

# Effects of Sugar-Sweetened Beverage Consumption on Microvascular and Macrovascular Function in a Healthy Population

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**Objective**—To assess vascular function during acute hyperglycemia induced by commercial sugar-sweetened beverage (SSB) consumption and its effect on underlying mechanisms of the nitric oxide pathway.

**Approach and Results**—In a randomized, single-blind, crossover trial, 12 healthy male participants consumed 600 mL (20 oz.) of water or a commercial SSB across 2 visits. Endothelial and vascular smooth muscle functions were assessed in the microcirculation using laser speckle contrast imaging coupled with iontophoresis and in the macrocirculation using brachial artery ultrasound with flow- and nitrate-mediated dilation. Compared with water, SSB consumption impaired microvascular and macrovascular endothelial function as indicated by a decrease in the vascular response to acetylcholine iontophoresis (208.3±24.3 versus 144.2±15.7%,  $P<0.01$ ) and reduced flow-mediated dilation (0.019±0.002 versus 0.014±0.002%/s,  $P<0.01$ ), respectively. Systemic vascular smooth muscle remained preserved. Similar decreases in endothelial function were observed during acute hyperglycemia in an in vivo rat model. However, function was fully restored by treatment with the antioxidants, *N*-acetylcysteine and apocynin. In addition, ex vivo experiments revealed that although the production of reactive oxygen species was increased during acute hyperglycemia, the bioavailability of nitric oxide in the endothelium was decreased, despite no change in the activation state of endothelial nitric oxide synthase.

**Conclusions**—To our knowledge, this is the first study to assess the vascular effects of acute hyperglycemia induced by commercial SSB consumption alone. These findings suggest that SSB-mediated endothelial dysfunction is partly due to increased oxidative stress that decreases nitric oxide bioavailability.

**Clinical Trial Registration**—URL: <https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=366442&isReview=true>. Australian New Zealand Clinical Trials Registry Number: ACTRN12614000614695.

**Visual Overview**—An online [visual overview](#) is available for this article. (*Arterioscler Thromb Vasc Biol.* 2017;37:1250-1260. DOI: 10.1161/ATVBAHA.116.308010.)

**Key Words:** acetylcholine ■ antioxidants ■ brachial artery ■ endothelium ■ hyperglycemia

Commercial sugar-sweetened beverages (SSB) are one of the most frequently consumed drinks worldwide.<sup>1,2</sup> As such, they represent a major source of added sugar in the modern diet. Habitual consumption of SSB induces frequent episodes of acute hyperglycemia and is linked to an increased risk of developing obesity, metabolic syndrome, and type 2 diabetes mellitus.<sup>2-4</sup> Furthermore, excessive SSB consumption is also associated with a higher incidence of advanced forms of cardiovascular disease (CVD),<sup>2</sup> which remains the single leading cause of death representing 31% of the global mortality rate.<sup>5</sup> Impaired vascular function is considered to be a main precursor to the pathogenesis of CVD and may be present long before atherosclerotic vascular changes occur.<sup>6</sup>

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Normal vascular function involves a continuous interaction between the endothelium and vascular smooth muscle (VSM) that is regulated by numerous vasodilators and vasoconstrictors.<sup>7,8</sup> In a recent systematic review and meta-analysis, our research group provided evidence that acute hyperglycemia induced by an oral sugar load transiently impairs endothelial function not only in patients with cardiometabolic disease, but also in those who are considered healthy.<sup>9</sup> It has been suggested that such endothelial dysfunction may be attributed to increased oxidative stress mediated by acute hyperglycemia.<sup>10,11</sup> Indeed, nitric oxide (NO) bioavailability in the vascular wall is highly sensitive to redox modulation of the cellular

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**Nonstandard Abbreviations and Acronyms**

<b>CVD</b>	cardiovascular disease
<b>eNOS</b>	endothelial NO synthase
<b>NO</b>	nitric oxide
<b>SSB</b>	sugar-sweetened beverages
<b>VSM</b>	vascular smooth muscle

environment.<sup>12,13</sup> However, due to a limited availability of microcirculatory data and discrepant reporting of macrovascular data, the impact of acute hyperglycemia on vascular function remains inconclusive and the underlying mechanisms not fully understood.

In this context and considering the surge in commercial SSB consumption, as well as its potential link to a sustained epidemic of CVD, this study aimed to provide a comprehensive assessment of the effect of SSB-mediated acute hyperglycemia on microvascular and macrovascular function in a healthy population. In addition, considering that experimental exploration of healthy vascular tissue in humans is difficult to perform, due to ethical barriers, this study also aimed to further investigate the underlying mechanisms of the interaction between acute hyperglycemia and endothelial function via an *in vivo* and *ex vivo* rat model.

## Materials and Methods

Materials and Methods are available in the [online-only Data Supplement](#).

## Results

Twelve healthy male participants were recruited and completed this randomized, single-blind, crossover trial between June 9, 2014 and July 4, 2014 (Figure 1). To examine the acute vascular effects of commercial SSB consumption in humans, variables of blood glucose concentration, heart rate, arterial pressure, and vascular function were assessed after the ingestion of 600 mL (20 oz.) of a commercial SSB and compared with values measured after water consumption (Figure 2). Nutritional information for each beverage is presented in Table 1.

### Participant Characteristics

Participants included in the study were aged  $31 \pm 1.9$  years with a body mass index of  $24.68 \pm 0.71$  kg/m<sup>2</sup>. At each visit, before the consumption of the test beverage, basal measurements of fasting blood glucose ( $4.95 \pm 0.15$  versus  $4.78 \pm 0.16$  mmol/L,  $P > 0.05$ ), heart rate ( $64.08 \pm 3.1$  versus  $62.25 \pm 3.2$  bpm,  $P > 0.05$ ), and mean arterial pressure ( $80.50 \pm 1.66$  mmHg versus  $79.86 \pm 1.48$  mmHg,  $P > 0.05$ ) were found to be similar (Figure 3).

