Title: The acute and postprandial effects of sugar moiety on vascular and metabolic health outcomes in adolescents

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Abstract:

This study explored the cardiometabolic responses to sugar moieties acutely, and following a subsequent mixed meal tolerance test (MMTT). Twenty-one healthy adolescents (N=10 female, 14.3±0.4 years) completed three experimental and one control condition, in a counterbalanced order. These consisted of different drinks to compare the effect of 300 mL of water (control), or 300 mL of water mixed with 60 g of glucose, fructose or sucrose, on vascular function (flow-mediated dilation; FMD, microvascular reactivity (total hyperaemic response; TRH); and cerebrovascular reactivity; CVR), and blood samples for [uric acid], [glucose], [triglycerides] and [lactate]. FMD increased 1 hour after glucose and sucrose ($P<0.001$, ES≥0.92) but was unchanged following fructose and water ($P\geq0.19$, ES≥0.09). CVR and TRH were unchanged 1 hour following all conditions ($P>0.57$, ES>0.02). Following the MMTT, FMD was impaired in all conditions ($P<0.001$, ES>0.40) with no differences between conditions ($P>0.13$, ES<0.39). Microvascular TRH was increased in all conditions ($P=0.001$, ES=0.88), and CVR was preserved in all conditions post MMTT ($P=0.87$, ES=0.02). Blood [uric acid] was elevated following fructose consumption and the MMTT ($P<0.01$, ES>0.40). Consumption of a sugar sweetened beverage did not result in vascular dysfunction in healthy adolescents, however the vascular and metabolic responses were dependent on sugar moiety.

Highlights:

- Glucose consumption acutely increases peripheral vascular function in healthy adolescents.
- Acute sugar sweetened beverage consumption (sucrose) does not result in adverse vascular outcomes.
- Elevations in uric acid are observed with fructose consumption, which may have implications over repeated exposure.

Keywords: sugar sweetened beverage, fructose, hyperglycaemia, cardiovascular disease, sucrose, endothelial function, glucose, asolescents, FMD, vascular function

Introduction

Clinically overt cardiovascular disease (CVD) typically presents in adulthood, however, subclinical manifestations of the atherosclerotic process are present in childhood (McGill et al., 2000), and are positively related to risk factor exposure (Raitakari et al., 2003). Children and adolescents who present with CVD risk factors have impaired macro-, micro- and cerebro-vascular function (Lande et al., 2012, Celermajer et al., 1992, Khan et al., 2003, Settakis et al., 2003), which may have implications for future cardiovascular and cerebrovascular health.

The consumption of sugar sweetened beverages (SSBs) in adolescents has received interest for their potential role in elevating future CVD risk, independently of weight status (Ambrosini et al., 2013). Adolescents consume the highest amounts of SSB (Bleich et al., 2018) and frequent SSB consumption in youth is associated with CVD risk factors such as decreased insulin sensitivity (Basu et al., 2013), hypertension (Nguyen et al., 2009), dyslipidaemia (Vos et al., 2017) and future body weight gain (Ludwig et al., 2001). The high consumption of SSB in adolescents is concerning given that this may provide routine exposure to acute episodes of oxidative stress, hyperglycaemia (Loader et al., 2017) elevations in uric acid (Carran et al., 2016), and triglyceride (TAG) concentrations (Malik and Hu, 2015), which are implicated in the atherosclerotic process. Furthermore, it is suggested that SSB consumption may elicit repeated, acute episodes of endothelial dysfunction (Loader et al., 2015), which is a prerequisite for unfavourable vascular remodelling (Juonala et al., 2004). However, the impact of acute SSB consumption on vascular function in youth is unclear.

A meta-analysis concluded that acute glucose consumption transiently impairs peripheral vascular function across child and adult populations (Loader et al., 2015). However, only two paediatric trials were available to be included in these analyses, which recruited normal and overweight adolescents (Dengel et al., 2007), and adolescents with type 1 diabetes (Dye et al., 2012). In contrast to adult data, hyperglycaemia acutely *elevated* vascular function, whilst a further study not included in the review identified no change in vascular function one hour following an oral glucose tolerance test in obese children and adolescents (Metzig et al., 2011).

