Vascular endothelial function and oxidative stress are related to dietary niacin intake among healthy middle-aged and older adults

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Kaplon RE, Gano LB, Seals DR. Vascular endothelial function and oxidative stress are related to dietary niacin intake among healthy middle-aged and older adults. J Appl Physiol 116: 156–163, 2014. First published December 5, 2013; doi:10.1152/japplphysiol.00969.2013.—We tested the hypothesis that vascular endothelial function and oxidative stress are related to dietary niacin intake among healthy middle-aged and older adults. In 127 men and women aged 48–77 yr, brachial artery flow-mediated dilation (FMD) was positively related to dietary niacin intake [%change (Δ): r = 0.20, P < 0.05; mmΔ: r = 0.25, P < 0.01]. In subjects with above-average dietary niacin intake (≥22 mg/day, NHANES III), FMD was 25% greater than in subjects with below-average intake (P < 0.05). Stepwise linear regression revealed that dietary niacin intake (above vs. below average) was an independent predictor of FMD (%Δ: β = 1.8; mmΔ: β = 0.5, both P < 0.05). Plasma oxidized low-density lipoprotein, a marker of systemic oxidative stress, was inversely related to niacin intake (r = −0.23, P < 0.05) and was lower in subjects with above- vs. below-average niacin intake (48 ± 2 vs. 57 ± 2 mg/dl, P < 0.01). Intravenous infusion of the antioxidant vitamin C improved brachial FMD in subjects with below-average niacin intake (P < 0.001, n = 33), but not above-average (P > 0.05, n = 20). In endothelial cells sampled from the brachial artery of a subgroup, dietary niacin intake was inversely related to nitrotyrosine, a marker of peroxynitrite-mediated oxidative damage (r = −0.30, P < 0.05, n = 55), and expression of the prooxidant enzyme, NADPH oxidase (r = −0.44, P < 0.01, n = 37), and these markers were lower in subjects with above- vs. below-average niacin intake [nitrotyrosine: 0.39 ± 0.05 vs. 0.56 ± 0.07; NADPH oxidase: 0.38 ± 0.05 vs. 0.53 ± 0.05 (ratio to human umbilical vein endothelial cell control), both P < 0.05]. Our findings support the hypothesis that higher dietary niacin intake is associated with greater vascular endothelial function related to lower systemic and vascular oxidative stress among healthy middle-aged and older adults.

flow-mediated dilation; NADPH oxidase; oxidized low-density lipoprotein

AGING IS THE PRIMARY RISK factor for cardiovascular diseases, and this is attributable in part to the development of vascular endothelial dysfunction, as indicated by impaired endothelium-dependent dilation (EDD) (7, 31, 44). EDD is reduced even in healthy middle-aged and older (MA/O) adults compared with young adult controls (10, 44, 51). However, substantial intra-individual variability in EDD exists among healthy MA/O adults (10, 23, 44, 57). As such, identifying both lifestyle and biological factors that contribute to this variability is an important biomedical goal, as it may lead to strategies to prevent and/or reverse vascular dysfunction in this at-risk group.

Our laboratory has shown previously that EDD is related to specific dietary factors among MA/O adults (24). One dietary factor that may influence EDD in this group is niacin intake. Niacin is a vitamin precursor of nicotinamide adenine dinucleotide and as such is involved in the regulation of diverse cellular functions, including reduction-oxidation reactions and stress resistance (8, 60). Indeed, higher amounts of niacin in the diet protect against reactive oxygen species (oxidative stress)-mediated impairments in EDD in rabbits (59). Oxidative stress is a fundamental process mediating endothelial dysfunction with age (17, 44, 50), and reducing oxidative stress could be an important mechanism by which niacin exerts beneficial effects on the vascular endothelium. Consistent with this, clinical studies have demonstrated that dietary interventions incorporating increased niacin intake reduce circulating markers of oxidative stress (1, 33). Evidence from preclinical models suggests that niacin may suppress oxidative stress by decreasing the expression and activity of the principal oxidant-producing enzyme, NADPH oxidase (4, 11, 48), the expression of which increases in the vascular endothelium with age, even in healthy adults (17, 40).

