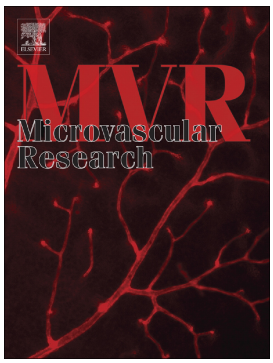

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Increased systemic endothelial-dependent microvascular reactivity after ingestion of a high-carbohydrate snack in young, healthy volunteers

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Running title: high carbohydrate snack increases systemic microvascular reactivity

Research Highlights

- Study with a homogeneous population of young and healthy volunteers, paired according to gender.
- The effects of a high-carbohydrate snack on microvascular function were compared to water ingestion.
- A single snack consisting on an acute oral load of carbohydrates determined a significant increase of systemic endothelial-dependent microvascular reactivity.

ABSTRACT

Objective: This study evaluated the acute effect of a high-carbohydrate snack (HCS) on systemic microvascular function of healthy, young volunteers, using laser speckle contrast imaging (LSCI).

Methods: Cutaneous microvascular blood flow was assessed in the forearm with LSCI coupled to iontophoresis of acetylcholine, using increasing anodal currents, before and after (25 min) the ingestion of a HCS or water (control). Twenty volunteers (10 male) received a single HCS (70 g of carbohydrates) in the fasting state in the morning.

Results: The area under the curve (AUC) of acetylcholine-induced microvascular vasodilation increased from 17,847 ± 4,539 to 20,315 ± 7,168 arbitrary perfusion units/sec (P=0.03) after ingestion of a HCS, but was unchanged after the ingestion of water (P=0.22).

Conclusion: A single snack consisting on an acute oral load of carbohydrates induced a significant increase of endothelium-dependent microvascular vasodilation in healthy, young subjects.

Keywords: laser-based methods; microvascular endothelial function; high carbohydrate snack; laser speckle contrast imaging.

Introduction

For many years, the consumption of lipids was claimed guilty in the pathophysiology of atherosclerosis and coronary artery disease (CAD) (Jakobsen et al., 2009, Mozaffarian et al., 2010, Siri-Tarino et al., 2010). This concept influenced dietary recommendations for the prevention of CAD worldwide (Hu and Willett, 2002), and possibly drove eating patterns towards increasing carbohydrate (CHO) intake (Malik et al., 2010). More recently, the link between fat consumption and atherosclerosis has been put into question, and the focus has been shifted to the detrimental cardiovascular effects of CHO (Dehghan et al., 2017, Li et al., 2015). In the current scenario of "unhealthy" eating habits and the epidemics of obesity and diabetes (Benjamin et al., 2019, Pereira et al., 2005), further knowledge upon the cardiovascular effects of the ingestion of high-CHO loads is desirable.

As endothelial dysfunction is an early marker of atherosclerosis (Verma et al., 2003), high-CHO drinks, or snacks (HCS) or meals have been employed to assess the acute influence of CHO on endothelial function, both in large vessels and in the microvasculature; however, most studies have small and heterogeneous patient samples (Kawano et al., 1999, Loader et al., 2015, Varsamis et al., 2018, Williams et al., 1998). Considering that the systemic microcirculation can be readily and noninvasively assessed on the skin by way of laser speckle contrast imaging (LSCI)(Cordovil et al., 2012), the impact of high-CHO oral ingestion on endothelial function may be further investigated. Therefore, this study sought to evaluate the acute effect of a HCS on skin microvascular function of healthy, young volunteers, using LSCI.

Methods

The present study was undertaken in accordance with the Helsinki Declaration of 1975, revised in 2000, and was approved by the Institutional Review Board (IRB) of

the National Institute of Cardiology, Rio de Janeiro, Brazil, under protocol # CAAE 86854318.8.0000.5272 and registered at ClinicalTrials.gov (NCT03515460). Once deemed eligible to participate in this study, all subjects read and signed an informed consent document approved by the IRB.

The study included twenty young, healthy volunteers. Inclusion criteria were age between 18 and 35 years with body mass index (BMI) between 18.5 and 29.0 kg/m^2. Exclusion criteria included diabetes mellitus or fasting plasma glycemia ≥100 mg/dL; fasting plasma triglycerides ≥150 mg/dL; low-density lipoprotein-cholesterol ≥160 mg/dL; arterial blood pressure>140/90 mmHg or antihypertensive treatment; smoking and pregnancy or breastfeeding.

Venous blood samples were obtained for the biochemical testing in the morning after a 12-hour fasting. The volunteers ingested a snack (total CHO content 70 g) consisting of six sweet cookies (54 g, Piraquê®, Turiaçu, Rio de Janeiro, Brazil), 30 g of fruit jelly (Ritter®, Cachoeirinha, Rio Grande do Sul, Brazil) and 200 mL of fruit juice (Tial Kids®, Belo Horizonte, Minas Gerais, Brazil), and skin microvascular reactivity was evaluated 25 minutes thereafter. On a second visit within 1-4 weeks, the same subjects underwent microvascular testing after the ingestion of 200 mL of water. Capillary blood glucose was measured using a digital monitor (Freestyle® Optium Neo Portable Blood Glucose Monitoring System, Abbott Diabetes Care Ltd., United Kingdom).