### SSB Consumption Increased Blood Glucose Concentration With No Major Effect on Heart Rate or Blood Pressure

Ingestion of the SSB significantly elevated blood glucose concentrations above basal values 20 minutes ( $7.77 \pm 0.38$  versus  $4.78 \pm 0.16$  mmol/L,  $P < 0.05$ ) after the beginning of consumption, with peak hyperglycemia ( $8.85 \pm 0.36$

mmol/L,  $P < 0.05$ ) recorded at 40 minutes (Figure 3A). A progressive decrease in blood glucose concentrations was observed between 40 and 75 minutes thereafter. However, blood glucose concentrations at 75 minutes were still significantly greater than basal blood glucose values ( $6.39 \pm 0.3$  versus  $4.78 \pm 0.16$  mmol/L,  $P < 0.05$ ), and all blood glucose values measured over time after water consumption. Blood glucose concentrations did not deviate from basal measurements after water consumption. Sugar-sweetened beverage-mediated acute hyperglycemia had no major effect on mean arterial pressure. However, a slight but significant reduction in mean arterial pressure was observed during peak hyperglycemia when compared with that at the baseline measurement ( $P < 0.05$ ; Figure 3B). No variations in heart rate were observed during acute hyperglycemia (Figure 3C).

### SSB Consumption Decreased Microvascular and Macrovascular Endothelial Function

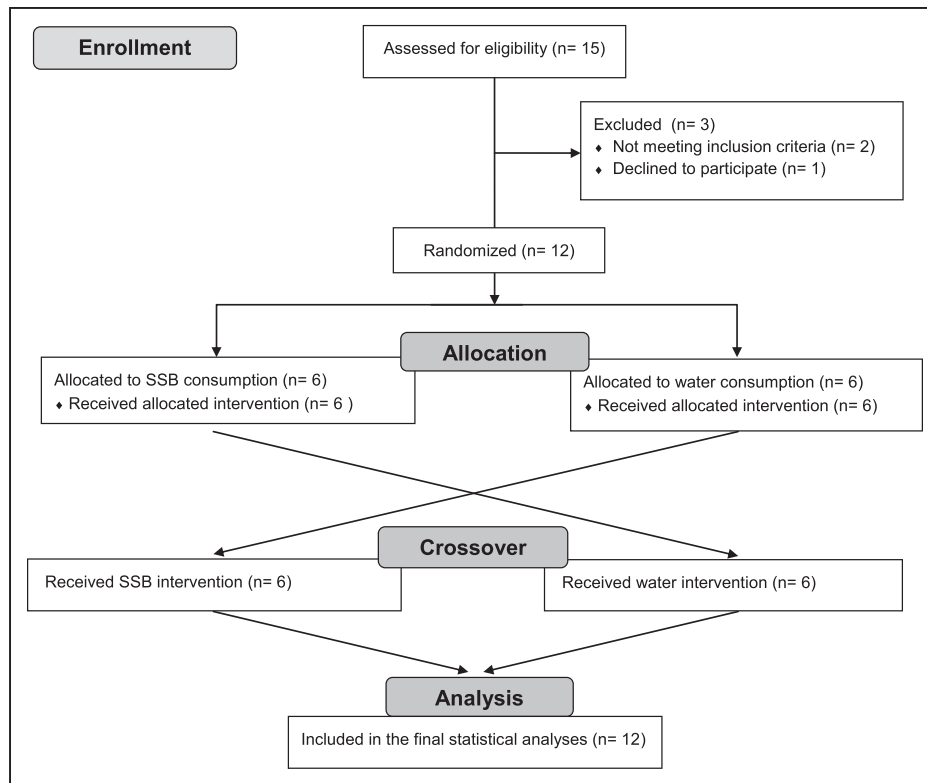
During acute hyperglycemia, all assessments of microvascular and macrovascular function were completed between 20 and 75 minutes after the beginning of SSB consumption. Vascular function was assessed in the same time period after water consumption to allow for comparison between the 2 test beverages.

#### Assessment of Cutaneous Microvascular Function

Cutaneous microvascular endothelial and VSM functions were assessed using laser speckle contrast imaging in conjunction with iontophoresis of acetylcholine and sodium nitroprusside, respectively. Before the beginning of iontophoresis, there were no differences in baseline measurements of basal cutaneous blood flux between each visit ( $23.6 \pm 1.8$  versus  $26.4 \pm 1.6$  perfusion units,  $P = 0.23$ ; Table 2). An increase in cutaneous blood flux was observed after iontophoresis of acetylcholine and sodium nitroprusside (Figure 4A). However, the relative percentage increase in cutaneous blood flux in response to acetylcholine iontophoresis was significantly lower during SSB-mediated acute hyperglycemia when compared with that during normoglycemia after water consumption ( $129.76 \pm 11.18$  versus  $196.78 \pm 20.61\%$ , respectively,  $P < 0.01$ ; Figure 4B). Even after accounting for differences in blood pressure between visits by converting cutaneous blood flux from perfusion units to cutaneous vascular conductance, the relative increase in cutaneous blood flux mediated by acetylcholine iontophoresis was still lower after ingestion of the SSB than that measured after water consumption ( $144.2 \pm 15.7$  versus  $208.3 \pm 24.3\%$ , respectively,  $P < 0.01$ ). In contrast, the vascular responses to iontophoresis of sodium nitroprusside were similar after the consumption of each test beverage.