Thus, the insight from the available paediatric data is limited and equivocal compared to the wider adult data, and the response in healthy adolescents warrants further study. In addition, the available paediatric investigations used glucose or dextrose, which are not representative of a commercially available SSB in which the main sugar is sucrose or high fructose containing sugars (Malik and Hu, 2015). Unlike glucose, fructose is metabolised independently of insulin (Chong et al., 2007), with insulin promoting vasodilation, via increases in nitric oxide (NO) bioavailability (Steinberg et al., 1994). Fructose metabolism promotes lipogenesis and increases blood uric acid concentrations, which impair vascular function and are implicated in the development of CVD (Malik and Hu, 2015). Currently, the impact of sugar moiety on vascular function and metabolic outcomes in healthy adolescents are unknown.

The available literature in youth solely focusses on peripheral macrovascular function (Dengel et al., 2007, Metzig et al., 2011) following a glucose load. This is an important limitation of current data, as it is known that the earliest impairments in vascular function occur in the microvasculature (Pinkney et al., 1997), which demonstrate only a weak association with macrovascular function (Dhindsa et al., 2008). Additionally, adolescents with CVD risk factors also present with impaired cerebrovascular function (Settakis et al., 2003), yet no study has provided any data regarding the acute micro- or cerebro-vascular response to sugars moieties constituting a SSB in adolescents.

The primary aim of this study was to identify the acute macro-, micro- and cerebro-vascular responses to glucose, fructose and sucrose consumption in ostensibly healthy adolescents. Additionally, the deleterious cardiometabolic effects of SSB consumption may be related to an exaggerated lipaemic response to a subsequent meal (Malik and Hu, 2015). Specificaly, the postprandial lipaemic excursion is more profound following the consumption of fructose compared to glucose (Chong et al., 2007). Therefore, the secondary aim was to investigate whether sugar moiety influenced the metabolic and vascular responses to a subsequent standardised meal challenge. It was hypothesised that sugar moiety would differentially

influence vascular function, with impairments following the fructose condition, concomittant with increases in uric acid and TAG concentrations.

Materials and methods

Participants

Twenty-four ostensibly healthy 13 to 15-year old adolescents volunteered to take part in this study. Three participants withdrew from the study due to time commitments, providing a final sample size of 21 (11 male, mean ± SD, age male = 14.4 ± 0.4 years, female = 14.2 ± 0.2 years). Maturity status was determined by self-assessment of secondary sexual characteristics using adapted drawings of the five stages of pubic hair development Morris and Udry (1980) and were as follows: (stage 2: n=2, stage 3: n=2, stage 4: n=15, stage 5: n=2). Written informed participant assent and parental consent were obtained following approval from the University of Exeter Sport and Health Sciences ethics committee (171206/B/07). Participants were initially screened for the study exclusion criteria, which were any contraindications to exercise, the use of any supplement or medication known to influence vascular function, and current or previous metabolic, cardiovascular, or cerebrovascular disease.

Experimental procedures

Participants completed a total of five visits to the laboratory, with each visit separated by approximately 1 week. Visit 1 was a preliminary familiarisation visit. Visits 2-5 were the experimental visits, completed in a counterbalanced order. To standardise responses between conditions, participants were asked to replicate their diet in the 24 hours preceding each visit. In addition, participants were asked to avoid vigorous physical activity for 24 hours prior to each condition. The four experimental visits consisted of a different test drink to compare the effects of: (1) 300 mL of water (control) or 300 mL of water mixed with 60 g of: (2) glucose, (3) fructose or (4) sucrose on vascular function and whole blood uric acid concentration ([uric acid]) and plasma [glucose], [TAG] and [lactate].

