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Postprandial Hyperglycemia Impairs Vascular Endothelial Function in Healthy Men by Inducing Lipid Peroxidation and Increasing Asymmetric Dimethylarginine:Arginine[1–3]

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Abstract

Postprandial hyperglycemia induces vascular endothelial dysfunction (VED) and increases future cardiovascular disease risk. We hypothesized that postprandial hyperglycemia would decrease vascular function in healthy men by inducing oxidative stress and proinflammatory responses and increasing asymmetric dimethylarginine:arginine (ADMA:arginine), a biomarker that is predictive of reduced NO biosynthesis. In a randomized, cross-over design, healthy men (n = 16; 21.6 ± 0.8 y) ingested glucose or fructose (75 g) after an overnight fast. Brachial artery flow-mediated dilation (FMD), plasma glucose and insulin, antioxidants, malondialdehyde (MDA), inflammatory proteins, arginine, and ADMA were measured at regular intervals during the 3-h postprandial period. Baseline FMD did not differ between trials ($P > 0.05$). Postprandial FMD was reduced following the ingestion of glucose only. Postprandial MDA concentrations increased to a greater extent following the ingestion of glucose compared to fructose. Plasma arginine decreased and the ratio of ADMA:arginine increased to a greater extent following the ingestion of glucose. Inflammatory cytokines and cellular adhesion molecules were unaffected by the ingestion of either sugar. Postprandial $AUC_{0–3\ h}$ for FMD and MDA were inversely related (r = −0.80; $P < 0.05$), suggesting that hyperglycemia-induced lipid peroxidation suppresses postprandial vascular function. Collectively, these findings suggest that postprandial hyperglycemia in healthy men reduces endothelium-dependent vasodilation by increasing lipid peroxidation independent of inflammation. Postprandial alterations in arginine and ADMA: arginine also suggest that acute hyperglycemia may induce VED by decreasing NO bioavailability through an oxidative stress-dependent mechanism. Additional work is warranted to define whether inhibiting lipid peroxidation and restoring arginine metabolism would mitigate hyperglycemia-mediated decreases in vascular function. J. Nutr. 141: 1961–1968, 2011.

Introduction

Estimates indicate that 11% of Americans > 20 y of age suffer from diabetes and at least 35% of adults have prediabetes (1). Diabetes increases CVD[7] risk by 3-fold and is the primary cause of diabetes-related mortality (2). Postprandial hyperglycemia has been suggested to better predict CVD risk than fasting glucose (3). VED, as evidenced by reduced endothelium-dependent vasodilation, is a leading factor contributing to CVD and is well associated with traditional CVD risk factors, including age, smoking, dyslipidemia, hypertension, and diabetes (4). Moreover, postprandial hyperglycemia, even at levels below the diagnostic criteria for diabetes, suppresses FMD (5–7), a noninvasive approach to assess endothelium-dependent vasodilation in humans (8).

The mechanism by which postprandial hyperglycemia induces VED remains poorly understood, but suppression of FMD responses suggests that NO bioavailability is reduced. This may be due to decreases in NO biosynthesis, greater uncoupling of eNOS, and greater consumption of NO by reactive oxygen species (9–11). Hyperglycemia-induced oxidative stress also decreases the activity of DDAH, which degrades ADMA, an endogenously produced competitive inhibitor of eNOS (12). Indeed, a higher ratio of ADMA:arginine is associated with

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[3] Supplemental Tables 1 and 2 are available from the "Online Supporting Material" link in the online posting of the article and from the same link in the online table of contents at jn.nutrition.org.
[7] Abbreviations: AAPH, 2,2′-azobis-2-methyl-propanimidamide; ADMA, asymmetric dimethylarginine; CAT, cationic transporter; γ-CEHC, γ-carboxyethyl-hydroxychroman; CVD, cardiovascular disease; DDAH, dimethylarginine dimethylaminohydrolase; DTPA, diethylenetriaminepentaacetic acid; eNOS, endothelial NO synthase; FMD, flow-mediated dilation of the brachial artery; FRAP, ferric-reducing ability of plasma; MDA, malondialdehyde; OGTT, oral glucose tolerance test; ORAC, oxygen radical absorbance capacity; PCA, perchloric acid; VCAM-1, vascular adhesion molecule 1; VED, vascular endothelial dysfunction.
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greater CVD risk (13). Chronic oxidative stress also increases CVD risk by inducing lipid peroxidation (6), which contributes to VED by reducing eNOS activity, increasing endothelial cell apoptosis and decreasing vascular endothelial integrity (14). Likewise, oxidized LDL accumulation in the vascular intima stimulates the secretion of proinflammatory cytokines and adhesion molecules and increases neutrophil recruitment, which further exacerbates VED (15).

Clearly, studies in vitro have shown that vasodilation is impaired in a hyperglycemia-dependent manner (16,17). However, the relation between postprandial hyperglycemia and vascular function in humans is somewhat equivocal, with most studies indicating suppression of FMD following an OGTT (6,7,10,18–20), whereas others have shown that FMD is unaffected (21) or improves in response to an oral glucose challenge (22). Another complicating factor is that an OGTT in humans does not consistently increase biomarkers of oxidative stress (6,7,10), nor is it known whether acute hyperglycemia increases inflammatory responses or disrupts postprandial levels of arginine and ADMA, an index of $NO^{\cdot}$ homeostasis. Thus, these inconsistencies and the limited information regarding the mechanistic relation among oxidative stress, inflammation, and altered NO homeostasis during hyperglycemia-mediated VED precludes our ability to effectively manage the risk of developing CVD. We therefore hypothesized in the present study that postprandial hyperglycemia in young men free of comorbidities would transiently suppress vascular endothelial function in a NO-dependent manner by inducing oxidative stress and inflammatory responses. To better define the mechanisms by which postprandial hyperglycemia affects vascular function in healthy college-aged men, we examined postprandial FMD, circulating arginine, and ADMA as surrogate biomarkers of NO homeostasis, antioxidant status, and oxidative stress and inflammatory markers in response to an oral glucose or fructose challenge.

Methods and Materials

Participants. The study protocol was approved by the Institutional Review Board for the protection of human participants at the University of Connecticut and participants provided written consent before enrolling. Healthy, nonsmoking men ($n = 16$) were recruited on the basis of age (18–35 y), stable BMI (>2 mo), adiposity (8–20%), nonuser of dietary supplements (>2 mo), fasting total cholesterol (<5.18 mmol/L) and glucose (<5.55 mmol/L), and resting blood pressure (<140/90 mm Hg). Young men were specifically enrolled to mitigate known variations in FMD responses between gender and due to aging (8). Participants had stable exercise patterns (<5 h/wk aerobic activity), were free of diabetes and other metabolic diseases, and were not using any medications or dietary agents known to affect vasodilation. Waist circumference was determined at the level of the umbilicus. Body density was estimated from skinfolds at 7 sites (23) and fat mass was calculated using appropriate equations for race (24,25).