The microcirculatory tests were performed in an undisturbed quiet room with a stable temperature (23 ± 1°C) after a 20-minute rest period in the supine position. Microvascular reactivity was evaluated using an LSCI system with a laser wavelength of 785 nm (PeriCam PSI system, Perimed, Järfälla, Sweden), and continuous recordings of cutaneous microvascular perfusion changes were measured in arbitrary perfusion

units (APU). The images were analyzed using PIMSoft software (Perimed, Järfälla, Sweden). One skin site on the ventral surface of the forearm was randomly chosen for the recordings. Hair, broken skin, areas of skin pigmentation and visible veins were avoided, and two drug-delivery electrodes were installed using adhesive discs (LI 611, Perimed, Järfälla, Sweden). Acetylcholine (Ach) 2% w/v (Sigma Chemical CO, St. Louis, USA) iontophoresis was performed using a micropharmacology system (PF 751 PeriIont USB Power Supply, Perimed, Sweden) using increasing anodal currents of 30, 60, 90, 120, 150 and 180 μA, which were administered for 10-second intervals spaced 1 minute apart. The total charges for the above currents were 0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mC, respectively. The dispersive electrode was attached approximately 15 cm from the electrophoresis chamber. Skin blood flow measurements in APU were divided by mean arterial pressure values to yield cutaneous vascular conductance (CVC) in APU/mmHg.

Statistical analysis

The results are presented as the mean ± SD or medians (25^{th} - 75^{th} percentiles). Statistical analyses of plasma glycemic curves were performed using repeated measures ANOVA followed by Dunnett's multiple comparisons test. The areas under the curve of microvascular vasodilation and serum glucose values were analyzed using the two-tailed paired *t* test. P values <0.05 were considered statistically significant. All statistical analyses were performed using Prism, version 6.0 (GraphPad Software Inc. La Jolla, CA, USA).

Results

Twenty subjects, with a male/female proportion of 1:1, were studied. The clinical and anthropometric characteristics of the study volunteers are shown in Table 1. Mean age was 26.9 years and mean body mass index was 24.1 kg/m^2. All participants had normal fasting glycemia, serum glycated hemoglobin and insulin. Serum glucose

increased in response to the HCS (Figure 1), starting at 5 minutes and peaking approximately at 25 minutes. Serum glycemia decreased from the baseline values of 91.6 ± 9.5 to 83.1 ± 9.4 mg/dL (P=0.0002) in the control group (water ingestion) 25 min after ingestion.

Microvascular reactivity

Figure 2 shows CVC curves in response to Ach, before and after the HCS (panel A) and before and after water intake (panel B). Of note, CVC progressively increased in response to incremental doses of Ach in all settings (fasting, post-water intake and post-HCS). Panel A shows that there was a more pronounced increase of CVC after the HCS (red curve) than in the fasting state (blue curve). In panel B, no increase in CVC is seen in response to Ach after water intake (red curve), when compared to the fasting state (blue curve). Panel C depicts the area under the curve (AUC) of microvascular vasodilation before (SNACK-PRE) and after (SNACK-POST) ingestion of the HCS, or before (H_2O-PRE) and after (H_2O-POST) ingestion of water. A significant increase of AUC was observed after the HCS (P=0.03), while no difference was found after water ingestion (P=0.22).

Discussion

In this study, the consumption of a single HCS did not impair systemic microvascular reactivity evaluated on forearm skin with LSCI. The postprandial microvascular response to the acute CHO load was, in fact, of vasodilation, contrary to what might be expected. Indeed, Ach-induced increases in microvascular conductance were significantly more pronounced after the ingestion of a HCS, when compared to water consumption, used as a reference.

Prior studies have demonstrated different vascular responses according to CHO administration as a meal or as an “isolated” oral load. The oral glucose tolerance test has

been shown to promote significant reductions of flow-mediated vasodilation (Title et al., 2000, Watanabe et al., 2011). Nonetheless, Russel et al (Russell et al., 2018) have shown a stimulatory effect of a mixed meal challenge on brachial artery flow, muscle blood flow and blood volume in skeletal muscle of healthy people, while orally ingested glucose actually decreased muscle blood flow and volume (impaired microvascular responses) despite maintaining brachial artery flow. Recent data have shown that the consumption of 50 g of sucrose, with or without 160 mg of vitamin C, did not influence post-occlusive microvascular reactivity in healthy young adults (West et al., 2019).