Visit 1: Preliminary anthropometric and familiarisation visit Body mass (Hampel XWM-150K, Hampel Electronics Co. Taiwan) and stature (SEC-225, Seca, Germany) were measured to

the nearest 0.1 kg and 0.1 cm, respectively. Percentage body fat was measured using the gold standard (Lowry and Tomiyama, 2015) method of air displacement plethysmography, shown to be reproducible in an adolescent population (Silva et al., 2013) (BodPod®, Life Measurement Instruments, USA).

Participants were familiarised with all testing procedures for the subsequent visits before completing a ramp incremental cycle test to exhaustion (Lode, Excalibur Sport, The Netherlands) to determine peak oxygen consumption ($\dot{V}O_{2peak}$) (MedGraphics Ltd, UK) (Barker et al., 2011) as as a descriptive characteristic.

Visits 2-5: Experimental visits An overview of the experimental protocol is illustrated in Figure 1. Following a 12 hour overnight fast, participants were collected and transported to the laboratory for 08:00. Participants rested in the supine position in a darkened and temperature-controlled (24°C) room for 15 minutes prior to baseline measurements of vascular function (including measurement of flow-mediated dilation (FMD), microvascular function (LDF) and cerebrovascular reactivity (CVR)). Blood pressure was then taken in a seated position. This was followed by collection of fasting fingertip capillary blood samples for analysis of [uric acid] and plasma [glucose], [TAG] and [lactate]. Participants then consumed one of the counterbalanced test drink conditions (glucose, sucrose, fructose or water) within 10 minutes. Fingertip blood samples for [uric acid], [glucose] and [lactate] were taken at 30 minute intervals for the following two hours, with the final blood sample also analysed for [TAG]. All vascular outcomes were reassessed within 40 - 70 minutes following drink consumption (Figure 1).

Participants consumed a mixed meal tolerance test (MMTT) 120 minutes following drink consumption, which provided 60 g fat, 45 g of sugar, 1316 kcal, and consisted of pizza (310 g), ice cream (125 g) and a chocolate pudding (95 g). The macronutrient composition of the MMTT has previously been shown to impair peripheral macrovascular and microvascular functions in adolescents (Bond et al., 2015b). Fingertip capillary blood samples were assessed at 60 minute intervals during the three hour postprandial period for [uric acid], [glucose] and [lactate], with the final blood sample also analysed for [TAG]. A final assessment of blood

pressure and vascular function was performed starting at 160 minutes after the MMTT to coincide with the elevation in [TAG] and the fall in peripheral vascular function reported previously in adolescents (Bond et al., 2015b).

Vascular function

Flow mediated dilation. Flow mediated dilation (FMD) was measured using high-resolution ultrasonography in duplex mode (Sequoia 512, Acuson, USA) using a 12- to 14-MHz linear array transducer in accordance with recent guidelines (Thijssen et al., 2019b) and our earlier work (Bond et al., 2015a). Baseline and post-occlusion brachial artery diameter was assessed during end-diastole using validated ECG-gating software (Medical Imaging Applications LLC, USA) by an investigator who was blind to the condition (Corretti et al., 2002, Mancini et al., 2002). Baseline arterial diameter was measured for 1.5 minutes. Endothelium-dependent vasodilation was calculated as the percentage increase in arterial diameter after a 5-minute ischemic stimulus induced by rapid forearm pneumatic cuff inflation (Hokanson, USA) to 220 mmHg (Thijssen et al., 2019b). When performed in this manner, the FMD response reflects NO mediated endothelial function (Doshi et al., 2001, Green, 2005). The area under the curve for estimated shear rate was calculated from the point of cuff deflation until the time of peak dilation (SR_{AUC}) (Thijssen et al., 2019a). As observed elsewhere (Thijssen et al., 2009) FMD was not consistently related to SR_{AUC}, so FMD was not normalised to this outcome. FMD was allometrically scaled for baseline diameter in order to address concerns about the ratio-scaled FMD statistic (Atkinson and Batterham, 2013). The within and between day coefficient of variation (CV) in adolescents was 4.5% and 9.7% respectively for FMD, in line with our previous work (Bond et al., 2017).