Materials. HPLC-grade solvents were purchased from Fisher Scientific as were the following chemicals: 2, 3, 5-triphenyltetrazol-2-ium chloride, acetic acid, ascorbic acid BHT, DTPA, lithium perchlorate, PCA, potassium hydroxide, potassium phosphate, sodium acetate, NaOH, Q12 ion pairing reagent, sodium acetate, trichloroacetic acid, and uric acid. β-Glucuronidase type HP-2S, $FeCl_3$, fluorescein, $NaPO_4$, tetramethoxypropane, thiobarbituric acid, Trolox, and vitamin E (α- and -γ-tocopherol) were from Sigma Aldrich. AAPH was from Cayman Chemical.

Study design. Participants were enrolled in a randomized, crossover, single-blind study in which they visited the study center in the fasting state (10–12 h) on 2 occasions separated by ≥7 d. During each visit, participants either completed a standardized clinical OGTT (75 g glucose dissolved in 240 mL water) or ingested an equal molar solution of fructose. Blood samples were collected at baseline (t = 0 min) and following the ingestion of glucose or fructose at 30, 60, 90, 120, 150, and 180 min. FMD was also measured at baseline (t = 0 min) and 30-min intervals postprandially during each visit, as described below, to assess vascular endothelial function.

Sample handling. A flexible catheter was inserted in the antecubital vein of the left arm and blood samples were collected into evacuated tubes containing EDTA, sodium heparin, or lithium heparin. Plasma was obtained by centrifugation (4°C, 15 min, 1500 × *g*) and snap-frozen in liquid nitrogen. For measurements of vitamin C and uric acid, sodium heparinized plasma was mixed (1:1) with 10% PCA containing 1 mmol/L DTPA. Following centrifugation (5 min, 15,000 × *g*, 4°C), the supernatant was snap-frozen in liquid nitrogen. All samples were stored at −80°C until analysis.

Dietary analysis. Participants completed a 3-d dietary record before each visit and all records were reviewed with participants for accuracy with a registered dietitian. Dietary intakes were analyzed using Food Processor SQL (ESHA Research).

Flow-mediated dilation. FMD was assessed by high-frequency ultrasonographic imaging as described (26), with minor modification. Briefly, the transducer was placed above the antecubital crease of the right arm and secured using a stereotactic clamp. Preocclusion brachial artery diameter was recorded for 30 heart beats. To better assess endothelial-dependent vasodilation, a blood pressure cuff was placed on the right forearm immediately distal to the olecranon process (27). The cuff was rapidly inflated to occlude the brachial artery (200 mm Hg, 5 min, Hokanson E20). Arterial diameter was assessed continuously for 300 heartbeats (~5 min) postocclusion. Brachial artery images were obtained using a 13-MHz linear array transducer and Aspen cardiac ultrasound system (Acuson). All images were electrocardiogram-gated and image analysis was performed using edge-detection software (Medical Imaging Applications). Postocclusion peak diameter was calculated by identifying peak dilation and averaging vessel diameters ± 5 frames surrounding the peak. Brachial artery FMD (%) was calculated as follows: [postocclusion peak diameter (mm) – preocclusion diameter (mm)]/preocclusion diameter (mm) · 100. FMD measurements were performed by the same technician and image analysis was assessed independently by 2 technicians who were unaware of the treatments. Analyzed FMD responses between technicians did not differ ($P > 0.05$) and were therefore averaged.

Clinical chemistries. Plasma TG, total cholesterol, and glucose were measured spectrophotometrically on a SpectraMax M2 microplate reader (Molecular Devices) according to the manufacturer's instructions (Pointe Scientific). Plasma insulin was measured by ELISA (Diagnostic Systems Laboratories).

MDA. Circulating MDA exists in free form and bound to protein (28) and both forms would be expected to reflect oxidative damage contributing to VED. Thus, to more fully assess the magnitude of lipid peroxidation, total (free and bound) MDA was measured by HPLC as described (29), with minor modifications. Briefly, plasma was mixed with BHT and NaOH and incubated for 30 min at 60°C before adding 5% trichloroacetic acid and incubating on ice for 10 min. After centrifugation, the supernatant was mixed with 0.6% (wt:v) thiobarbituric acid, incubated (30 min, 95°C), and then rapidly chilled before extracting with butanol. Following centrifugation, the supernatant was injected on a Shimadzu LC-20XR system equipped with a RF-10AXL fluorescence detector set to 532/553 nm (excitation/emission). HPLC separation was performed isocratically at 0.8 mL/min on a C_{18} separation column (250 × 4.6 mm i.d., 5 μm; Phenomenex) using 50:50 methanol and 25 mmol/L phosphate buffer (pH 6.5) as the mobile phase. MDA was quantified from standards prepared in parallel from tetramethoxypropane.

Arginine and ADMA. Arginine and ADMA were simultaneously measured by HPLC as described (30), with minor modifications. Briefly, arginine and ADMA were extracted from plasma (100 μL) by solid-

phase extraction on a polymeric cation-exchange column (Hypersep Retain-CX SPE column; 30 mg, 1 mL; Fisher Scientific) using ammonia: water:methanol (10:40:50, v:v:v). HPLC separation was performed isocratically at 1.1 mL/min on the aforementioned HPLC system programmed to 340/455 nm (excitation/emission) and a Shim-Pack XR-ODS column (50 × 3.0 mm i.d., 2.2 μm; Shimadzu). *Ortho*-phthalaldehyde-derivatives of arginine and ADMA were eluted from the column with 50 mmol/L potassium phosphate buffer (pH 6.5) and 6.5% (v:v) acetonitrile. Different sensitivity settings were used to enable simultaneous detection of arginine and ADMA. The column was washed with 50% acetonitrile for 2 min and the system was equilibrated for 2 min before the next injection. Analytes were quantified on the basis of peak area relative to internal standard (methylmonoarginine).

Inflammatory markers. Plasma MPO and high-sensitivity C-reactive protein (CRP) were measured using separate ELISA kits (BioCheck). Plasma IL-6, IL-10, TNFα, soluble intracellular adhesion molecule 1, VCAM-1, and E-selectin were measured using xMAP technology on a Luminex IS200 system with corresponding antibodies (Millipore).