In the present study, we tested the hypothesis that higher dietary niacin intake is associated with greater brachial artery flow-mediated dilation (FMD), a commonly used measure of EDD (9), and lower oxidative stress in MA/O adults free from clinical disease. To test this hypothesis, we determined dietary niacin intake and FMD in a group of 127 healthy adults aged 48–77 yr, as well as in subgroups with dietary niacin intake greater than or equal to vs. less than 22 mg/day, i.e., at or above compared with below the average dietary niacin intake in adults 40–80 yr of age in the Third National Health and Nutrition Examination Survey (NHANES III) (2). Circulating oxidized low-density lipoprotein (LDL) was assessed to characterize systemic oxidative stress (29, 38). To determine the role of dietary niacin on vascular oxidative stress, FMD was assessed in the presence and absence of a supraphysiological dose of the potent antioxidant, vitamin C, in subsets of subjects with above- and below-average dietary niacin intake. We also measured the abundance of the marker of peroxynitrite-mediated oxidative damage, nitrotyrosine, and expression of the oxidant-producing enzyme, NADPH oxidase, in endothelial cells obtained from the brachial artery of subgroups varying in dietary niacin intake. Finally, we determined the expression and estimated activation of the nitric oxide-producing enzyme, endothelial nitric oxide synthase (eNOS), in biopsied endothelial cells to better characterize the molecular events underlying a potential relation between dietary niacin intake and EDD.

METHODS

Subjects. A cohort from our laboratory database (n = 127) previously assessed for endothelial function were used for this analysis. All subjects were healthy, nonsmoking men and postmenopausal women between the ages of 48 and 77 yr, who were free of clinical disease as

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assessed by medical history, physical examination, blood chemistries, electrocardiogram, resting blood pressure, and cardiovascular responses to a graded exercise test. Subjects were not taking medications and refrained from all dietary supplements for 2 wk before participation in the study. All procedures were approved by the Institutional Review Board at the University of Colorado at Boulder. The nature, risks, and benefits of all study procedures were explained to volunteers, and their written, informed consent was obtained before participation in the study.

Procedures. All testing was performed at the Clinical Translational Research Center (CTRC) at the University of Colorado Boulder following a 12-h fast from food and caffeine and 24-h abstinence from exercise and alcohol. Postmenopausal status was confirmed by absence of menstruation for >12 mo and follicular stimulating hormone levels >40 IU/L (12).

Subject clinical characteristics and blood assays. Blood pressure was measured over the brachial artery during seated rest using a semiautomated device (Dynamap pro 100, GE, Health Care). The nature, risks, and benefits of all study procedures were explained to volunteers, and their written, informed consent was obtained before participation in the study.

Dietary analysis. Dietary niacin intake was estimated from the Block 1998 food-frequency questionnaire (NHANES III food-intake database, below-average niacin intake n = 21, above-average niacin intake n = 25) or a 3-day diet record (The Food Processor 8.2, ESHA Research, below-average niacin intake n = 55, above-average niacin intake n = 26) and analyzed by a CTRC bionutritionist, as previously described by our laboratory (19, 39). Niacin intake from the food-frequency questionnaire was summed over all foods based on the US Department of Agriculture Food and Nutrient Database for Dietary Studies. Niacin intake from 3-day diet records was calculated as niacin (mg) + [tryptophan (mg)/60]. Individuals estimated to consume greater than or equal to vs. less than 22 mg/day were divided into above-average (n = 51) and below-average (n = 76) dietary niacin subgroups, respectively, based on the NHANES III database (2). The nomenclature for FMD and endothelium-independent dilation. Duplex ultrasonography (Power Vision 6000, Toshiba; multifrequency linear-array transducer) was used to assess brachial artery FMD and endothelium-independent dilation (brachial artery dilation in response to 0.4-mg sublingual nitroglycerin), as described previously by our laboratory (19, 23, 24). To ensure subject safety, endothelium-independent dilation was only assessed in individuals who had a resting supine systolic blood pressure >100 mmHg. In a subset of subjects (below-average niacin intake n = 33, above-average niacin intake n = 20), FMD measurements were made during saline infusion (control) and during supraphysiological intravenous infusion of vitamin C, as described previously by our laboratory (19). Brachial artery FMD is reported as absolute (mm²) and percent change (%Δ) in accordance with recent guidelines (15, 21). In a subset of subjects (n = 38), time-averaged peak hemodynamic shear rate was calculated as (8 × time-averaged peak velocity)/occlusion diameter, based on a large, centered sample volume from the first 10 velocity envelopes (first 15 s) following cuff release per recent recommendations (21).