Microvascular function. Microvascular function was simultaneously assessed during the FMD protocol via laser Doppler flowmetry (LDF; Moor Instruments Ltd, UK) (Bond et al., 2017). Two low power laser probes (785 nm at 2.5 mW) were affixed to reproducible points on the distal third of the forearm free from freckles, scars or hair, and these positions were outlined in pen to facilitate repeat assessments. Perfusion data were collected at 40 Hz and then interpolated

to 1 second averages before being smoothed using a 5 second moving average. Peak reactive hyperaemia (PRH) was defined as the percentage increase in perfusion following cuff deflation. The total hyperaemic response (TRH) was calculated by determining the incremental area under the post-occlusive reactive hyperaemic curve multiplied by the time taken for reactive hyperaemia to return to baseline (Wong et al., 2003). When calculated in this manner, TRH is known to be NO independent (Wong et al., 2003) and accounts for any differences in baseline skin perfusion. The within and between day CV were 24% and 25% for PRH, and 33% and 34% for TRH, respectively.

Cerebrovascular reactivity. Middle cerebral artery blood velocity (MCAv) was measured as the mean of the MCAv envelope per heartbeat, measured by transcranial Doppler ultrasonography using a 2 MHz pulsed Doppler ultrasound system (DWL ®, Doppler-Box^{TM}X, Compumedics, Germany). Insonation of the right MCA was initiated at a depth of ~ 50 mm, and then optimised prior to locking the probe in place using a size adjustable headset for unilateral measurement of the right MCA (DWL ®, DiaMon ®, Compumedics, Germany). Resting MCAv was measured as the average over 60 seconds, with participants lying in the supine position. Cerebrovascular reactivity (CVR) was determined as the percentage increase in MCAv from the resting baseline to the peak in the 10 seconds following a maximal breath-hold of up to 30 seconds (Koep et al., 2020). Three breath-holds were performed with a 60 second recovery between each (Bright and Murphy, 2013). We have published reliability data in this sample of adolescents previously (Koep et al., 2020) and observed a within and between day CV of 10.8% and 15.3% respectively. Beat-by-beat blood pressure was continuously measured by finger plethysmography (Finometer PRO, Netherlands). The change in mean arterial pressure (MAP) from baseline during the last 5 seconds of the breath-hold was calculated, to determine the presence of any Valsalva manoeuvre. One participant was removed from analysis due to a substantial Valsalva manoeuvre (>15mmHg, and visual inspection from two independent researchers). Therefore, CVR data are reported in a sample of n=20. All data were acquired continuously at 200 Hz using an analogue-to-digital converter

(Powerlab; model - 8/30, ADInstruments, USA). Data were stored at 200 Hz for subsequent analysis using commercially available software (Lab Chart version 8, ADInstruments).

Blood pressure. Brachial artery blood pressure was assessed in a seated position following quiet rest using an automated device (Dinamap Pro V100) prior to commencement of vascular function measures. Arterial systolic blood pressure (SBP), diastolic blood pressure (DBP) were measured in triplicate, and interpreted as the lowest value recorded.

Blood outcomes

Prior to each blood sample, a water bath (~39°C) was used to warm participants hand for five minutes prior to pressing a safety lancet firmly to the surface of participants finger tip to puncture the skin (Blade length 1.5mm, Saerstedt, Ltd, UK). ~300 µL of blood was collected into lithium-heparin coated microvette (TAG) and a further ~300 µL of blood was collected into a heparin-fluorine coated microvette (glucose and lactate) (CB 300 Tubes, Saerstedt, Ltd, UK). If a sufficient sample could not be obtained a second finger tip puncture was made on a different finger. All samples were centrifuged immediately at 13,000 *g* for 15 minutes. Plasma was then aliquoted and either stored at -80 °C for [TAG] analysis or analysed immediately for plasma [glucose] and [lactate] (YSI 2300 Stat Plus Glucose analyser, USA). Plasma [TAG] was quantified in duplicate by enzymatic, calorimetric methods using an assay kit according to the manufacturer's guidelines (Cayman Chemical Company, USA). The CV for the inter error assay for plasma [TAG] was 5.8%. Uric acid was analysed in capillary whole blood from a single finger stick blood sample (UASure, Apex Biotechnology Corp., Taiwan). The intra-assay coefficients of CVs of the UASure® have been reported as 4.8% at uric acid levels of 5.8 mg/dL.