Total antioxidant status. Total antioxidant status was assessed using the ORAC and FRAP assays. The ORAC assay was performed as described (31) and is based on the principle that antioxidants scavenge reactive oxygen species formed by an AAPH-induced peroxyl radical-generating system. Briefly, plasma or Trolox standard diluted in phosphate buffer was mixed with fluorescein and AAPH. The fluorescence decay of flourescein was monitored at 485/520 nm (excitation/emission) on a SpectraMax M2 microplate reader (Molecular Devices). The AUC for the decay curve was calculated using the trapezoidal method and ORAC concentrations were determined by linear regression. The FRAP assay was measured as described (32), with minor modifications, and is based on the principle that plasma antioxidants, particularly those that are hydrophilic, reduce ferric iron to ferrous iron. Briefly, plasma or Trolox (standard) was mixed with FRAP reagent and incubated (15 min, 37°C) and the absorbance was measured at 593 nm.

Vitamin E and γ-CEHC. Plasma α- and γ-tocopherol were measured by HPLC-Coularray (ESA Inc.), as described (33). γ-CEHC, the physiological metabolite of γ-tocopherol, was measured by HPLC-Coularray with minor modifications (34). Briefly, a methanolic extract of plasma was dried under nitrogen, reconstituted in water, and subjected to enzymatic hydrolysis with β-glucuronidase (800 U) and sulfatase (60 U). Samples were extracted with ethyl acetate, dried under nitrogen, dissolved in 70% methanol, and separated (1 mL/min) on a Phenomenex Luna C_{18} column (250- × 4.6-mm i.d., 5 μm) and detected at potential settings of −100, −50, 200, and 350 mV. The mobile phase was 35:65 acetonitrile: water containing 10 mmol/L ammonium acetate (pH 4.3). γ-CEHC was quantified using internal standard (Trolox).

Ascorbic and uric acids. Ascorbic and uric acids were analyzed by HPLC-Coularray from PCA-treated plasma as described (33). Samples were separated at 1 mL/min on a Supelcosil LC-8 column (150- × 4.6-mm i.d., 3 μm, Supelco) and detected at oxidation potentials of 150, 275, 400, and 525 mV. The mobile phase was 7.5% methanol containing 40 mmol/L sodium acetate, 0.5 mmol/L DTPA, and 1.5 mmol/L Q-12 ion pairing reagent.

Statistical methods. All data are expressed as means ± SEM and were analyzed using SPSS version 15.0. Treatment, time, and treatment × time interaction effects for postprandial responses for FMD and plasma biomarkers were evaluated using 2-way repeated-measures ANOVA with Bonferroni correction to evaluate pairwise differences. Differences between trials for dietary intakes, postprandial $AUC_{0-3\ h}$, or baseline values were evaluated using a Student's paired *t* test. The $AUC_{0-3\ h}$ was calculated using the trapezoidal rule over the 3-h postprandial period. Correlation coefficients (*r*) were calculated using multiple linear regression controlling for repeated measures within each participant, as described by Bland and Altman (35). All measurements were initially evaluated for normality using Shapiro-Wilk's W-test. Biomarkers that were not normally distributed were insulin, CRP, VCAM-1, E-selectin, IL-6, and IL-10. These were log-transformed using the natural base to achieve normality. Statistical analyses were performed on transformed and untransformed data in parallel. Because there were no qualitative differences in the results, untransformed values are reported with their significance levels, because they permit more meaningful interpretations than transformed data. An α-level of $P < 0.05$ was considered significant for all analyses.

Results

Participants and dietary intakes. Participants had resting blood pressure and fasting plasma glucose, total cholesterol, and TG concentrations that were within normal clinical limits (**Table 1**). Despite participants having BMI indicative of being overweight, adiposity was 17 ± 2%, which is within established healthy limits (36) and consistent with studies indicating that direct measures of adiposity are better for predicting obesity-related CVD risk compared to BMI (37). Dietary intakes of macronutrients and selected micronutrients did not differ between trials (**Supplemental Table 1**), indicating that participants maintained similar dietary patterns throughout the study.

Plasma glucose and insulin. Fasting glucose and insulin concentrations did not significantly differ between trials (**Fig. 1**). Postprandial glucose increased by 30 min following glucose ingestion and returned to baseline concentrations at 150 min. In contrast, fructose ingestion increased plasma glucose by only 10–12% at 30–60 min and this increase was substantially less than that caused by glucose ingestion (Fig. 1*A*). Following glucose or fructose ingestion, plasma insulin also increased by 30 min and returned to baseline concentrations at 180 min, although plasma insulin concentrations increased to a greater extent following ingestion of glucose compared to fructose (Fig. 1*B*).

FMD. Fasting brachial FMD responses did not significantly differ between trials (**Fig. 2***A*). Additionally, the preocclusion brachial artery diameter did not differ throughout the postprandial period for either treatment ($P > 0.05$) (data not shown), suggesting that changes in postprandial FMD responses were unaffected by repetitive hyperemia. In contrast, FMD responses

TABLE 1 Participant characteristics[1]

Clinical measurements	
Age, *y*	21.6 ± 0.8
Height, *m*	1.80 ± 0.01
Weight, *kg*	93.1 ± 6.5
Fat mass, *% total mass*	17.4 ± 0.2
Systolic blood pressure, *mm Hg*	117 ± 1
Diastolic blood pressure, *mm Hg*	79 ± 1
Waist circumference, *cm*	88.7 ± 3.6
Plasma glucose, *mmol/L*	5.33 ± 0.11
Plasma insulin, *pmol/L*	147 ± 24
Plasma TG, *mmol/L*	0.76 ± 0.08
Total plasma cholesterol, *mmol/L*	3.59 ± 0.18
Plasma arginine, *μmol/L*	85.8 ± 4.5
Plasma ADMA[2], *nmol/L*	521 ± 10
Plasma ADMA:arginine, *nmol/μmol*	6.40 ± 0.46
Baseline brachial artery diameter, *mm*	4.00 ± 0.11
Peak brachial artery dilation, *mm*	4.26 ± 0.14

[1] Data are mean ± SEM, *n* = 16.
[2] ADMA, asymmetric dimethylarginine.