Vascular endothelial cell protein markers. Nitrotyrosine (1:300; Abcam, Cambridge, MA) and NADPH oxidase-p47phox (1:1,500; Millipore, Billerica, MA), eNOS (1:650; BD Biosciences, San Jose, CA), and phosphorylated eNOS (p-eNOS, serine 1177; 1:650; Millipore, Billerica, MA) protein expression was assessed in subsets of subjects (nitrotyrosine: n = 55; NADPH oxidase-p47phox: n = 37, eNOS/p-eNOS: n = 21) by quantitative immunofluorescence in endothelial cells obtained from brachial artery sampling, as described previously by our laboratory (16–18). All protein expression data are reported as human endothelial cell intensity relative to human umbilical vein endothelial cell (HUVEC) intensity. Normalization to HUVEC protein expression provides a control to account for potential variations in staining intensity between staining sessions.

Data analysis. Statistical analyses were performed in SPSS (IBM SPSS Statistics 19). Bivariate relations were assessed by Pearson correlation analysis and between-group differences were determined by t-tests for independent samples. Repeated-measures ANOVA was performed to determine the group (below- or average-average dietary niacin intake) by condition (saline or vitamin C) interaction for FMD, and paired t-tests were used to assess within-group differences in the case of a significant interaction. Backwards multiple linear regression was used to evaluate the independent relation between dietary niacin intake and FMD, while controlling for clinical and dietary characteristics (P > 0.10 for elimination). The Kolmogorov-Smirnov test was used to assess normality. Nonnormal variables were square root or log-transformed, depending on the level of positive skew. Because transforming nonnormal variables did not change the nature or significance of any bivariate relations or group differences, these data are presented in standard units. Statistical significance was set at P < 0.05 for all analyses.

RESULTS

Clinical and dietary characteristics. Clinical characteristics for all subjects and below- and above-average dietary niacin groups are presented in Table 1. The below- and above-average dietary niacin groups did not differ in systolic blood pressure; body mass; body mass index; total, HDL, or LDL cholesterol; triglycerides; or fasting glucose (all P > 0.05). There were small (5–6%) group differences in age and diastolic blood pressure (P < 0.01), and the above-average dietary niacin group was more physically active and had a higher percentage of men.

Macronutrient intake for all subjects and below- and above-average dietary niacin groups are presented in Table 2. The below- and above-average dietary niacin groups did not differ in relative carbohydrate, protein, or fat intake (all P > 0.05).

Table 1. Clinical characteristics of subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Below-Average Intake</th>
<th>Above-Average Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>127</td>
<td>76</td>
<td>51</td>
</tr>
<tr>
<td>Sex (men/women)</td>
<td>71/56</td>
<td>36/40</td>
<td>35/16*</td>
</tr>
<tr>
<td>Age, yr</td>
<td>60±1</td>
<td>61±1</td>
<td>58±1‡</td>
</tr>
<tr>
<td>Systolic BP, mmHg</td>
<td>119±1</td>
<td>118±1</td>
<td>120±2</td>
</tr>
<tr>
<td>Diastolic BP, mmHg</td>
<td>74±1</td>
<td>72±1</td>
<td>76±1</td>
</tr>
<tr>
<td>Body mass, kg</td>
<td>72±1</td>
<td>71±2</td>
<td>74±2</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>24.3±0.3</td>
<td>24.4±0.4</td>
<td>24.2±0.5</td>
</tr>
<tr>
<td>Physical activity, MET h/wk</td>
<td>50±4</td>
<td>42±5</td>
<td>62±7*</td>
</tr>
<tr>
<td>Total cholesterol, mg/dl</td>
<td>199±2</td>
<td>190±3</td>
<td>197±4</td>
</tr>
<tr>
<td>HDL cholesterol, mg/dl</td>
<td>59±1</td>
<td>60±2</td>
<td>57±2</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>118±2</td>
<td>117±3</td>
<td>118±3</td>
</tr>
<tr>
<td>Triglycerides, mg/dl</td>
<td>105±4</td>
<td>101±5</td>
<td>110±7</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>89±1</td>
<td>89±1</td>
<td>90±1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of subjects; BP, blood pressure; MET, metabolic equivalent; HDL, high-density lipoprotein; LDL, low-density lipoprotein. *P < 0.05 and †P < 0.01 vs. low-niacin group.
had lower levels of circulating oxidized LDL compared with the group also had higher intake of potassium, sodium, iron, zinc, vitamin A, vitamin E, and vitamin B6 (all \( P < 0.01 \)). There were no group differences in calcium or vitamin C intake (\( P > 0.05 \)).