There may be several reasons for these findings. These responses may be linked to insulin, which has well-known vasodilating effects (Rajendran et al., 2013). On the other hand, acute hyperglycemia impairs nitric oxide (NO)-induced vasodilation, due to a decrease of NO bioavailability, among other possible mechanisms (Loader et al., 2017). Animal and *in vitro* studies have also shown increased oxidative stress and production of vasoconstrictors such as endothelin-1 and prostanoids in response to hyperglycemia (Ceriello et al., 1998, Mah and Bruno, 2012, Mah et al., 2011).

The present study takes advantage of a very homogeneous population of young and healthy volunteers, paired according to gender, and who underwent both interventions (HCS and water ingestion). Thus, several biases were probably overcome. Nonetheless, due to the myriad of possible factors intervening in the relationship between CHO ingestion and vascular function, further investigation is needed to elucidate the complex metabolic pathways which regulate this relationship.

Conclusions

A single snack consisting on an acute oral load of carbohydrates determined a significant increase of endothelium-dependent microvascular vasodilation. This finding

adds evidence to other data and challenges the widely held belief that carbohydrates are always detrimental to vascular function.

ACKNOWLEDGEMENTS

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DISCLOSURES

The authors have no conflicts to disclose.

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Table 1: Clinical characteristics of the healthy volunteers (n=20).

Age (years)	26.9 ± 4.6
Body weight (kg)	71.5 ± 10.4
Male n (%)	10 (50)
Body mass index (kg/m²)	24.1 ± 2.6
Waist circumference (cm)	77.8 ± 7.2
Fasting glucose (mg/dL)	85.5 ± 5.9
Glycated hemoglobin (HbA1c) (%)	5.3 ± 0.3
Fasting insulin (µUI/mL)	7.6 (5.8 - 12.1)
Total cholesterol (mg/dL)	164.8 ± 28.6
LDL-Cholesterol (mg/dL)	110.9 ± 31.4
HDL-Cholesterol (mg/dL)	53 (46 - 62)
Triglycerides (mg/dL)	79 (64 - 92)
Creatinine (mg/dL)	0.9 ± 0.2
Uric acid (mg/dL)	5.2 ± 1.3
SAP (mmHg)	117.8 ± 11.9
DAP (mmHg)	72.7 ± 8.6
MAP (mmHg)	87.8 ± 8.5

The results are presented as the mean ± SD. Values that did not follow a Gaussian distribution are presented as medians (25^{th} - 75^{th} percentiles; Shapiro-Wilk normality test).

Abbreviations; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; MAP, mean arterial pressure.

LEGENDS TO FIGURES

FIGURE 1: Mean plasma glucose concentrations after the ingestion of a single high-carbohydrate snack in healthy volunteers (n=5).

The results are shown as the mean ± SD.

Statistical analyses were performed using repeated measures ANOVA followed by Dunnett's multiple comparisons test.

*P<0.05 and **P<0.01 vs. basal values.

FIGURE 2: Effects of increasing currents of forearm skin acetylcholine (Ach) iontophoresis on cutaneous microvascular conductance (expressed as arbitrary perfusion units (APU) divided by mean arterial pressure in mmHg) in healthy volunteers before (SNACK-PRE) and after (SNACK-POST) ingestion of a single high-carbohydrate snack (A) or before (H_2O-PRE) and after (H_2O-POST) water ingestion (B) (control curves).

The results are shown as the mean ± standard deviation.

(C) Areas under the curve of microvascular vasodilation induced by acetylcholine (Ach) iontophoresis. Values are expressed as box and whisker plots where the center line denotes the median value, the box contains the 25th to 75th percentiles of dataset and whiskers mark the maximum and minimum values, and were analyzed using the two-tailed paired *t* test.

FIGURE 1

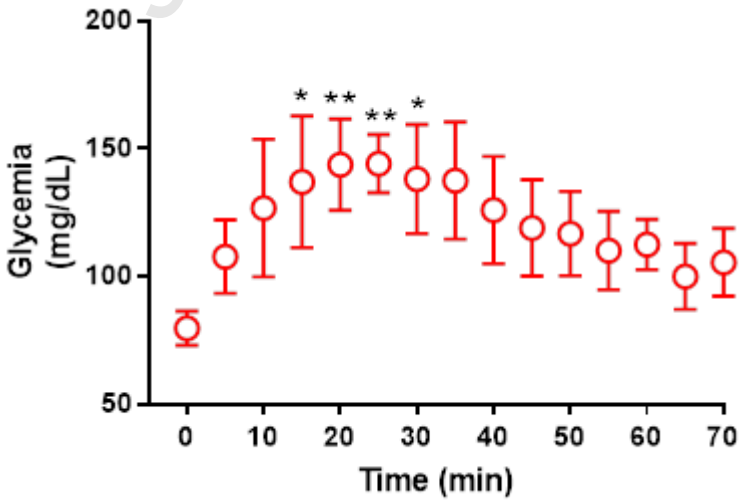

FIGURE 2