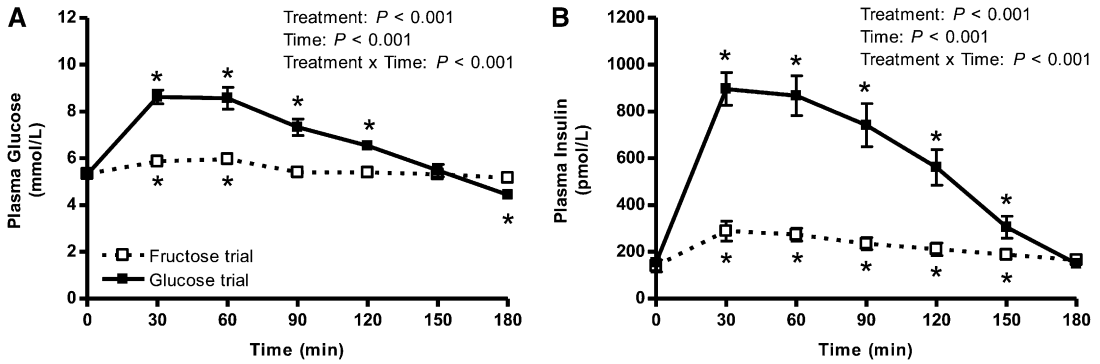

FIGURE 1 Postprandial glucose (*A*) and insulin (*B*) responses following fructose and glucose ingestion by men. Data are means ± SEM, $n = 16$. *Different from baseline, $P < 0.05$.

decreased at 30 min and returned to baseline levels at 120 min following glucose ingestion. Postprandial FMD responses were unaffected by fructose ingestion. Multiple linear regression, controlling for within-participant repeated measure, indicated a relation between FMD $AUC_{0–3\ h}$ and glucose $AUC_{0–3\ h}$ (mean slope = −0.05, $r = -0.82$; $P < 0.05$), supporting that the magnitude of postprandial hyperglycemia is associated with impairments in vascular function.

Markers of antioxidant status and oxidative stress. We measured major plasma antioxidants and oxidative stress biomarkers to better define whether postprandial vascular function is regulated in an oxidative stress-dependent manner. Fasting ORAC, FRAP, tocopherols, γ-CEHC, vitamin C, and uric acid did not significantly differ between trials (**Table 2**). Postprandial AUC did not differ between trials for ORAC, tocopherols, or γ-CEHC and no main effects for treatment, time, or their interaction were observed. In contrast, the $AUC_{0–3\ h}$ of uric acid and FRAP were greater ($P < 0.05$) following the ingestion of fructose compared to glucose. This was expected, because fructose increases the production of uric acid (38), the primary predictor of FRAP (32). Plasma uric acid concentrations increased at 30 min and remained elevated throughout the postprandial period, whereas uric acid did not change in the glucose trial (treatment × time interaction, $P < 0.05$). Although hyperuricemia is associated with CVD (39,40), uric acid levels remained within normal limits (150–480 μmol/L) (41), suggesting that it may be functioning consistent with its antioxidant properties (40). Lastly, a treatment × time interaction effect was observed for vitamin C ($P < 0.01$), but post hoc analysis did not detect any significant time-dependent changes during the postprandial period for either trial, nor were there any differences between treatments.

Plasma MDA was measured to examine whether hyperglycemia induces lipid peroxidation. Baseline (t = 0 min) MDA concentrations did not differ between trials but increased by 32–66% at 30–90 min following the glucose challenge (Fig. 2*B*). Consistent with the small but transient increase in plasma glucose following fructose ingestion (Fig. 1*A*), plasma MDA also increased by 10–20% at 30–90 min but clearly to a lesser extent compared to glucose ingestion (Table 2). Multiple linear regression analysis also indicated a relation between MDA $AUC_{0–3\ h}$ and glucose $AUC_{0–3\ h}$ (mean slope = 93.7, $r = 0.87$; $P < 0.05$) and that the MDA $AUC_{0–3\ h}$ was highly associated with FMD $AUC_{0–3\ h}$ (mean slope = −5.52, $r = -0.80$; $P < 0.05$) (Fig. 2*C*). Collectively, these findings suggest that impairments in vascular function, mediated by hyperglycemia, occurs in an oxidative stress-dependent manner through a mechanism involving lipid peroxidation.

Assessment of NO homeostasis. To better define whether hyperglycemia-mediated suppression of FMD is NO dependent, plasma arginine and ADMA were measured as markers of NO homeostasis. Fasting plasma arginine and ADMA did not differ

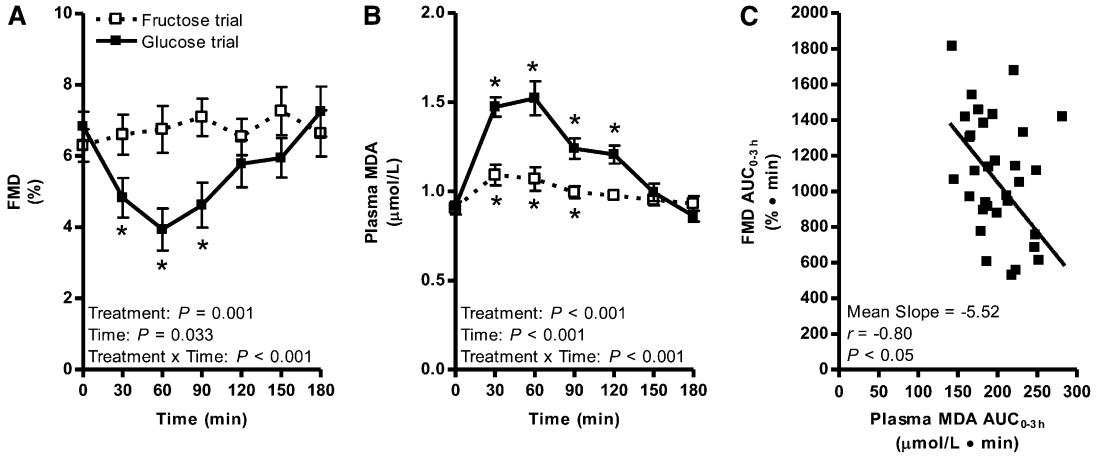

FIGURE 2 Postprandial brachial artery FMD (*A*), postprandial plasma MDA (*B*), and the relation between FMD and MDA $AUC_{0–3\ h}$ (*C*) following fructose and glucose ingestion by men. Data are means ± SEM, $n = 16$. Multiple linear regression, controlling for repeated measures was used to calculate correlation coefficients (*r*). *Different from baseline, $P < 0.05$. FMD, flow-mediated dilation; MDA, malondialdehyde.