**Dietary niacin intake and brachial artery FMD.** In all subjects, brachial artery FMD was positively related to dietary niacin intake (\( \Delta \): \( r = 0.20, P < 0.05 \); mm\( \Delta \): \( r = 0.25, P < 0.01 \)). Dietary niacin intake remained a significant independent predictor of FMD when controlling for clinical and dietary characteristics (\( \Delta \): \( r = 0.23, P < 0.05 \); mm\( \Delta \): \( r = 0.28, P < 0.01 \), Table 4). In subjects with above-average dietary niacin intake, FMD was 25\% (mm\( \Delta \)) higher compared with the below-average dietary niacin group (both \( P < 0.05 \), Fig. 1). Group differences in brachial artery FMD also remained significant when controlling for clinical and dietary characteristics (\( \Delta \): \( \beta = 1.8 \); mm\( \Delta \): \( \beta = 0.05 \), both \( P < 0.05 \), Table 4).

Endothelium-independent dilation was not related to dietary niacin intake (\( P > 0.05 \)) and did not differ between below- and above-average dietary niacin groups (\( \Delta \): 25 \pm 1 vs. 27 \pm 1; mm\( \Delta \): 0.89 \pm 0.03 vs. 0.95 \pm 0.04, both \( P > 0.05 \)). Similarly, baseline brachial artery diameter and time-averaged peak shear rate were not related to dietary niacin intake (\( P > 0.05 \)) and did not differ between below- and above-average dietary niacin groups (baseline diameter: 3.5 \pm 0.1 vs. 3.6 \pm 0.1 mm; shear rate: 804 \pm 43 vs. 889 \pm 63 s\(^{-1} \)), both \( P > 0.05 \).

**Dietary niacin intake and circulating oxidized LDL.** Oxidized LDL was inversely related to dietary niacin intake (\( r = -0.23, P < 0.05 \)). Similarly, the above-average niacin group had lower levels of circulating oxidized LDL compared with the below-average niacin group (48 \pm 2 vs. 57 \pm 2 mg/dl, \( P < 0.01 \); Fig. 2).

**Dietary niacin intake and oxidative stress-related suppression of FMD.** In the subset of subjects in whom supraphysiological concentrations of the antioxidant vitamin C were infused to determine the influence of oxidative stress, baseline brachial FMD was \( \sim 50\% \) higher in the above- vs. below-average niacin intake group (\( P < 0.01 \), Fig. 3). Vitamin C infusion improved brachial FMD in subjects with below-average dietary niacin intake by 30\% (mm\( \Delta \)) to 32\% (mm\( \Delta \)) (both \( P < 0.001 \)), but had no effect on FMD in subjects with above-average dietary niacin intake (\( P > 0.05 \); \( P < 0.05 \) for group \( \times \) condition interaction, repeated-measures ANOVA). Based on these responses to vitamin C infusion, oxidative stress-related suppression of FMD accounted for 56\% (mm\( \Delta \)) to 60\% (mm\( \Delta \)) of the difference in baseline FMD between the below- and above-average dietary niacin subgroups.