#### Macrovascular Measurements

Endothelial and VSM functions were then assessed in the macrocirculation using ultrasound of the brachial artery in conjunction with flow-mediated dilation and nitrate-mediated dilation, respectively. After the consumption of each test beverage, there were no differences in basal brachial artery diameter or blood flow between visits ( $4.7 \pm 0.1$  versus  $4.7 \pm 0.1$  mm,  $P = 0.68$  and  $48.0 \pm 5.7$  versus  $53.0 \pm 6.5$  mL/

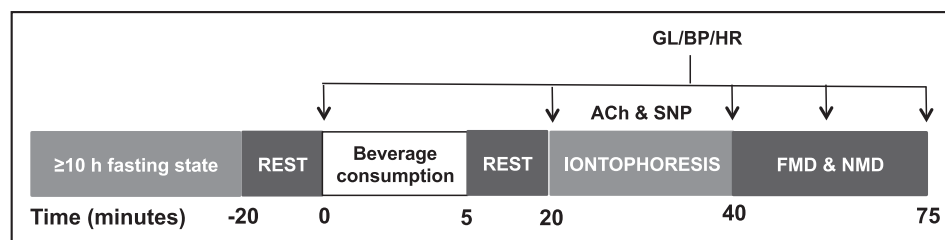


**Figure 1.** Flow diagram illustrating participant recruitment and randomized allocation to sugar-sweetened beverage (SSB) or water consumption interventions; and the participant retention to the conclusion of the cross-over trial.

min,  $P=0.67$ , respectively; Table 2). Increases in brachial blood flow and the  $\Delta$ shear rate induced during hyperemia were also similar between each visit ( $408.3\pm 27.6$  versus  $445.1\pm 34.5$  mL/min,  $P=0.19$  and  $484.3\pm 32.8$  versus  $501.6\pm 37.1$  %/s,  $P=0.51$ , respectively). However, flow-mediated dilation was significantly reduced ( $-23.86\pm 5.33\%$ ) during SSB-mediated acute hyperglycemia when compared with that during normoglycemia after water consumption; when expressed solely as the percentage change in diameter ( $6.53\pm 0.61$  versus  $8.56\pm 0.54\%$ , respectively,  $P<0.01$ ; Figure 5) and as the percentage change in diameter with respect to the change in shear rate ( $0.014\pm 0.002$  versus  $0.019\pm 0.002$  %/s, respectively,  $P<0.01$ ). In contrast, there were no differences in the responses to nitrate-mediated dilation between each visit.

### Endothelial Dysfunction After SSB Consumption May Be Associated With an Acute Hyperglycemic-Mediated Increase in Oxidative Stress That Decreases NO Bioavailability

Given that both flow-mediated dilation and the vascular response to acetylcholine iontophoresis were reduced during SSB-mediated acute hyperglycemia, while the vascular responses to nitrate administration were preserved, clinical findings clearly suggest that acute hyperglycemia impairs microvascular and macrovascular function via an endothelium-dependent pathway. However, considering that further experimental exploration within vascular tissues of healthy humans is, for ethical reasons, difficult to perform, the underlying mechanisms by which acute hyperglycemia mediates endothelial dysfunction were evaluated further, both in vivo



**Figure 2.** Study design: sequence of testing. In a fasted state ( $\geq 10$  hours) participants rested for 20 minutes before consuming 600 mL (20 oz.) of water or sugar-sweetened beverage (SSB) within a 5-minute period. Participants then rested for a further 15 minutes before iontophoresis of acetylcholine (ACh) and sodium nitroprusside (SNP) were performed. Immediately after the conclusion of iontophoresis, flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD) were also performed. Blood glucose (GL), blood pressure (BP), and heart rate (HR) were measured after the 20 minutes resting period, 15 minutes after consuming the test beverage, at the end of iontophoresis, at the end of FMD and at the end of NMD. All 12 participants were administered water or the commercial SSB in a randomized order.

**Table 1. Nutrient Composition of Each Test Beverage**

	Water	SSB
Volume, mL	600	600
Energy, kJ	0	1200
Protein, g	0	0.30
Fat		
Total, g	0	0
Saturated, g	0	0
Carbohydrate, g	0	72.4
Sugars, g	0	72.4
Calcium, mg	18	N/A
Magnesium, mg	6	N/A
Sodium, mg	24	108
Zinc, mg	0.06	N/A
Ingredients	Tap water	Carbonated water, sugar, reconstituted lemon juice (5%), food acids (330, 331, natural flavor, preservatives (211, 223), natural color (safflower extract)

Nutrient composition of water was obtained from the US Department of Agriculture Nutrient Database Standard Reference Release 27.<sup>14</sup> Nutrient composition for the commercial SSB was obtained from the nutrition information label on packaging. Participants consumed 600 mL of water or commercial SSB 15 min before the beginning of vascular assessment. Participants completed trials on separate days for each test beverage in a randomized order. SSB indicates sugar-sweetened beverage.

and ex vivo, using an experimental rat model (Figure I in the [online-only Data Supplement](#)). Furthermore, as it is not possible to administer rats with an intraperitoneal injection of commercial SSB, this study used an intraperitoneal injection of glucose to examine the underlying mechanisms of SSB-mediated endothelial dysfunction. Importantly, previous research has revealed that acute changes in vascular function after SSB consumption are more related to the glucose, than the fructose, in sucrose<sup>15</sup>; and that fructose has a minimal effect on endothelial function in rats.<sup>16</sup> Moreover, the adverse vascular effects of glucose consumption in humans has been further demonstrated in this study with the [online-only Data Supplement](#) indicating that macrovascular endothelial function is decreased during glucose-mediated acute hyperglycemia, compared with that during normoglycemia, when expressed as the percentage change in diameter (6.17±0.66 versus 9.92±1.26%, respectively, *P*<0.05) and as the percentage change in diameter with respect to the change in shear rate (0.011±0.001 versus 0.018±0.003%/s, respectively, *P*=0.07; Figure II in the [online-only Data Supplement](#)).

