plaque tissue and degrades glutathione, thereby disrupting redox balance. Therefore, it is of interest that GGT activity was markedly reduced (−30%) on niacin treatment, suggestive of attenuated intra-plaque oxidation of atherogenic lipoproteins such as LDL.

There is abundant evidence to substantiate the role of elevation in plasma homocysteine as an independent, causal cardiovascular risk factor (Table 1), a role explained by both proatherogenic and prothrombotic actions. A unifying mechanistic hypothesis for such actions is however lacking, although available evidence implicates
endothelial dysfunction as a consequence of impaired nitric oxide activity.\textsuperscript{42}  

Considered together, the plasma biomarker profile re-inforces earlier evidence that ER niacin exerts a plethora of antiatherogenic and cardioprotective actions on the arterial wall and potentially on the atherosclerotic plaque. These effects are focused on key cell types integral to the vasculature, and notably endothelial cells, but equally attenuate the inflammatory potential of immunoinflammatory cell types, which may enter the intima at sites of endothelial dysfunction and lesion predilection, including monocytes and other leucocytes.

In contrast to the multiple cardioprotective effects of ER niacin, the earlier findings of Blond \textit{et al.}\textsuperscript{5} in this cohort documented induction of hepatic insulin resistance, with alteration in insulin signalling and accumulation of diacylglycerol in hepatocytes; the potential regulatory role of diacylglycerol on insulin action remains indeterminate. A possible dissociation of the effects of niacin on lipid metabolism and insulin action was however suggested. Insulin resistance is a multifacetted syndrome, but an integral feature is elevated cardiovascular risk driven by dysregulation of fatty acid metabolism in adipose tissue with translation into an atherogenic mixed dyslipidemia.\textsuperscript{43} This dimension of niacin action, considered together with the potentially deleterious induction of elevation in homocysteine levels, may counterbalance much of the potentially beneficial actions on the vascular system discussed previously.

With respect to the identification of biomarker clusters on niacin treatment, and of the possibility of differentiating niacin-receptor dependent from nonreceptor-dependent mechanisms, high-stringency analysis delineated 2 lipid-centered clusters, one involving a strong negative correlation between triglycerides and HDL-C and the second involving total cholesterol, LDL-C, apoB, and Lp(a). Recent evidence allowed exclusion of the HCAR2 receptor in these effects.\textsuperscript{15}

A cluster of HOMA-IR, insulin, and IL-6 suggested that despite minor, nonsignificant changes in plasma IL-6 levels, IL-6 was nonetheless intimately related to insulin levels and insulin resistance. A definitive role for IL-6 in the induction of insulin resistance in adipocytes by impairing insulin signalling, and action has been documented.\textsuperscript{44}

Of the remaining biomarkers identified by regression network analysis at high stringency, the adiponectin–cystatin C cluster was notable, particularly in view of the recent finding that these proteins are complexed in plasma.\textsuperscript{45} The finding that cystatin C is an inhibitor of adiponectin-mediated vasculoprotective effects suggests that the marked increment in adiponectin levels seen on niacin (72%), and thus its potential biological actions, were largely neutralized by complexation with cystatin C.\textsuperscript{45} This effect is of considerable relevance to the potential attenuating impact of niacin on the pathophysiology of cardiometabolic disease, as adiponectin is an insulin sensitizer, modulates inflammatory responses, and promotes macrophage polarization to the antiinflammatory M2 phenotype.\textsuperscript{46} Indeed, the induction of insulin resistance by ER niacin in this MetS cohort was not attenuated by major increment in adiponectin (Table 1).\textsuperscript{6}