**Dietary niacin intake and vascular endothelial cell protein markers of oxidative stress.** Dietary niacin intake was inversely related to the abundance of nitrotyrosine (\( r = -0.30, P < 0.05 \)) and NADPH oxidase expression (\( r = -0.44, P < 0.01 \)) in endothelial cells sampled from the brachial artery. Similarly, the above-average niacin intake group had lower endothelial cell abundance of nitrotyrosine (0.39 \pm 0.05 vs. 0.56 \pm 0.07 ratio to HUVEC control, \( P < 0.05 \)) and expression of NADPH oxidase (0.38 \pm 0.05 vs. 0.53 \pm 0.05 ratio to HUVEC control, \( P < 0.05 \); Fig. 4) compared with the below-average niacin intake group.

**Dietary niacin intake and vascular endothelial cell eNOS expression and activation.** Endothelial cell expression of eNOS and the ratio of p-eNOS to eNOS was not related to dietary niacin intake and did not differ between below- and above-average dietary niacin groups (eNOS: 0.37 \pm 0.07 vs. 0.41 \pm 0.06, p-eNOS:eNOS: 0.86 \pm 0.19 vs. 0.67 \pm 0.12, both \( P > 0.05 \)).

**DISCUSSION**

This is the first study to demonstrate that vascular endothelial function (EDD as assessed by brachial artery FMD) is related to dietary niacin intake among healthy MA/O adults. We also show that this relation is independent of vascular smooth muscle sensitivity to a nitric oxide donor (endothelium-independent dilation), indicating an effect of niacin specific to the vascular endothelium. Importantly, our findings implicate

### Table 2. Macronutrient intake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Below-Average Intake</th>
<th>Above-Average Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total kilocalories, kcal/day</td>
<td>2127 \pm 52</td>
<td>1932 \pm 63</td>
<td>2401 \pm 73*</td>
</tr>
<tr>
<td>Carbohydrate intake, %total kcal/day</td>
<td>50.4 \pm 0.8</td>
<td>51.1 \pm 1.0</td>
<td>49.5 \pm 1.2</td>
</tr>
<tr>
<td>Protein intake, %total kcal/day</td>
<td>15.9 \pm 0.3</td>
<td>15.5 \pm 0.4</td>
<td>16.5 \pm 0.4</td>
</tr>
<tr>
<td>Fat intake, %total kcal/day</td>
<td>33.7 \pm 0.6</td>
<td>33.4 \pm 0.8</td>
<td>34.0 \pm 1.0</td>
</tr>
</tbody>
</table>

Values are means \( \pm SE \). *\( P < 0.01 \) vs. low-niacin group.

### Table 3. Micronutrient intake

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>All</th>
<th>Below-Average Intake</th>
<th>Above-Average Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Niacin, mg/day</td>
<td>21.7 \pm 0.9</td>
<td>14.9 \pm 0.5</td>
<td>31.7 \pm 1.0*</td>
</tr>
<tr>
<td>Vitamin B6, mg/day</td>
<td>1.86 \pm 0.09</td>
<td>1.46 \pm 0.11</td>
<td>2.42 \pm 0.11*</td>
</tr>
<tr>
<td>Calcium, mg/day</td>
<td>1.062 \pm 0.46</td>
<td>1.028 \pm 0.64</td>
<td>1.112 \pm 0.64</td>
</tr>
<tr>
<td>Sodium, mg/day</td>
<td>2.995 \pm 114</td>
<td>2.730 \pm 153</td>
<td>3.390 \pm 155*</td>
</tr>
<tr>
<td>Potassium, mg/day</td>
<td>3.036 \pm 118</td>
<td>2.451 \pm 119</td>
<td>3.854 \pm 173*</td>
</tr>
<tr>
<td>Zinc, mg/day</td>
<td>10.4 \pm 0.5</td>
<td>8.0 \pm 0.4</td>
<td>13.7 \pm 0.7*</td>
</tr>
<tr>
<td>Iron, mg/day</td>
<td>15.9 \pm 0.7</td>
<td>13.3 \pm 0.7</td>
<td>19.5 \pm 1.1*</td>
</tr>
<tr>
<td>Vitamin A, IU/day</td>
<td>11,555 \pm 830</td>
<td>9,457 \pm 879</td>
<td>16,632 \pm 1,487*</td>
</tr>
<tr>
<td>Vitamin E, IU/day</td>
<td>11.7 \pm 0.8</td>
<td>9.6 \pm 1.1</td>
<td>14.9 \pm 1.0*</td>
</tr>
<tr>
<td>Vitamin C, mg/day</td>
<td>139 \pm 7</td>
<td>132 \pm 10</td>
<td>151 \pm 10</td>
</tr>
</tbody>
</table>

Values are means \( \pm SE \). *\( P < 0.01 \) vs. low-niacin group.