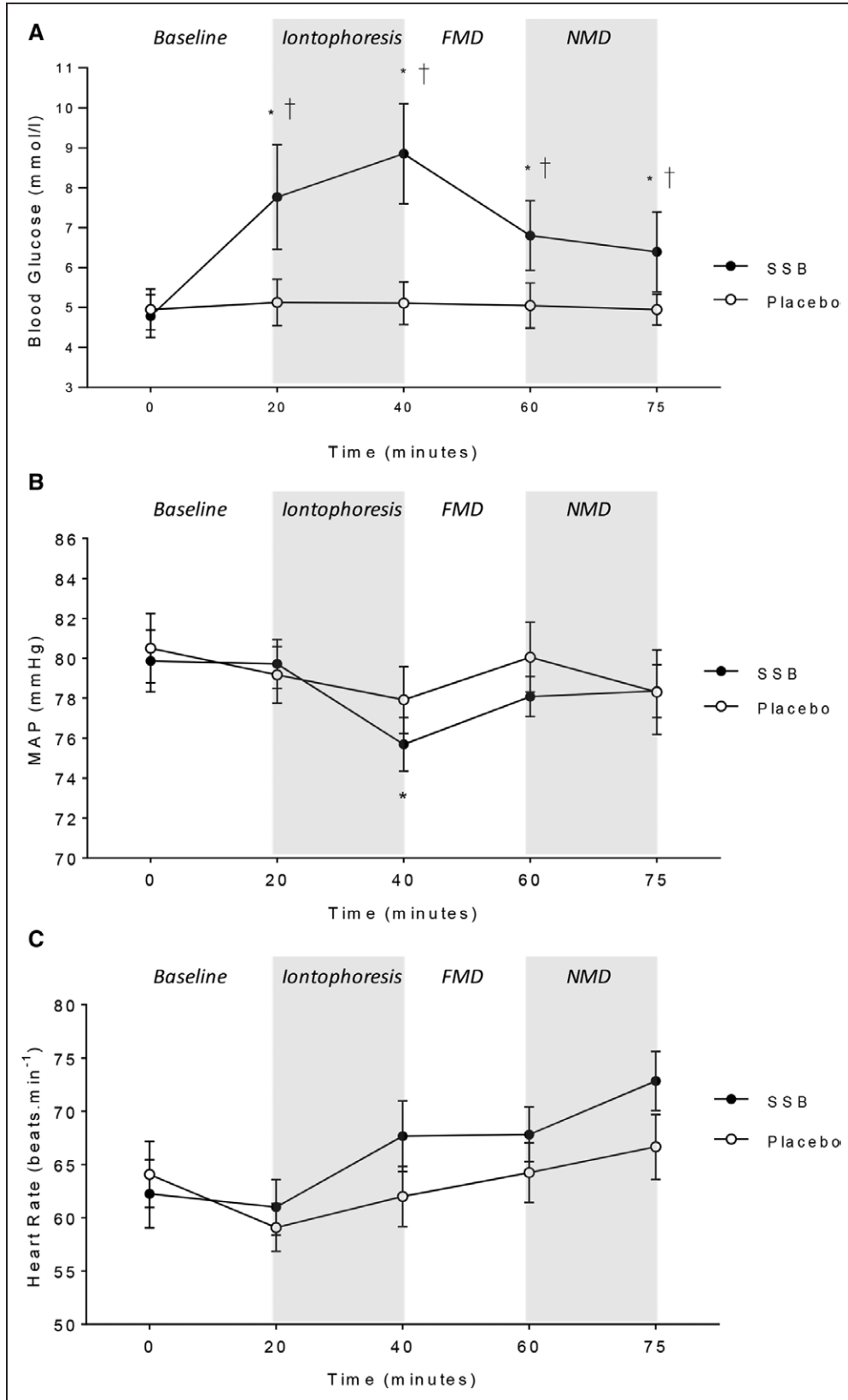
**In Vivo Experimental Exploration**

Acute hyperglycemia was induced in each rat using an intraperitoneal injection of glucose that mediated a hyperglycemic plateau between 10 and 20 minutes after administration, with peak hyperglycemia occurring at 15 minutes (Figure IIIA in the [online-only Data Supplement](#)). To allow for comparisons between the acute hyperglycemic and normoglycemic

conditions, a group of control rats received a placebo injection of sodium chloride 0.9% (saline). During the acute hyperglycemic plateau, iontophoresis of acetylcholine and sodium nitroprusside was performed inducing a significant increase in cutaneous microvascular blood flux (Figure 6A). However, similar to the findings obtained in human trials, the relative increase in cutaneous blood flux in response to iontophoresis of acetylcholine was decreased during acute hyperglycemia when compared with normoglycemic controls, while the vascular response to sodium nitroprusside iontophoresis remained preserved (Figure 6B). Such endothelial dysfunction may be primarily caused by an acute hyperglycemic-mediated increase in oxidative stress.<sup>17</sup> Considering this, a third group of rats were treated with a dose of the antioxidant, *N*-acetylcysteine, during normoglycemia and before the induction of acute hyperglycemia. Treatment with *N*-acetylcysteine had no effect on vascular function during normoglycemia. However, although the acute hyperglycemic responses to the intraperitoneal injection of glucose between *N*-acetylcysteine-treated and untreated rats were similar (Figure IIIB in the [online-only Data Supplement](#)), the increase in cutaneous blood flux in response to acetylcholine iontophoresis was fully restored in rats treated with the antioxidant (Figure 6B), supporting the implication of increased oxidative stress in acute hyperglycemia-mediated endothelial dysfunction.

**Ex Vivo Experimental Exploration**

Given that endothelial function is highly dependent on NO bioavailability, which itself is dependent on endothelial NO synthase (eNOS) and its activation by phosphorylation at serine 1177 (eNOS<sup>ser1177</sup>), ex vivo experiments were then performed on rat aortic tissue to investigate the interaction between oxidative stress and the NO pathway; and whether this interaction contributes to the endothelial dysfunction observed during acute hyperglycemia. Similar to that observed after SSB consumption in human trials and the intraperitoneal injection of glucose in in vivo experimental exploration, acute hyperglycemia induced by the hyperglycemic solution decreased the relaxation response to acetylcholine administration in precontracted aortic rings in a dose-dependent manner (Figure IVA and IVB in the [online-only Data Supplement](#)). However, pretreatment with the nonspecific antioxidant, *N*-acetylcysteine restored the relaxation response in aortic rings during acute hyperglycemia. Interestingly, similar results were observed when apocynin, a specific inhibitor of nicotinamide adenine dinucleotide phosphate hydrogen (NADPH) oxidase,<sup>18</sup> was administered (Figure 6C). Although these results were supported by electron paramagnetic resonance assessments, which measured an elevation in the production of reactive oxygen species during acute hyperglycemia when compared with the normoglycemic condition (Figure 6D), Western blotting revealed that acute hyperglycemia had no effect on the expression of eNOS or its activation in aortic rings (Figure 6E). Moreover, eNOS dimerization, evaluated by the SDS-resistant dimer/monomer ratio, was not affected by acute hyperglycemia (Figure 6F). Despite this, there was still a marked reduction in the bioavailability of NO in the aortic tissue, indicated by a lower concentration of nitrites, during acute hyperglycemia (Figure 6G).