Changes in whole blood [uric acid] and plasma [glucose] following test drink consumption (0-120 minutes), and following the MMTT (120-300 minutes) were quantified using total and incremental area under the curve (tAUC, iAUC). All AUC analyses were calculated using the trapezoid rule (GraphPad Prism, USA).

Statistical analyses

Statistical analyses were conducted using SPSS (version 25, IBM, USA) and data are presented as a mean ± SD. For allometically-adjusted FMD, brachial diameter changes on the logged scale were analysed using an ANCOVA model, with logarithmically-transformed baseline diameter as a covariate and with condition and time point as fixed factors (Atkinson and Batterham, 2013). Covariate adjusted means were then back-transformed and interpreted in the usual manner. Analysis of LDF, CVR, blood pressure, plasma [uric acid], [glucose], [TAG] and [lactate] were performed using a repeated measures ANOVA with condition (glucose, sucrose, fructose, water) and time (pre drink, post drink, post meal) as the main effects. Sex was initially included as a between subjects comparison for CVR, LDF and FMD, however, the inclusion of sex into the ANOVA model did not reveal a significant interaction effect and therefore these data were subsequently pooled. Analysis of tAUC and iAUC responses to the test drink, and to the MMTT were performed using a one-way repeated measures ANOVA. Normality of data was determined using the Shapiro-Wilk test and if violated (glucose, lactate, resting MCAv, TAG), statistical analyses were performed on log transformed data. For ease of interpretation, graphical presentation of variables that were log transformed are displayed as the raw data. Homogeneity of variance was determined using Mauchly's test of sphericity, with the Greenhouse-Geisser correction performed if required. Effect sizes for the ANOVA model were displayed as partial eta squared (η_p^2), and interpreted as <0.06 = small, 0.06-0.14 = moderate and >0.14 = large. In order to locate significant differences between conditions, post hoc analyses were run as pairwise comparisons between means and interpreted using the *P* value and standardised effect sizes (*d*) to document the magnitude of the effect using the following thresholds: small (0.2), moderate (0.5), and large (0.8) (Cohen, 1992). Statistical significance was accepted when $P<0.05$.

Results

Participant characteristics are shown in Table 1.

Flow mediated dilation. Allometrically-adjusted macrovascular responses are presented in Figure 2. FMD was greater compared to pre drink one hour following glucose ($P<0.001$,

d=0.92) and sucrose (*P*<0.001, *d*=0.93), but unchanged after fructose (*P*=0.19, *d*=0.34) and water (*P*=0.72, *d*=0.09). At the post drink time point, FMD was greater in the glucose condition compared to fructose (*P*=0.02, *d*=0.69) and water (*P*<0.001, *d*=1.18), whilst sucrose was greater to water only (*P*=0.001, *d*=0.96). FMD was lowered by the MMTT compared to the post drink time point (*P*<0.001 for all, *d*=0.40 to 1.45, Figure 2A), with no differences between conditions (*P*>0.13, *d*<0.39 for all).

SR_{AUC} was always reduced post drink (*P*=0.02, *d*=0.32) and greater post MMTT (*P*=0.02, *d*=0.45) compared to pre drink, with no difference between conditions.

Microvascular function. Microvascular PRH and TRH are presented in Figure 3. Compared to pre drink, PRH increased after glucose (*P*=0.03, *d*=0.70), fructose (*P*<0.001, *d*=1.18) and sucrose (*P*=0.01, *d*=0.40), but not water (*P*=0.55, *d*=0.18). At the post drink time point, all sugar conditions were significantly greater than water (*P*<0.04 for all, *d*=0.51 to 1.00), and fructose was also greater than sucrose (*P*=0.03, *d*=0.54). Post drink PRH was lowered by the MMTT following consumption of glucose (*P*<0.001, *d*=1.39), fructose (*P*=0.001, *d*=0.97) and sucrose (*P*<0.001, *d*=1.09), but not water (*P*=0.09, *d*=0.50). At the post MMTT time point, fructose was greater than all other conditions (*P*<0.03 for all, *d*=0.54 to 0.78).