### Table 4. Stepwise linear regression models assessing the independent relation between dietary niacin intake and FMD

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Model 1</th>
<th>Model 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log niacin, mg/day</td>
<td>4.22</td>
<td>1.77</td>
</tr>
<tr>
<td>LDL cholesterol, mg/dl</td>
<td>0.02</td>
<td>0.01</td>
</tr>
<tr>
<td>Carbohydrate intake, %total kcal/day</td>
<td>-0.09</td>
<td>0.04</td>
</tr>
<tr>
<td>Potassium, mg/day</td>
<td>-1 \times 10^{-3}</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

Backwards selection models. SE, standard error; \( r \), semipartial correlation coefficient. Model 1 dependent variable = flow-mediated dilation (FMD); \( R^2 = 0.17 \), intercept = 3.98. Model 2 dependent variable = \%FMD; \( R^2 = 0.17 \), intercept = 6.66. Variables entered were age, sex, systolic blood pressure, diastolic blood pressure, body mass, physical activity, HDL cholesterol, LDL cholesterol, fasting glucose, total kilocalories, carbohydrate intake, protein intake, vitamin B6, calcium, sodium, potassium, zinc, iron, vitamin A, vitamin E, vitamin C, and niacin (mg/day; model 1 only) or niacin group (above/below average, model 2 only). Variables excluded due to multicollinearity were body mass index, total cholesterol, triglycerides, and fat intake.
lower oxidative stress as a key mechanism of action underlying the favorable effects of higher niacin intake on vascular endothelial function in MA/O adults. Specifically, we show that above-average dietary niacin intake is associated with lower circulating oxidized LDL and oxidative-stress-mediated suppression of FMD. We also demonstrate an inverse relation between dietary niacin intake and markers of oxidative stress (nitrotyrosine abundance and NADPH oxidase expression) in endothelial cells obtained from the brachial artery of a subset of our subjects. Taken together, these findings suggest that higher dietary niacin intake is associated with greater vascular endothelial function in healthy MA/O adults, at least in part as a result of lower oxidative stress.

**Dietary niacin intake and vascular endothelial function in MA/O adults.** In the present study, brachial artery FMD, a well-established measure of EDD and vascular endothelial function, was positively related to dietary intake of niacin among MA/O adults free of clinical disease, and this relation was independent of other dietary and clinical characteristics. In addition, FMD was greater in subjects with daily niacin intake at or above vs. below the mean level for adults 40–80 yr of age based on NHANES III (2). Indeed, dietary niacin group status was one of the strongest independent predictors of FMD of all of the clinical and dietary characteristics assessed. Importantly, niacin intake was not related to brachial artery dilation in response to sublingual nitroglycerin, a measure of vascular smooth muscle sensitivity to nitric oxide. Together, these observations indicate that dietary niacin intake has a modulatory effect on EDD in healthy MA/O adults through an endothelium-specific mechanism.

These findings extend previous reports that niacin protects against endothelial dysfunction in rabbits induced by oxidative stress or an acute inflammatory stimulus, but has no effect on endothelium-independent dilation (59). Our results in healthy MA/O adults correspond in part with previous clinical studies reporting that chronic (≥12 wk) treatment with pharmacological doses of niacin improves brachial FMD in patients with dyslipidemia featuring low baseline HDL cholesterol (5, 30, 53, 58). However, in these clinical studies, niacin treatment significantly modified plasma lipid concentrations or fasting glucose levels, both of which can affect endothelial function independently of changes in dietary or other factors (14, 55).
contrast, the positive relation between dietary niacin intake and EDD in the present investigation was independent of lipid and glucose levels, suggesting alternative mechanisms. Our results are not consistent with part of an earlier report that niacin treatment failed to improve FMD in patients with normal HDL cholesterol levels (58). However, the findings are not directly comparable in that our subjects did not have clinical disease and were not taking medications known to have pleotropic effects on vascular function.