**Figure 3.** Changes in (A) blood glucose concentrations, (B) mean arterial pressure (MAP), and (C) heart rate over time in response to consumption of 600 mL of water or commercial sugar-sweetened beverage (SSB). \* $P < 0.05$  vs baseline; † $P < 0.05$  vs water. FMD indicates flow-mediated dilation; and NMD, nitrate-mediated dilation.

**Table 2. Microvascular and Macrovascular Function in Response to Water and Commercial SSB Consumption in Healthy Subjects**

n	Water	SSB	P-Value
	12	12	
<b>Microcirculation</b>			
Basal CBF, PU	23.6±1.8	26.4±1.6	P=0.231
Peak ACh CBF, PU	70.9±8.0	63.0±6.4	P=0.346
Peak SNP CBF, PU	101.2±6.0	101.9±7.4	P=0.934
ACh CVC increase, %	208.3±24.3	144.2±15.7*	P=0.008
SNP CVC increase, %	360.3±26.7	355.9±29.3	P=0.926
Skin resistance, Ω	399.4±41.3	395.6±30.2	P=0.872
<b>Macrocirculation</b>			
Basal brachial artery diameter, mm	4.7±0.1	4.7±0.1	P=0.684
Basal brachial blood flow, mL/min	48.0±5.7	53.0±6.5	P=0.673
Peak brachial blood flow, mL/min	408.3±27.6	445.1±34.5	P=0.194
Δshear rate, %/s	484.3±32.8	501.6±37.1	P=0.506
FMD/Δshear rate, %/s	0.019±0.002	0.014±0.002*	P<0.001
Brachial hyperemia, %	886.4±132.3	855.5±107.7	P=0.693

Values are mean±SEM. Δshear rate means peak brachial shear rate minus resting brachial shear rate. P-value was estimated by repeated-measures ANOVA followed by post hoc Tukey multiple comparison tests. Ach indicates acetylcholine; CBF, cutaneous blood flux; CVC, cutaneous vascular conductance; FMD, flow-mediated dilation; PU, perfusion units; SSB, sugar-sweetened beverage; and SNP, sodium nitroprusside.

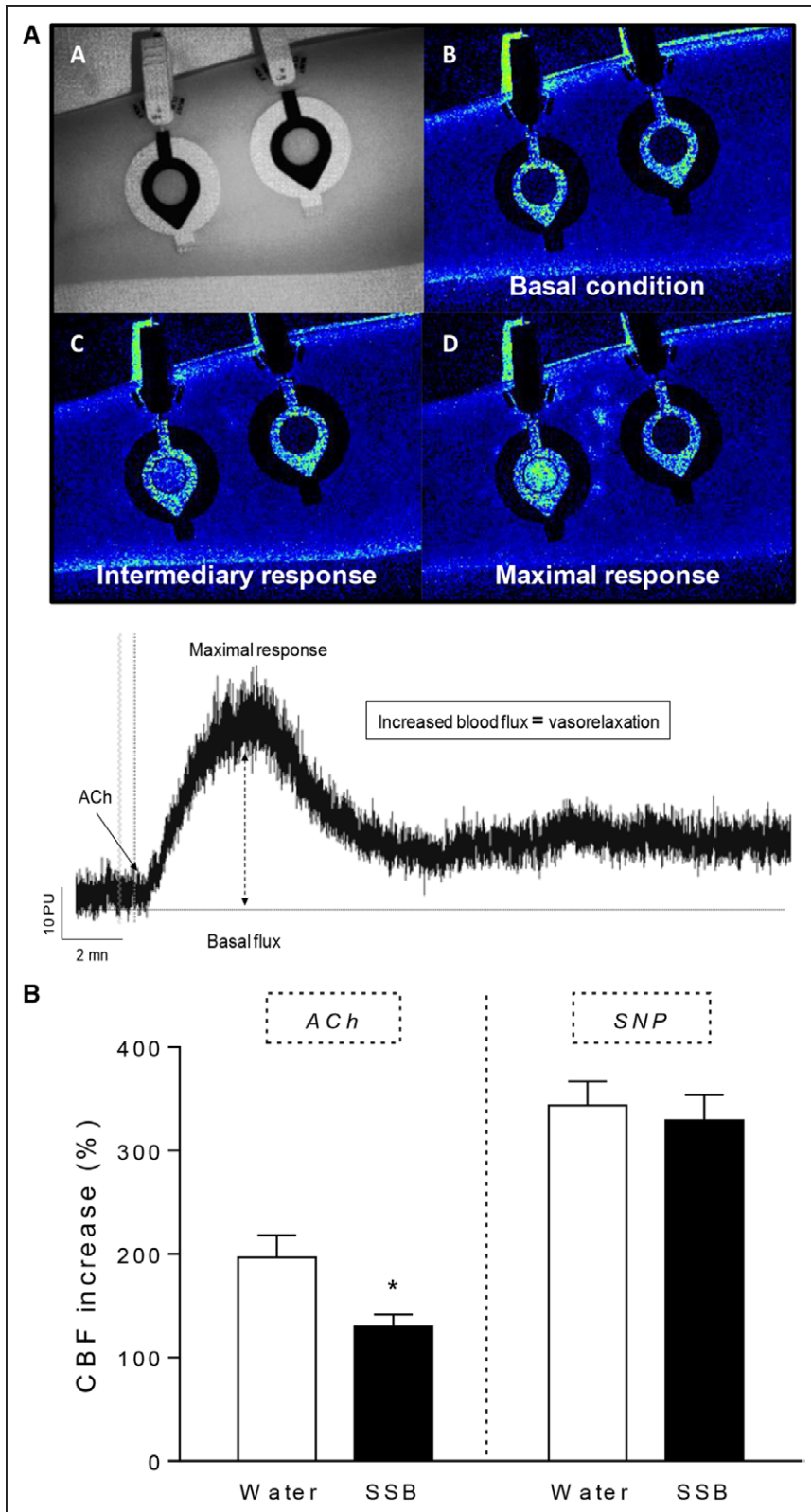
\*P<0.05 vs water.