TABLE 2 Baseline concentrations and $AUC_{0–3\ h}$ for plasma antioxidants and oxidative stress markers following the oral administration of glucose or fructose in men[1]

	Baseline			$AUC_{0–3\ h}$[3]		
	Fructose trial	Glucose trial	*P*	Fructose trial	Glucose trial	*P*
ORAC[2], *mmol/L Trolox eq.*	3.44 ± 0.10	3.58 ± 0.24	0.61	1090 ± 32	1070 ± 54	0.74
FRAP, *mmol/L Trolox eq.*	1.00 ± 0.03	0.967 ± 0.03	0.30	196 ± 7	175 ± 4	0.01
α-Tocopherol, *mmol/L*	0.014 ± 0.001	0.015 ± 0.001	0.32	2.60 ± 0.20	2.72 ± 0.19	0.11
γ-Tocopherol, μ*mol/L*	2.3 ± 0.3	2.1 ± 0.3	0.40	396 ± 43	356 ± 42	0.11
γ-CEHC, μ*mol/L*	0.30 ± 0.06	0.32 ± 0.07	0.65	48 ± 4	51 ± 9	0.93
Vitamin C, *mmol/L*	0.037 ± 0.004	0.034 ± 0.004	0.25	7.11 ± 0.79	5.96 ± 0.69	0.07
Uric acid, *mmol/L*	0.361 ± 0.017	0.351 ± 0.011	0.52	72.0 ± 3.0	63.5 ± 2.1	0.01
MDA, μ*mol/L*	0.91 ± 0.04	0.92 ± 0.03	0.63	180 ± 6	220 ± 8	<0.001

[1] Data are means ± SEM, $n = 16$.
[2] FRAP, ferric-reducing ability of plasma; γ-CEHC, γ-carboxyethyl-hydroxychroman; MDA, malondialdehyde; ORAC, oxygen radical absorbance capacity.
[3] Units for $AUC_{0–3\ h}$ = units for measured markers × min.

between trials. Plasma arginine decreased postprandially in both trials (**Fig. 3***A*) but to a greater extent following glucose ingestion ($AUC_{0–3\ h}$ 13.2 ± 0.83 vs. 12.3 ± 0.64 mmol/L·min; $P < 0.01$). Postprandial ADMA concentrations were not affected by either treatment (Fig. 3*B*), but a main effect for time was observed ($P < 0.01$). Post hoc analysis indicated no differences between any time points for ADMA concentrations and the ADMA $AUC_{0–3\ h}$ did not differ between trials ($AUC_{0–3\ h}$ 89.0 ± 1.67 vs. 89.4 ± 1.52 μmol/L·min; $P > 0.05$). In contrast, the ratio of ADMA:arginine, a risk factor for CVD and index of $NO^{\cdot}$ biosynthesis (13), increased in both trials, but to a greater extent following the ingestion of glucose compared to fructose (Fig. 3*C*; $AUC_{0–3\ h}$ 1.39 ± 0.09 vs. 1.32 ± 0.11 μmol/μmol·min; $P < 0.05$), consistent with greater glycemia following glucose ingestion. Collectively, these findings suggest that hyperglycemia may suppress NO biosynthesis by limiting arginine availability and increasing the proportion of ADMA relative to arginine, which would be expected to competitively inhibit binding of arginine to eNOS (42).

Markers of inflammation. We also examined whether inflammatory responses are associated with changes in postprandial vascular function (**Supplemental Table 2**). No significant differences were observed between trials at baseline for protein levels of CRP, MPO, IL-6, IL-10, TNFα, or the adhesion molecules intracellular adhesion molecule 1, VCAM-1, and E-selectin. We also observed no significant treatment, time, or treatment × time interaction effects for inflammatory markers, nor did the postprandial $AUC_{0–3\ h}$ of any of these inflammatory markers significantly differ between trials. This suggests that hyperglycemia-mediated VED is independent of inflammatory responses, at least in this model of acute hyperglycemia in healthy men.

Discussion

The findings of this study suggest that the acute ingestion of glucose suppresses vascular function in healthy young men by inducing lipid peroxidation and increasing ADMA:arginine independent of any changes in inflammation. FMD decreases following the ingestion of glucose, but not fructose, supporting that postprandial hyperglycemia impairs vascular function. Furthermore, FMD is inversely related to plasma glucose and maximal FMD suppression corresponds to peak plasma glucose, consistent with the work of others (6,10,18–20). Data also suggest that glucose ingestion decreases vascular function in an oxidative stress-dependent manner, as evidenced by greater postprandial MDA, a response that was highly correlated with glycemic responses and inversely related to postprandial FMD. We also provide novel evidence that glucose-induced impair-

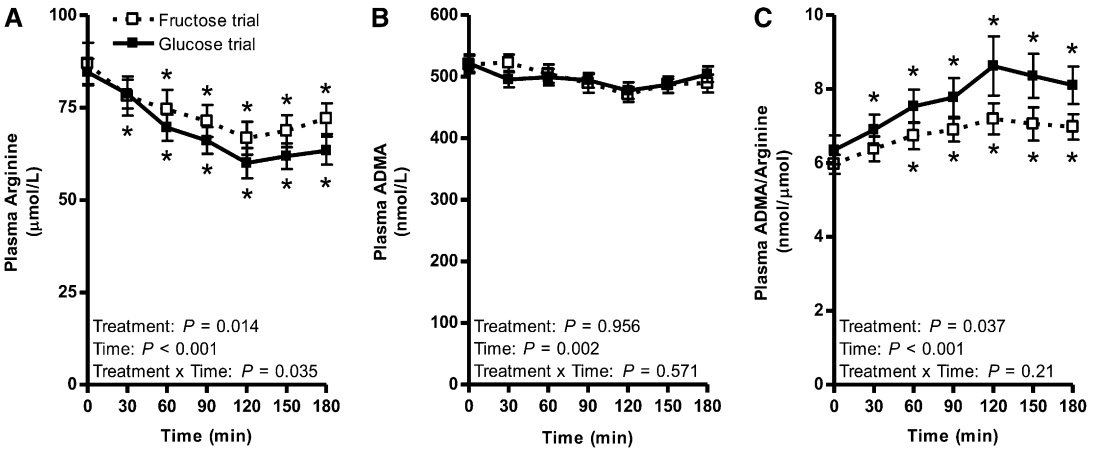

FIGURE 3 Postprandial concentrations of plasma arginine (*A*) and ADMA (*B*) and the ratio of ADMA:arginine (*C*) following the ingestion of fructose and glucose by men. Data are means ± SEM, $n = 16$. *Different from baseline, $P < 0.05$. ADMA, asymmetric dimethylarginine.

ments in vascular function may occur in a NO-dependent manner by reducing circulating arginine and increasing ADMA:arginine, which would be expected to not only limit substrate for NO biosynthesis but also competitively inhibit eNOS. Collectively, this study supports that postprandial hyperglycemia transiently impairs vascular function by inducing oxidative stress and likely decreasing NO bioavailability.