TRH was always greater post MMTT compared to the post drink time point (*P*=0.001, *d*=0.88).

Cerebrovascular function. Cerebrovascular outcomes are presented in Figure 4. Resting MCAv was lowered following the MMTT compared to post drink (*P*=0.01 *d*=0.23), with no differences between conditions.

Blood pressure

There was no significant condition by time interaction for SBP and DBP (*P*=0.37, η_p^2=0.05, and *P*=0.97, η_p^2=0.011 respectively). SBP and DBP were not significantly different between conditions (*P*=0.13, η_p^2=0.09, and *P*=0.1, η_p^2=0.1 respectively), nor was it significant between time points (*P*=0.26, η_p^2=0.27, and *P*=0.44, η_p^2=0.04 respectively).

Blood outcomes

Whole blood [uric acid] and plasma [glucose], [TAG] and [lactate] are presented in Figure 5.

Uric acid. Whole blood [uric acid] was elevated in fructose compared to all other conditions 60 and 120 minutes following drink consumption ($P \leq 0.02$, $d \geq 0.63$). Additionally, blood [uric acid] was greater in fructose compared to control 30 minutes ($P=0.009$, $d=0.67$) and 90 minutes ($P=0.002$, $d=0.56$) following drink consumption. Blood [uric acid] was elevated 60 minutes following MMTT consumption ($P<0.001$, $d=0.06$), and 180 minutes following MMTT ($P=0.04$, $d=0.43$) post fructose consumption compared to glucose. Blood [uric acid] was also significantly elevated following fructose compared to sucrose 90 minutes following drink consumption ($P=0.003$, $d=0.55$) and 60 and 120 minutes following MMTT ($P \leq 0.01$, $d \geq 0.37$).

The tAUC and iAUC for blood [uric acid] were both elevated following fructose consumption compared to all other conditions ($P<0.01$, $d>0.52$). There were no significant differences for post MMTT iAUC ($P=0.19$, $\eta_p^2=0.076$), however, post MMTT tAUC for blood [uric acid] was significantly higher in fructose than all other conditions ($P<0.008$, $d>0.40$).

Plasma glucose. Plasma [glucose] was higher following glucose consumption than all other conditions 30-120 minutes post drink consumption ($P \leq 0.005$, $d \geq 0.58$). Plasma [glucose] was higher in sucrose compared to fructose 30 minutes following drink consumption ($P<0.001$) and water ($P<0.001$, $d \geq 2.39$). Plasma [glucose] was elevated in glucose and sucrose conditions compared to water at 30-60 minutes post drink consumption ($P<0.05$, $d=0.82$). Following the MMTT, plasma [glucose] in glucose and sucrose conditions were significantly lower compared to the water condition for the following two hours ($P<0.001$, $d \geq 0.59$), and fructose was lower compared to water one hour following the MMTT ($P=0.001$, $d=1.01$), with no significant differences 3 hours following MMTT consumption between any conditions.

Post drink iAUC for plasma [glucose] was significantly greater in glucose and sucrose than water and fructose conditions ($P<0.001$, $d>1.00$). Post drink iAUC for plasma [glucose] was lower in water than all sugar conditions ($P<0.003$, $d>1.05$). Post drink tAUC for plasma [glucose] was significantly higher in glucose than all other conditions ($P<0.001$, $d>1.39$) and

higher in sucrose than fructose and water (P<0.001, d>1.43). Following the MMTT, plasma [glucose] iAUC was lower in glucose than all other conditions (P<0.006, d>0.77), and higher in water than all other conditions (P<0.001, d>0.69). The tAUC for post MMTT plasma [glucose] showed no significant differences between glucose, sucrose or fructose conditions (P>0.05, d>0.13), with water elevated compared to all conditions (P<0.005, d>0.72)

Plasma triglyceride. Plasma [TAG] was increased post MMTT compared to post drink across all conditions (P<0.001, d=1.54).