At intermediate stringency ($P < .01$), interaction was identified between individual clusters, which were independent at $P < .001$. Thus, the apoB/LDL-C cluster interacted significantly with the triacylglycerol/HDL-C cluster and was linked to the insulin resistance cluster through IL-6. IL-6 represents a focal point of the inflammatory cytokine cascade and was 1.5-fold elevated at both baseline and on treatment relative to control subjects. Consistent with this phenotype at baseline, CRP levels were more than 2.5-fold elevated relative to controls. One interpretation of these findings is that expansion of visceral adipose tissue led to secretion of large amounts of IL-6, and that in addition, apoB-containing particles, including those transporting triglycerides, were major drivers of IL-6 production by plaque macrophages upon cholesterol accumulation and transformation to foam cells.\textsuperscript{47,48} Furthermore, the minor cluster involving apoE and MMP-9 interacted with both major lipid clusters, a finding in part reflecting the association of apoE with both VLDL and HDL, but also perhaps the coproduction of apoE and MMP-9 by the proinflammatory macrophage.\textsuperscript{3,40}

Whereas apoC-III presented as an isolated cluster at $P < .001$, interaction with CRP, ICAM-1, TNF receptors, GGT, and E-selectin occurred at lower stringency. Furthermore, variability in the response to niacin was pronounced with respect to both apoC-III and CRP, which showed a negative correlation, despite significant mean decrements in each case. These findings are of interest given evidence for the promotion of inflammation and endothelial cell dysfunction by apoC-III.\textsuperscript{49} On the other hand, a putative contribution of the observed reduction in apoC-III to enhanced catabolism of triglyceride-rich lipoproteins and remnants on niacin treatment is indeterminate.

Our biomarker approach precludes definitive identification of the receptor-dependent or receptor-independent mechanisms underlying the niacin phenotype documented here in obese subjects with mixed dyslipidemia and the MetS. Nonetheless, there is an emerging evidence base for the direct action of niacin on a wide spectrum of cells and tissues via the HCAR2 receptor.\textsuperscript{1,22–26,29,30} However, we cannot exclude indirect cascade effects on vascular or other biomarkers as the consequences of an attenuated dyslipidemic profile.

\textbf{Conclusions and limitations}

The present bioarray approach to circulating biomarker status, in association with determination of the lipid/apolipoprotein profile, has generated valuable information regarding a multiorgan perspective of the action of ER niacin in a small group of obese, insulin-resistant males presenting MetS and an atherogenic mixed dyslipidemia; indeed, such a patient phenotype is well matched to niacin action.\textsuperscript{4} Our approach provided an extensive baseline
phenotype and allowed evaluation of therapeutic response. Its further development to include biomarkers indicative of adverse effects to this multifaceted agent on a personalized patient basis may be of interest given the distinct clinical profile of ER niacin therapy.

Our findings are based on a modelling strategy, and as such are hypothesis generating. A substantially larger study over a prolonged period of time in dyslipidemic subjects with an expanded panel of biomarkers is therefore now warranted, on the one hand to validate our findings, but equally to evaluate the potential relationships between biomarker changes on ER niacin monotherapy and significant side effects specific to such treatment. Indeed, such a study would likely further our understanding of side effects specific to ER niacin as compared to those observed when niacin is used either on a background of statin therapy (as in AIMP-HIGH), or a background of both a statin and laropiprant (as in HPS2-THRIVE).18,19

Any comment on the relevance of our present findings to the failure of the AIMP-HIGH and HPS2-THRIVE trials involving statin-niacin combinations to provide cardiovascular benefit would be premature. Nonetheless, biomarker profiling has revealed a distinct atheroprotective benefit/risk ratio for ER niacin, insulin resistance, and homocysteine elevation predominating in the risk component. One may predict that balance or imbalance in this ratio may be critical to any potential overall cardiovascular benefit derived from niacin treatment. Finally, it is poignant that insight into niacin-mediated improvement in HDL function and its relevance to vasculoprotection is critically lacking herein; moreover, HDL-C levels cannot be considered as surrogates for such functionality.28

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Authors’ contributions: MK and ML coordinated the clinical protocol. PR conducted all biochemical assays. MA performed statistical and network cluster analyses. All authors contributed to data interpretation. MJC, MA, and JB composed the manuscript. All authors have read and approved the final manuscript.

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Supplementary data

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