In humans, chronic and postprandial hyperglycemia is associated with increased oxidative stress (6,10,43). Although we observed no changes in plasma antioxidants following glucose ingestion, MDA increases substantially during the postprandial period in response to glucose ingestion, indicating that hyperglycemia-mediated decreases in vascular function may be partly attributed to lipid peroxidation. In contrast, postprandial MDA was unaffected in healthy young adults following an OGTT despite significant decreases in FMD responses (7). This discrepancy may be attributed to differences in methodologies in that we measured total MDA following alkaline hydrolysis, whereas free MDA was measured by others (7). Studies in vitro and in vivo demonstrate that hyperglycemia induces oxidative stress by activating NADPH oxidase from inflammatory cells (44) or increasing mitochondrial superoxide generation (45). Our observation that inflammatory markers are unaffected by either carbohydrate treatment likely reflects that our participants are young and free of morbidities, whereas individuals with diabetes having greater baseline inflammation also have exacerbated inflammatory responses due to postprandial hyperglycemia (46).

VED is an early event in the etiology of atherosclerosis (47) and its progression is commonly implicated by the oxidation of LDL in the subendothelial space. We observed that glucose ingestion increases MDA and the magnitude of lipid peroxidation is associated with decreases in vascular function. This is consistent with others demonstrating that FMD responses from insulin-resistant men are associated with MDA-modified LDL (48). Likewise, arginine treatment to endothelial cells restores NO synthesis that is otherwise inhibited by oxLDL (49), suggesting that oxLDL affects arginine bioavailability for NO production (49,50). Consequently, greater lipid peroxidation, such as that observed herein, likely suppresses NO bioavailability.

We also provide novel evidence that glucose ingestion increases ADMA:arginine by decreasing plasma arginine. Arginine, the amino acid required for eNOS-mediated NO biosynthesis, is transported into endothelial cells by CAT-1 and -2. CAT-1, eNOS, and caveolin are colocalized in plasma membrane caveolae (51) and directs arginine to eNOS for NO synthesis. That plasma arginine decreases to a greater extent following glucose ingestion suggests that increased plasma glucose may be stimulating intracellular arginine uptake by upregulating CAT-1 expression. However, mRNA expression of CAT-1 increases at $\geq$4 h in endothelial cells treated with glucose (51,52) and no studies to our knowledge have examined this effect at earlier time points. Furthermore, increased oxidative stress following glucose ingestion may upregulate arginase in endothelial cells, resulting in greater intracellular arginine degradation and may contribute to hyperglycemia-mediated impairments in vascular function. Although individuals with diabetes have greater arginase activity compared to healthy controls (53), it remains unknown whether arginase activity is upregulated within 3 h in response to glucose. Oxidative stress also inhibits DDAH-mediated ADMA degradation, which results in greater intracellular ADMA (54). Activity of purified human DDAH-1 decreased within 2 h following treatment with hydroxynonenal, a lipid hydroperoxide degradation product (54), further supporting that lipid peroxidation disrupts NO homeostasis. Thus, our findings suggest that hyperglycemia-induced alterations in arginine and ADMA affect vascular function and warrant further investigation to better define the mechanisms involved.

Because hyperglycemia increases plasma glucose and insulin, we cannot fully conclude that changes in FMD are solely the result of greater plasma glucose rather than insulin. Insulin has vasoactive properties (55), but more invasive work is needed to assess the effects of hyperglycemia independent of insulin. However, intra-arterial infusion of dextrose suppresses endothelium-dependent vasodilation with or without pharmacologic inhibition of insulin secretion (56). This suggests that hyperglycemia, but not hyperinsulinemia, is responsible for the suppression of FMD responses observed in our study. Harris et al. (57) also demonstrated that repeated FMD measurements at 30-min intervals do not confound FMD responses, which supports that our observed changes in postprandial FMD are associated with acute hyperglycemia. Although our measurement of FMD best reflects endothelium-dependent vasodilation (8), decreases in vascular function may be mediated through a mechanism independent of NO. Indeed, alterations in other endogenous vasodilators and vasoconstrictors are known to affect FMD (58).

Our observation that postprandial ADMA:arginine increases to a greater extent following glucose ingestion suggests that impairments in eNOS-mediated biosynthesis of NO may impair vascular function. However, direct measurements of NO in vivo were beyond the scope of this study, because its accurate assessment requires invasive NO sensors (59). Metabolites of NO could be measured, but this approach likely underestimates nitrate compared to more sensitive GC-MS methods (60). Additionally, plasma ADMA and arginine may not accurately reflect concentrations within endothelial cells, where ADMA and arginine would be expected to have the greatest impact. To better define whether these hyperglycemia-mediated effects occur in a NO-dependent manner, future studies involving infusion of pharmacological inhibitors of NOS such as N^G-monomethyl-L-arginine are needed. Because this study was limited to healthy, college-aged men, this precludes extrapolations to women, other age groups, or individuals having impaired glucose metabolism. However, the study design allowed us to investigate the acute effects of glucose ingestion on vascular endothelial function in the absence of confounding factors such as age, gender, and comorbidities. Finally, fructose was selected as an isocaloric control for the activation of the sympathetic nervous system by glucose ingestion, which can affect FMD responses (61). Although we observed minimal effects on vascular function following a single oral dose of fructose, our findings do not support fructose consumption as a strategy to mitigate VED. The consumption of pure fructose does not reflect typical dietary patterns. Indeed, most dietary fructose is derived from fructose-sweetened beverages and foods, and their chronic consumption has been implicated with greater CVD risk (62). Thus, additional work would be needed to assess whether chronic fructose ingestion affects vascular function.

In conclusion, this study demonstrates that the acute ingestion of glucose induces oxidative stress and likely attenuates vascular endothelial function in healthy young men in a NO-dependent manner. This suggests that repeated bouts of postprandial hyperglycemia may affect future CVD risk and controlling hyperglycemic responses may maintain vascular function. The close association between lipid peroxidation and vascular function also suggests that antioxidants capable of suppressing lipid peroxidation may improve vascular endothelial function otherwise suppressed by acute hyperglycemia.