Plasma lactate. [Lactate] was higher in fructose and sucrose than glucose and water 30 and 60 minutes post drink consumption ($P\leq$0.003, $d\geq$1.20). Plasma [lactate] remained higher in fructose compared to all other conditions 90 and 120 minutes following drink consumption (P<0.001, $d\geq$0.87). Plasma lactate remained elevated in sucrose and glucose 90 minutes following drink consumption compared to water (P<0.001, $d\geq$1.04), and sucrose remained higher than water at 120 minutes post drink consumption (P=0.001, d=0.85). Following the MMTT, [lactate] was significantly elevated in the control condition compared to glucose and sucrose conditions one hour following MMTT consumption ($P\leq$0.04, $d\geq$0.27), with [lactate] remaining elevated compared to glucose two hours post MMTT ($P\leq$0.04, $d\geq$0.52). Plasma [lactate] was elevated in sucrose and fructose compared to glucose 3 hours following the MMTT ($P<0.05$, $d\geq$0.26).

Discussion

This is the first study to identify the acute effect of sugar moiety on a range of vascular and metabolic outcomes in healthy adolescents. The findings of this study demonstrate that glucose and sucrose acutely increased FMD relative to the control condition, whilst fructose had no effect on FMD. Parameters of microvascular function were increased following sucrose, fructose and glucose consumption. Following a mixed meal challenge, FMD was impaired compared to baseline, with no difference between conditions. Microvascular TRH was increased following the MMTT in all conditions. Microvascular PRH however, decreased following sucrose, glucose and fructose consumption compared to the control condition of water. Cerebrovascular function was preserved in all conditions following both the drink and the MMTT. Sugar moiety did not increase the lipaemic response to a test meal, however elevations in [uric acid] were observed which may be a potential concern, as chronic elevations in [uric acid] via repeat exposure to SSB are implicated in the development of atherosclerosis and metabolic disease (Malik and Hu, 2015).

Acute post drink response

Previous studies in adults report an impairment in FMD following acute SSB consumption (Loader et al., 2015, Loader et al., 2017), likely due to hyperglycaemia-induced oxidative stress and an accompanying decrease in NO bioavailability (Ceriello et al., 2002). In contrast, the current observation of an increase in FMD following glucose and sucrose consumption is in line with other adolescent data (Dengel et al., 2007, Dye et al., 2012), indicating that the FMD response may differ in paediatric groups compared to the aforementioned adult studies. However, this is yet to be formally examined within a single study. Such disparate findings between adolescents and adults may be partly explained by known differences in the insulin and redox response to a glucose load in adolescents. Firstly, pubertal maturation is associated with transient insulin-resistance (Ball et al., 2006), and adolescents are characterised by an exaggerated insulin response to an oral glucose challenge compared to adults (Tricò et al., 2018). Insulin is a potent vasodilator, with >65% of insulin-mediated dilation shown to be NO

dependent (Steinberg et al., 1994). Although not measured in the current study, it is plausible that a greater elevation in insulin responses to an oral glucose challenge confers a transient increase in FMD in adolescents. This mechanism is supported by fructose ingestion resulting in no change in FMD, unlike sucrose and glucose consumption, presumably due to a lower insulin response following fructose consumption (Chong et al., 2007). Secondly, the available data in adolescents indicates that oxidative stress is not acutely increased following glucose consumption (Metzig et al., 2011). Thus, the potentially greater insulin-mediated NO production in adolescents would be preserved, leading to an increase in NO bioavailability and enhanced FMD response. The unchanged FMD response following fructose consumption is also indicative of a preserved redox state. However, it was beyond the scope of this study to directly measure insulin concentrations or markers of oxidative stress, so this requires further investigation.