### Discussion

This study aimed to assess the effect of acute hyperglycemia induced by the ingestion of a commercial SSB on vascular function in healthy sedentary participants. It was found that SSB consumption decreased both microvascular and macrovascular endothelial function, whereas VSM function remained unaffected during acute hyperglycemia. Considering these findings, in vivo and ex vivo explorations were then performed in an experimental rat model to further investigate the interaction between acute hyperglycemia and vascular function and examine the underlying mechanisms that may mediate such endothelial dysfunction. Similar to that observed in human trials, acute hyperglycemia mediated a decrease in endothelial function in both in vivo and ex vivo experimental rat models. Interestingly, endothelial function was fully restored during acute hyperglycemia in rats that were pretreated with the antioxidant, N-acetylcysteine, and in aortic rings that were pretreated with either N-acetylcysteine or a specific inhibitor of NADPH oxidase, apocynin. Further to this, it was found that acute hyperglycemia was associated with increased production of arterial reactive oxygen species and reduced bioavailability of NO. Collectively, these findings suggest that acute hyperglycemia induces endothelial dysfunction by mediating an increase in oxidative stress that disrupts the NO pathway.

It is currently unknown how acute hyperglycemia induced by commercial SSB consumption affects vascular function because of limited and discrepant data. Previous research has most commonly assessed vascular function during acute hyperglycemia induced by a typical oral glucose load using

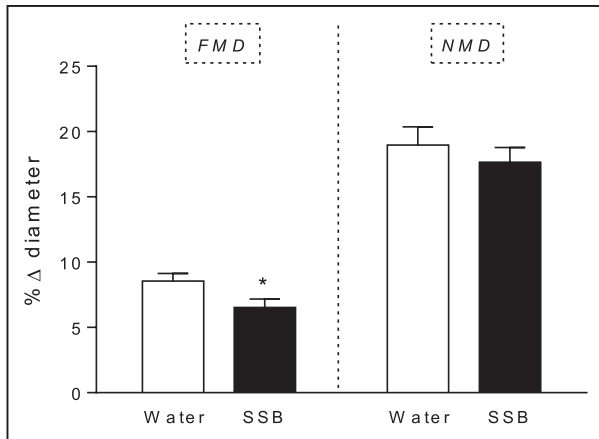
measures of macrovascular reactivity, such as brachial artery ultrasound with flow-mediated dilation.<sup>9</sup> However, emerging evidence suggests that coronary microvascular disease may explain the occurrence of myocardial ischemia, heart failure, and CVD mortality after myocardial infarction without apparent coronary macrovascular disease,<sup>19</sup> highlighting the need to assess the microcirculation, which represents most of the arterial vascular network and exerts dominant control over local blood flow,<sup>20</sup> in conjunction with the macrocirculation when investigating mechanisms that contribute to the pathogenesis of CVD. The results of this study are consistent with those in previous research, which demonstrated that commercial SSB consumption mediates a decrease in macrovascular endothelial function as indicated by reduced flow-mediated dilation during acute hyperglycemia.<sup>21</sup> Importantly, it should be noted that this previous study induced acute hyperglycemia by administering a commercial SSB in conjunction with a high caloric commercial candy bar. In contrast, the microcirculatory findings contradict a separate study, which found that consumption of commercial SSB enhances microvascular endothelial function, as indicated by an increased cutaneous blood flux response to iontophoresis of acetylcholine during acute hyperglycemia.<sup>22</sup> However, in this study, acute hyperglycemia was induced using a commercial SSB that is defined as an energy drink containing caffeine, which is an ingredient that has been found to increase microvascular reactivity in healthy participants.<sup>23</sup> Considering the possible confounding vascular effects of these additional foods or ingredients, this present research is, to our knowledge, the first to examine the effects of acute hyperglycemia induced by commercial



**Figure 4.** Microvascular function in healthy participants after consumption of 600 mL of water or a commercial sugar-sweetened beverage (SSB). **A, Top,** Laser speckle contrast imaging electrode position on the right forearm. **Bottom,** Representative curve of cutaneous blood flow (CBF) during acetylcholine (ACh) iontophoresis. **B,** The percentage increase from baseline in CBF in response to iontophoresis of ACh and sodium nitroprusside (SNP). \* $P < 0.01$  vs water.

SSB consumption alone on microvascular and macrovascular function. The results of this study also contribute to clarifying the effect of acute hyperglycemia on systemic vascular endothelial function, which in a recent systematic review and meta-analysis was found to be inconclusive because of

limited microvascular data and discrepant reporting of shear stress data in studies that used flow-mediated dilation to assess changes in vascular function from normoglycemic to hyperglycemic states.<sup>9</sup> Given that this present study found a decrease in flow-mediated dilation during acute hyperglycemia with no



**Figure 5.** Macrovascular function in healthy participants after consumption of 600 mL of water or commercial sugar-sweetened beverage (SSB). The percentage change from baseline brachial artery diameter in response to flow-mediated dilation (FMD) and nitrate-mediated dilation (NMD). \* $P < 0.01$  vs water.