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Literature Cited

1. CDC. National diabetes fact sheet: national estimates and general information on diabetes and prediabetes in the United States. In: U.S. Department of Health and Human Services, editor. Atlanta: CDC; 2011.
2. Lloyd-Jones D, Adams RJ, Brown TM, Carnethon M, Dai S, De Simone G, Ferguson TB, Ford E, Furie K, Gillespie C, et al. Executive summary: heart disease and stroke statistics–2010 update: a report from the American Heart Association. Circulation. 2010;121:948–54.
3. DECODE Study Group, the European Diabetes Epidemiology Group. Glucose tolerance and cardiovascular mortality: comparison of fasting and 2-hour diagnostic criteria. Arch Intern Med. 2001; 161:397–405.
4. Ogita H, Liao J. Endothelial function and oxidative stress. Endothelium. 2004;11:123–32.
5. Ceriello A, Quagliaro L, Piconi L, Assaloni R, Da Ros R, Maier A, Esposito K, Giugliano D. Effect of postprandial hypertriglyceridemia and hyperglycemia on circulating adhesion molecules and oxidative stress generation and the possible role of simvastatin treatment. Diabetes. 2004;53:701–10.
6. Kawano H, Motoyama T, Hirashima O, Hirai N, Miyao Y, Sakamoto T, Kugiyama K, Ogawa H, Yasue H. Hyperglycemia rapidly suppresses flow-mediated endothelium-dependent vasodilation of brachial artery. J Am Coll Cardiol. 1999;34:146–54.
7. Title LM, Cummings PM, Giddens K, Nassar BA. Oral glucose loading acutely attenuates endothelium-dependent vasodilation in healthy adults without diabetes: an effect prevented by vitamins C and E. J Am Coll Cardiol. 2000;36:2185–91.
8. Harris RA, Nishiyama SK, Wray DW, Richardson RS. Ultrasound assessment of flow-mediated dilation. Hypertension. 2010;55:1075–85.
9. Cai S, Khoo J, Mussa S, Alp NJ, Channon KM. Endothelial nitric oxide synthase dysfunction in diabetic mice: importance of tetrahydrobiopterin in eNOS dimerisation. Diabetologia. 2005;48:1933–40.
10. Ceriello A, Taboga C, Tonutti L, Quagliaro L, Piconi L, Bais B, Da Ros R, Motz E. Evidence for an independent and cumulative effect of postprandial hypertriglyceridemia and hyperglycemia on endothelial dysfunction and oxidative stress generation: effects of short- and long-term simvastatin treatment. Circulation. 2002;106:1211–8.
11. Du XL, Edelstein D, Dimmeler S, Ju Q, Sui C, Brownlee M. Hyperglycemia inhibits endothelial nitric oxide synthase activity by posttranslational modification at the Akt site. J Clin Invest. 2001;108: 1341–8.
12. Sydow K, Munzel T. ADMA and oxidative stress. Atheroscler Suppl. 2003;4:41–51.
13. Bode-Böger SM, Scalera F, Ignarro LJ. The L-arginine paradox: Importance of the L-arginine/asymmetrical dimethylarginine ratio. Pharmacol Ther. 2007;114:295–306.
14. Holvoet P, Collen D. Oxidized lipoproteins in atherosclerosis and thrombosis. FASEB J. 1994;8:1279–84.
15. Libby P. Inflammation in atherosclerosis. Nature. 2002;420:868–74.
16. Ellis A, Cheng ZJ, Li Y, Jiang YF, Yang J, Pannirselvam M, Ding H, Hollenberg MD, Triggle CR. Effects of a Western diet versus high glucose on endothelium-dependent relaxation in murine micro- and macro-vasculature. Eur J Pharmacol. 2008;601:111–7.
17. Reyes-Toso CF, Linares LM, Ricci CR, Aran M, Pinto JE, Rodriguez RR, Cardinali DP. Effect of melatonin on vascular reactivity in pancreatectomized rats. Life Sci. 2004;74:3085–92.
18. Wascher TC, Schmoelzer I, Wiegratz A, Stuehlinger M, Mueller-Wieland D, Kotzka J, Enderle M. Reduction of postchallenge hyperglycaemia prevents acute endothelial dysfunction in subjects with impaired glucose tolerance. Eur J Clin Invest. 2005;35:551–7.
19. Xiang GD, Sun HL, Zhao LS, Hou J, Yue L, Xu L. The antioxidant alpha-lipoic acid improves endothelial dysfunction induced by acute hyperglycaemia during OGTT in impaired glucose tolerance. Clin Endocrinol (Oxf). 2008;68:716–23.
20. Zhu W, Zhong C, Yu Y, Li K. Acute effects of hyperglycaemia with and without exercise on endothelial function in healthy young men. Eur J Appl Physiol. 2007;99:585–91.
21. Siafarikas A, Watts K, Beye P, Jones TW, Davis EA, Green DJ. Lack of effect of oral glucose loading on conduit vessel endothelial function in healthy subjects. Clin Sci (Lond). 2004;107:191–6.
22. Major-Pedersen A, Ihlemann N, Hermann TS, Christiansen B, Dominguez H, Kveiborg B, Nielsen DB, Svendsen OL, Kober L, Torp-Pedersen C. Effects of oral glucose load on endothelial function and on insulin and glucose fluctuations in healthy individuals. Exp Diabetes Res. 2008;2008:672021.
23. Jackson AS, Pollock ML. Generalized equations for predicting body density of men. Br J Nutr. 1978;40:497–504.
24. Schutte JE, Townsend EJ, Hugg J, Shoup RF, Malina RM, Blomqvist CG. Density of lean body mass is greater in blacks than in whites. J Appl Physiol. 1984;56:1647–9.
25. Siri W. Body composition from fluid spaces and density: analysis of methods. In: Brozek J, Hanschel A, editors. Techniques for measuring body composition. Washington, DC: National Academy of Sciences; 1961. p. 223–45.
26. Ballard KD, Bruno RS, Seip RL, Quann EE, Volk BM, Freidenreich DJ, Kawiecki DM, Kupchak BR, Chung MY, Kraemer WJ, et al. Acute ingestion of a novel whey-derived peptide improves vascular endothelial responses in healthy individuals: a randomized, placebo controlled trial. Nutr J. 2009;8:34.
27. Doshi SN, Naka KK, Payne N, Jones CJ, Ashton M, Lewis MJ, Goodfellow J. Flow-mediated dilatation following wrist and upper arm occlusion in humans: the contribution of nitric oxide. Clin Sci (Lond). 2001;101:629–35.
28. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11:81–128.
29. Hong YL, Yeh SL, Chang CY, Hu ML. Total plasma malondialdehyde levels in 16 Taiwanese college students determined by various thiobarbituric acid tests and an improved high-performance liquid chromatography-based method. Clin Biochem. 2000;33:619–25.
30. Teerlink T, Nijveldt RJ, de Jong S, van Leeuwen PAM. Determination of arginine, asymmetric dimethylarginine, and symmetric dimethylarginine in human plasma and other biological samples by high-performance liquid chromatography. Anal Biochem. 2002;303:131–7.
31. Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Prior RL. High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. J Agric Food Chem. 2002; 50:4437–44.
32. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem. 1996;239:70–6.
33. Bruno RS, Dugan CE, Smyth JA, DiNatale DA, Koo SI. Green tea extract protects leptin-deficient, spontaneously obese mice from hepatic steatosis and injury. J Nutr. 2008;138:323–31.
34. Freiser H, Jiang Q. Optimization of the enzymatic hydrolysis and analysis of plasma conjugated gamma-CEHC and sulfated long-chain carboxychromanols, metabolites of vitamin E. Anal Biochem. 2009; 388:260–5.
35. Bland JM, Altman DG. Calculating correlation coefficients with repeated observations: part 1: correlation within subjects. BMJ. 1995; 310:446.
36. Gallagher D, Heymsfield SB, Heo M, Jebb SA, Murgatroyd PR, Sakamoto Y. Healthy percentage body fat ranges: an approach for developing guidelines based on body mass index. Am J Clin Nutr. 2000; 72:694–701.
37. Lee CM, Huxley RR, Wildman RP, Woodward M. Indices of abdominal obesity are better discriminators of cardiovascular risk factors than BMI: a meta-analysis. J Clin Epidemiol. 2008;61:646–53.
38. Emmerson BT. Effect of oral fructose on urate production. Ann Rheum Dis. 1974;33:276–80.