Dietary niacin intake and systemic oxidative stress. In the present study, dietary niacin intake was inversely related to oxidized LDL, and individuals with above-average dietary niacin intake had lower circulating levels of oxidized LDL compared with individuals with below-average intake. These findings are consistent with previous studies that have demonstrated improvements in systemic markers of oxidative stress following chronic pharmacological niacin treatment in dyslipidemic individuals (20) and dietary interventions that incorporate increased intake of niacin (1, 33). As such, our findings contribute to mounting evidence that niacin intake may modulate systemic oxidative stress.

Dietary niacin intake and oxidative stress-linked suppression of EDD. Increasing preclinical evidence demonstrates that niacin protects vascular cells against oxidative stress in response to various stressors in vitro (22, 28). In the present study, we provide novel evidence that extends this general concept to MA/O humans. Specifically, we found that infusion of supraphysiological concentrations of the antioxidant vitamin C (ascorbic acid) improved brachial artery FMD in subjects with below-average niacin intake, but not in subjects with above-average intake. This finding suggests that the lower baseline brachial artery FMD observed in healthy MA/O adults with below-average niacin intake is mediated in part by oxidative stress. Based on the FMD responses to vitamin C infusion, this oxidative-stress mediated suppression of FMD accounted for a little more than one-half of the difference in FMD between our dietary niacin subgroups. The partial restoration of FMD with vitamin C infusion suggests that additional

**Fig. 3.** FMD (%Δ) following intravenous infusion of saline and vitamin C (Vit C) in a subset of subjects with below- (<22 mg/day; n = 33) and above-average (≥22 mg/day; n = 20) dietary niacin intake. NS, nonsignificant. ‡P < 0.001 vs. saline condition.

**Fig. 4.** A and B: relation between dietary niacin intake and arterial endothelial nitrotyrosine abundance (A) and NADPH oxidase protein expression (B). Representative immunofluorescent images are displayed below each graph. C and D: differences in nitrotyrosine abundance (C) and NADPH oxidase expression (D) between individuals with below- (<22 mg/day; n = 36 and n = 29, respectively) and above-average (≥22 mg/day; n = 19 and n = 8, respectively) dietary niacin intake. Values are expressed as human endothelial cell intensity relative to human umbilical vein endothelial cell intensity. *P < 0.05 vs. low-niacin group.
factors contributed to the baseline difference in FMD between the below- and above-average dietary niacin subgroups. Such factors may include inflammatory signaling or chronic vascular damage that cannot be reversed by acute antioxidant treatment.

Endothelial cell oxidative stress and expression of NADPH oxidase. Impaired FMD in MA/O adults compared with young controls is associated with greater vascular endothelial cell oxidative stress (17). Here we report for the first time an inverse relation between dietary niacin intake and vascular endothelial cell nitrotyrosine abundance, providing evidence for modulation of endothelial cell oxidative stress by dietary niacin intake in MA/O adults. NADPH oxidase is the most important oxidant-producing enzyme in the vasculature (42, 52) and is linked to oxidative stress in multiple pathophysiological settings, including aging (32, 34). NADPH oxidase produces superoxide, which reacts with nitric oxide to form peroxynitrite (36, 42) and promote subsequent nitrotyrosine formation (3). Niacin treatment decreases NADPH oxidase expression and activity in vitro and in other preclinical models (4, 11, 48), and this may be an important mechanism underlying the relation between dietary niacin intake and endothelial cell oxidative stress in the present investigation. Indeed, we observed an inverse relation between dietary niacin intake and endothelial cell NADPH oxidase expression. Together, these findings suggest that dietary niacin intake may decrease endothelial cell oxidative stress in part through reduced expression of NADPH oxidase.

Endothelial cell eNOS expression and activation. To further investigate the molecular mechanisms underlying the relation between dietary niacin intake and FMD, we assessed endothelial cell expression of the principal nitric oxide-producing enzyme, eNOS, as well as the ratio of p-eNOS to eNOS, a marker of enzyme activation. Indeed, preclinical studies have demonstrated a loss of the vascular-protective effects of niacin when eNOS is genetically inhibited (22). In contrast, we did not observe any relation between dietary niacin intake and the expression of eNOS or the ratio of p-eNOS to eNOS, suggesting that dietary levels of niacin intake may modulate EDD through an eNOS-independent mechanism, perhaps through increasing nitric oxide bioavailability secondary to decreased oxidative stress.