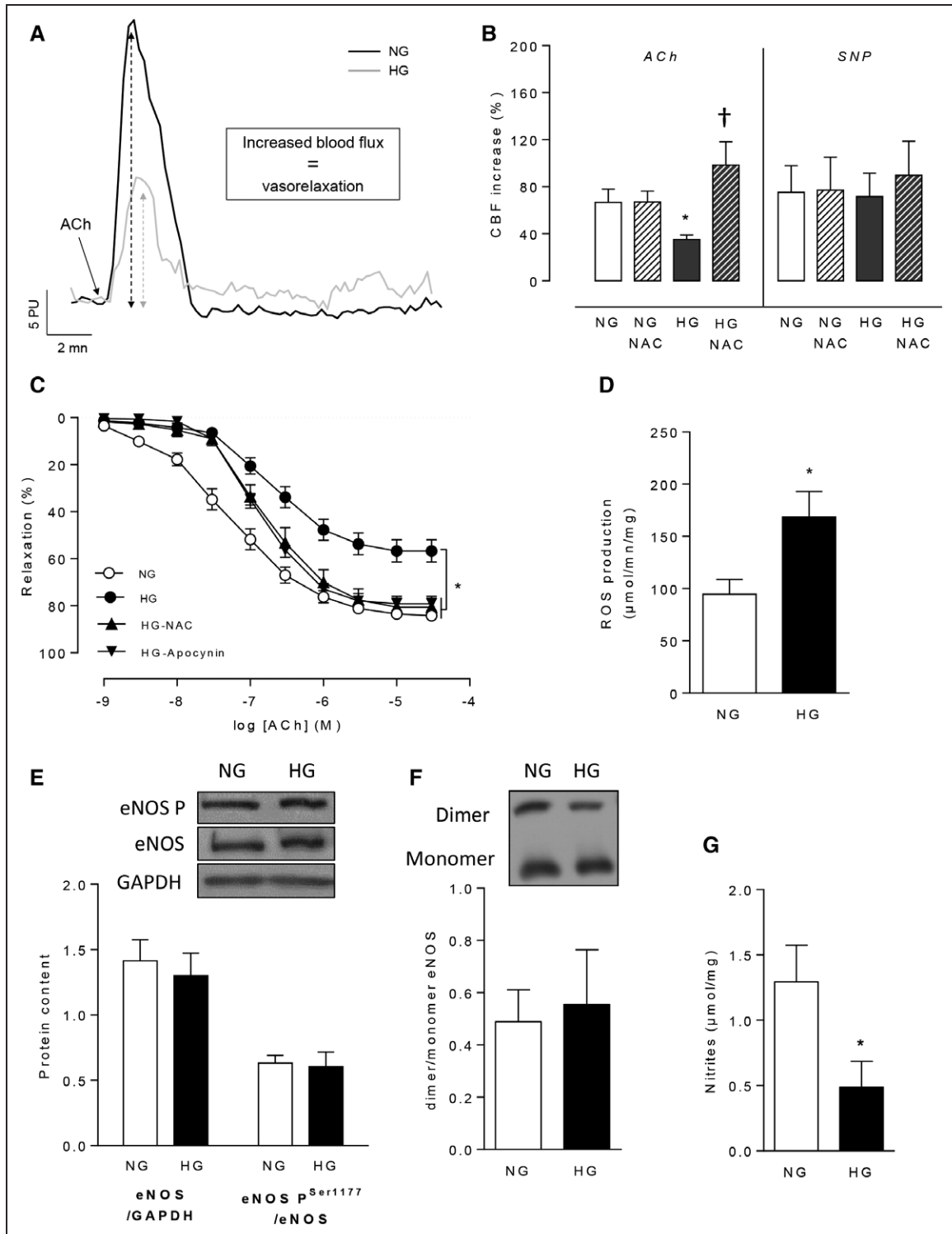
reduction in shear stress, a disruption of the NO pathway may be implicated in SSB-mediated endothelial dysfunction.<sup>24,25</sup>

Current evidence suggests that acute hyperglycemia induces endothelial dysfunction by mediating an abnormal elevation in oxidative stress that disturbs normal underlying mechanisms of NO synthesis.<sup>26,27</sup> In the postprandial state, oxidative metabolism initiates oxidative phosphorylation of adenosine triphosphate at the electron transport chain of the mitochondria, which via the phenomenon of electron leakage causes superoxide generation of reactive oxygen species.<sup>28</sup> Moreover, it has been clearly reported that increased glycemia is responsible for the activation of NADPH oxidase, which also contributes to production of the superoxide anion.<sup>10</sup> Although these reactive oxygen species are normally readily detoxified, elevated activity within this mechanism such as that after commercial SSB consumption increases production to a rate beyond suppressive capabilities of the antioxidant systems.<sup>29</sup> The implication of oxidative stress in acute hyperglycemia-mediated endothelial dysfunction is further supported by *ex vivo* findings in this study, which demonstrated that acute hyperglycemia increased reactive oxygen species in rat aortic rings. Moreover, treatment with the antioxidants, *N*-acetylcysteine and Apocynin, were found to attenuate the impaired relaxation response to acetylcholine iontophoresis observed during acute hyperglycemia. Importantly, the applicability of this oxidative stress-dependent mechanism to acute hyperglycemia-mediated endothelial dysfunction in living organisms was also demonstrated in this study, which revealed for the first time in an *in vivo* experimental rat model that antioxidant treatment also fully restores cutaneous microvascular endothelial function during acute hyperglycemia. This study also found that eNOS<sup>ser1177</sup> and the eNOS dimer/monomer ratios remained preserved during acute hyperglycemia, whereas the concentration of nitrites was decreased. Such experimental findings provide evidence that SSB-mediated endothelial dysfunction is due, at least in part, to decreased bioavailability of NO that is not caused by a disruption to the synthesis of NO via the eNOS pathway. Indeed, the reaction between NO and free radical superoxide results in the

formation of peroxynitrite, a potent cytotoxic molecule.<sup>30</sup> This nitro-oxidative stress could be a primary mechanism responsible for the decrease in NO bioavailability that was observed in our model of acute hyperglycemic stress.