39. Fang J, Alderman MH. Serum uric acid and cardiovascular mortality the NHANES I epidemiologic follow-up study, 1971–1992. National Health and Nutrition Examination Survey. JAMA. 2000;283:2404–10.
40. Lippi G, Montagnana M, Franchini M, Favaloro EJ, Targher G. The paradoxical relationship between serum uric acid and cardiovascular disease. Clin Chim Acta. 2008;392:1–7.
41. Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Case records of the Massachusetts General Hospital. Weekly clinicopathological exercises. Laboratory reference values. N Engl J Med. 2004;351:1548–63.
42. Böger RH. The emerging role of asymmetric dimethylarginine as a novel cardiovascular risk factor. Cardiovasc Res. 2003;59:824–33.
43. Santini SA, Marra G, Giardina B, Cotroneo P, Mordente A, Martorana GE, Manto A, Ghirlanda G. Defective plasma antioxidant defenses and enhanced susceptibility to lipid peroxidation in uncomplicated IDDM. Diabetes. 1997;46:1853–8.
44. Quagliaro L, Piconi L, Assaloni R, Martinelli L, Motz E, Ceriello A. Intermittent high glucose enhances apoptosis related to oxidative stress in human umbilical vein endothelial cell: the role of protein kinase C and NAD(P)H-oxidase activation. Diabetes. 2003;52:2795–804.
45. Nishikawa T, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, et al. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. Nature. 2000;404:787–90.
46. Esposito K, Giugliano D, Nappo F, Marfella R. Regression of carotid atherosclerosis by control of postprandial hyperglycemia in type 2 diabetes mellitus. Circulation. 2004;110:214–9.
47. Versari D, Daghini E, Virdis A, Ghiadoni L, Taddei S. Endothelial dysfunction as a target for prevention of cardiovascular disease. Diabetes Care. 2009;32 Suppl 2:S314–21.
48. Mizuno T, Matsui H, Imamura A, Numaguchi Y, Sakai K, Murohara T, Okumura K. Insulin resistance increases circulating malondialdehyde-modified LDL and impairs endothelial function in healthy young men. Int J Cardiol. 2004;97:455–61.
49. Vergnani L, Hatrik S, Ricci F, Passaro A, Manzoli N, Zuliani G, Brovkovych V, Fellin R, Malinski T. Effect of native and oxidized low-density lipoprotein on endothelial nitric oxide and superoxide production: key role of L-arginine availability. Circulation. 2000;101:1261–6.
50. Jay MT, Chirico S, Siow RC, Bruckdorfer KR, Jacobs M, Leake DS, Pearson JD, Mann GE. Modulation of vascular tone by low density lipoproteins: effects on L-arginine transport and nitric oxide synthesis. Exp Physiol. 1997;82:349–60.
51. Mann GE, Yudilevich DL, Sobrevia L. Regulation of amino acid and glucose transporters in endothelial and smooth muscle cells. Physiol Rev. 2003;83:183–252.
52. Vásquez R, Farias M, Vega JL, Martin RS, Vecchiola A, Casanello P, Sobrevia L. D-glucose stimulation of L-arginine transport and nitric oxide synthesis results from activation of mitogen-activated protein kinases p42/44 and Smad2 requiring functional type II TGF-beta receptors in human umbilical vein endothelium. J Cell Physiol. 2007;212:626–32.
53. Kashyap SR, Lara A, Zhang R, Park YM, DeFronzo RA. Insulin reduces plasma arginase activity in type 2 diabetic patients. Diabetes Care. 2008;31:134–9.
54. Forbes SP, Druhan LJ, Guzman JE, Parinandi N, Zhang L, Green-Church KB, Cardounel AJ. Mechanism of 4-HNE mediated inhibition of hDDAH-1: implications in no regulation. Biochemistry. 2008;47:1819–26.
55. Baron AD. Hemodynamic actions of insulin. Am J Physiol. 1994;267:E187–202.
56. Williams SB, Goldfine AB, Timimi FK, Ting HH, Roddy MA, Simonson DC, Creager MA. Acute hyperglycemia attenuates endothelium-dependent vasodilation in humans in vivo. Circulation. 1998;97:1695–701.
57. Harris RA, Padilla J, Rink LD, Wallace JP. Variability of flow-mediated dilation measurements with repetitive reactive hyperemia. Vasc Med. 2006;11:1–6.
58. Deanfield JE, Halcox JP, Rabelink TJ. Endothelial function and dysfunction: testing and clinical relevance. Circulation. 2007;115:1285–95.
59. Davies IR, Zhang X. Nitric oxide selective electrodes. Methods Enzymol. 2008;436:63–95.
60. Tsikas D. Methods of quantitative analysis of the nitric oxide metabolites nitrite and nitrate in human biological fluids. Free Radic Res. 2005;39:797–815.
61. Järvisalo MJ, Jartti L, Marniemi J, Ronnemaa T, Viikari JS, Lehtimaki T, Raitakari OT. Determinants of short-term variation in arterial flow-mediated dilatation in healthy young men. Clin Sci (Lond). 2006;110:475–82.
62. Johnson RK, Appel LJ, Brands M, Howard BV, Lefevre M, Lustig RH, Sacks F, Steffen LM, Wylie-Rosett J. Dietary sugars intake and cardiovascular health: a scientific statement from the American Heart Association. Circulation. 2009;120:1011–20.