Experimental considerations. We observed differences in both age and physical activity between our below- and above-average dietary niacin groups. Although FMD declines with advancing age (10), and aerobic exercise protects against age-associated vascular dysfunction (6, 13, 40, 41), both the inverse relation between niacin intake and FMD, as well as the above- vs. below-average niacin intake group differences in FMD, remained significant after statistically controlling for age and physical activity. These findings suggest that differences in age and physical activity did not account for the observed relation between niacin intake and FMD in the present study.

The above-average niacin intake group had a higher percentage of men than the below-average niacin intake group. However, covarying for sex did not account for the positive relation between niacin intake and FMD or the niacin intake-related group differences in brachial FMD. In addition, we did not observe group differences in baseline brachial artery diameter or peak shear rate, which can differ between men and women and influence or even account for sex differences in FMD (27). As such, we do not believe that differences in sex composition of the groups influenced our results and key conclusions.

Due to the retrospective nature of the present study, we were limited to dietary assessment of niacin intake to estimate niacin status. Estimation of niacin status from dietary assessments can be difficult due to the influence of protein consumption on the biosynthesis of niacin from tryptophan and limited bioavailability of bound forms of niacin (25, 43). However, urinary excretion of niacin metabolites correlates with estimates of niacin intake from diet records in free-living adults (45, 54), demonstrating that dietary assessment of niacin intake is reflective of niacin status.

Two methods of dietary assessment were used by our CTRC bionutritionists to quantify dietary niacin intake over the period encompassing our analysis. The agreement between food frequency questionnaires and 3-day diet records varies, depending on the study cited (26, 35, 37, 47). However, these methods similarly classify subjects into quintiles of dietary niacin intake (37), and all relations between dietary niacin intake and our physiological outcomes were confirmed by determining group differences in individuals with below- and above-average dietary niacin intake.

In the present investigation, we were not able to assess all NADPH oxidase isoforms implicated in endothelial dysfunction, as we recover a limited number of cells with our endothelial biopsy technique. However, the NADPH oxidase subunit p47phox has been previously associated with oxidative stress and endothelial dysfunction with age (17, 40), suggesting that it is a strong marker of these phenotypes. Furthermore, due to immediate fixation of biopsied cells, we are not able to directly assess NADPH oxidase activity. To address this limitation, we measured a marker of cell oxidative damage, a downstream event of NADPH oxidase activation.

Finally, the absolute correlation coefficients for the relation between niacin intake and brachial artery FMD in the overall group (n = 127) were modest (r = 0.20–0.25). However, the true physiological relation between dietary niacin intake and FMD is likely to be much stronger than reflected by the correlation coefficients because of the inherent measurement errors of these variables, particularly the estimate of niacin from diet records. The ~25% difference in FMD between groups with above-compared with below-average niacin intake further supports the significance of the association. Moreover, increases in FMD as small as 1% and 1 SD have been associated with a decreased risk for cardiovascular disease events and mortality in healthy adults (46, 61), suggesting that even small variations in FMD have important clinical implications.

Conclusions. Here we demonstrate for the first time that dietary niacin intake is positively related to brachial artery FMD in MA/O adults without clinical disease. We also provide novel evidence that higher dietary niacin intake is associated with lower oxidized LDL, oxidative stress-mediated suppression of FMD, and vascular endothelial cell nitrotyrosine and NADPH oxidase. These findings provide unique translational in vivo (functional) and ex vivo (molecular) support for the idea that niacin intake may be an important dietary factor that favorably influences vascular endothelial function in MA/O adults via inhibition of oxidative stress.

REFERENCES


44. Pierce GL, Eskurza I, Walker AE, Fay TN, Seals DR.

43. Ray R, Shah AM.

42. Pierce GL, Donato AJ, LaRocca TJ, Eskurza I, Silver AE, Seals DR.