Despite not being affected in this study, previous research has demonstrated decreased VSM function mediated by VSM cell proliferation in as little as 6 hours after the induction of hyperglycemia in animal and *in vitro* studies,<sup>31</sup> suggesting a need to extend the typical assessment period of vascular function after SSB consumption in future research. Moreover, considering the global rate of commercial SSB consumption and its role in transient endothelial dysfunction, even in a healthy population, research must also quantify the relative loading of SSB over time that mediates significant vascular remodeling and contributes to the pathogenesis of CVD in humans.<sup>9</sup> In addition to examining the underlying mechanisms of NO-mediated microvascular function, future research may also assess the effect of SSB consumption on other main vasoactive mediators such as prostaglandin I<sub>2</sub>, endothelium-derived hyperpolarizing factor, and endothelin-1, all of which have varying influence between the microvascular and macrocirculation.<sup>7,11,32</sup> Finally, these studies need to be conducted across a variety of ethnicities, some of which have previously demonstrated decreased vascular function even at rest and, therefore, may be more severely affected by the deleterious vascular-related effects of acute hyperglycemia than that observed in white populations.<sup>33,34</sup>

Several inherent limitations must be considered when interpreting this data. Whereas the main sugar in SSB is sucrose, which is comprised of glucose and fructose, only glucose was used to induce acute hyperglycemia in the experimental rat model that aimed to explain the effects of SSB consumption on underlying mechanisms of vascular function. However, it has been suggested that acute deleterious cardiovascular effects of sucrose are more related to glucose rather than fructose,<sup>11,15</sup> with previous findings also demonstrating that fructose has no significant effect on endothelial function in rats.<sup>16</sup> Nevertheless, although normal VSM function remained preserved after SSB consumption in this study, previous research has found that fructose can decrease the VSM relaxation response to the administration of sodium nitroprusside in rats,<sup>16</sup> and therefore, it cannot be completely discounted that fructose may also contribute, to some extent, to the observed SSB-mediated endothelial dysfunction. It must also be considered that ingredients other than sucrose that comprise commercial SSB were not evaluated individually in this research and, therefore, it is not known how they may contribute to the observed SSB-mediated endothelial dysfunction. Indeed, increases in plasma sodium may also contribute to a decrease in NO bioavailability.<sup>35</sup> In addition, it was not possible to blind participants to the intervention by using a sugar-free placebo such as a commercial diet soda because of previous research suggesting that even artificial sweeteners may interact with taste receptors stimulating insulin secretion, which may induce a vascular response.<sup>36</sup> Given that changes in blood insulin concentration in response to SSB consumption were not monitored in this study, it was not possible to explore what effect SSB consumption may have on mechanisms of



**Figure 6.** Implication of oxidative stress in endothelial dysfunction during acute hyperglycemia. **A**, Representative curve of cutaneous microvascular blood flux (CBF) during normoglycemia (NG) and acute hyperglycemia (HG) in response to acetylcholine (ACh) iontophoresis in rats. **B**, The percentage increase from baseline in CBF in response to iontophoresis of acetylcholine and sodium nitroprusside after administration of sodium chloride 0.9% (saline) in NG rats and after the induction of HG in rats pretreated with saline to examine the effect of HG alone, or in rats pretreated with *N*-acetylcysteine (NAC) to evaluate implication of oxidative stress in endothelial dysfunction. **C**, A dose–response curve to ACh during NG and HG (100 mmol/L) in precontracted aortic rings and during HG (100 mmol/L) in precontracted rings preincubated in NAC (20 mmol/L) or in Apocynin (100 μmol/L). **D**, Production of reactive oxygen species (ROS) evaluated by electron paramagnetic resonance in NG aortic preparations; and aortic preparations preincubated with HG Krebs solution (glucose concentration: 30 mmol/L). **E**, Expression and activation of endothelial nitric oxide synthase (eNOS) by phosphorylation (P) at serine 1177 analyzed by Western blotting in NG aortic preparations and aortic preparations preincubated in HG Krebs solution (100 mmol/L). **F**, eNOS dimer/monomer ratio analyzed by detecting SDS-resistant eNOS dimers using low-temperature SDS–PAGE in the aortas of NG and HG rats. **G**, Nitrite concentration in NG aortic preparations and aortic preparations preincubated with HG Krebs solution or HG (100 mmol/L). \**P*<0.05.

insulin-mediated vasodilation. Finally, vascular function was assessed in a focussed sample and, therefore, the effect of SSB consumption may vary across health groups, ethnicities, and genders.

In conclusion, this study is, to our knowledge, the first to assess vascular function during acute hyperglycemia induced by SSB consumption alone. The findings of this study demonstrate that commercial SSB consumption induces microvascular and macrovascular endothelial dysfunction in a healthy population. Furthermore, data from the experimental rat model suggest that this commercial SSB-mediated endothelial dysfunction is partly because of increased oxidative stress, which reduces NO bioavailability. Ultimately, these results inform international public health policy on the adverse effects of both commercial SSB and general excess sugar consumption, and how they contribute so acutely, even in those considered healthy, to the upregulation of mechanisms that are the primary precursors to the pathogenesis of CVD.<sup>37</sup>

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### Disclosures

None.

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### Highlights

- To our knowledge, this study is the first to examine the effects of acute hyperglycemia induced by consumption of a commercial sugar-sweetened beverage alone on vascular function.
- Consumption of commercial sugar-sweetened beverages induces acute hyperglycemia that mediates a transient endothelial dysfunction in the microvascular and macrocirculation, even in those considered to be healthy.
- Findings from an in vivo and ex vivo experimental rat model suggest that acute hyperglycemia is associated with an increase in oxidative stress that decreases nitric oxide bioavailability that subsequently induces endothelial dysfunction.