In contrast to FMD, the mechanisms underpinning the total reactive hyperaemic response in the cutaneous circulation are NO independent (Wong et al., 2003). The present data demonstrated no change in TRH microvascular function following all sugar conditions, which is consistent with our previous observations in healthy adults (West et al., 2019). However, PRH was acutely increased following sucrose, fructose and glucose consumption compared to water. These data are the first of their kind, to indicate that microvascular function is not impaired following SSB consumption in adolescents.

A novelty of this investigation is that cerebrovascular function remained unaltered following sucrose, fructose and glucose consumption. Although evidence exists highlighting peripheral macrovascular and cerebrovascular function share a common NO mediated pathway (Lavi et al., 2006), the current data are the first to indicate peripheral arterial changes may not extend to the cerebrovasculature in healthy adolescents. However, this inference is made with caution. The present study utilised a breath-hold protocol, as this method is sensitive to paediatric disease risk (Settakis et al., 2003), and offers a predominantly CO_2 based stimulus, with two thirds of the response related to alterations in arterial CO_2 in adults (Przybylowski et

al., 2003). However, no data are available regarding whether this breath-hold method is sensitive to acute changes in cerebrovascular function. Furthermore, the relationship between peripheral and cerebrovascular reactivity has recently been questioned from a mechanistic standpoint (Carr et al., 2020, Weston, 2020). Our investigation offers initial insight regarding whether the cerebrovasculature is defended against postprandial changes, but this remains an interesting area of further study.

In support of examining both the independent and combined effect of the different sugar moieties constituting a SSB, the metabolic responses significantly differed with consumption of glucose, fructose and sucrose. Most markedly, the consumption of fructose, but not sucrose or glucose, increased [uric acid]. Elevated uric acid concentrations have been shown elsewhere to acutely impair vascular function and are implicated in the progression of hypertension, through insulin resistance and reduced NO production (Choi et al., 2014). In contrast to rodent data (Choi et al., 2014), no acute changes in FMD or blood pressure following fructose consumption were observed in the current study, which corroborates other evidence in adults (Bidwell et al., 2010). However, the effect that repeat exposure to elevations in [uric acid] through habitual SSB consumption might have remains unknown, and this may be a pertinent health concern for this age group (Alper et al., 2005). Similarly, we did not observe any differences in [TAG] between experimental conditions, which is thought to be a means by which SSB consumption may increase cardiometabolic disease risk (Malik and Hu, 2015). It is possible that the greater lactate excursion post fructose partly accounts for the lack of [TAG] increase (Sun and Empie, 2012). However, acute postprandial elevations in [TAG] with fructose consumption might take longer to manifest than our three hour observation used in the current study (Chong et al., 2007). As such, this influence of prior SSB consumption remains an interesting and as yet unanswered question. Additionally, it is feasible that such alterations in postprandial [TAG] might be reserved to less healthy participants, which is an important future direction of this work.

Interactions of sugar moiety with the MMTT

The inclusion of a subsequent MMTT is a strength of the current study design, and provides novel insight into the acute consequences of SSB consumption than the observation following a test drink alone. In the present study, consumption of the MMTT resulted in similar impairments in FMD in all conditions, despite differences in blood [uric acid]. This was contrary to our hypothesis, based on data in adults (Brown et al., 2008), and animal models (Khosla et al., 2005), which highlights acute endothelial dysfunction and hypertension following elevated uric acid concentrations. Given that postprandial vascular function and blood pressure were not different between conditons in the current study, it appears adolescents are protected from this dysfunction following fructose ingestion. Similar to the acute post-drink response observed, cerebrovascular function was preserved following the MMTT in all conditions which indicates that the cerebrovasculature may be able to defend against postprandial lipaemia. Impairments in PRH following a MMTT have been previously reported in an adolescent population (Bond et al., 2015a), and the current findings are the first to demonstrate an elevated response to the MMTT following prior sugar intake, with no differences between conditions.

Sugar moiety had no effect on the lipaemic response to the subsequent test meal as similar increases in [TAG] were observed across all conditions compared to the post drink time point. The current investigation is unable to identify the contributions of specific lipoproteins to the total TAG pool for further insight. However, the lack of difference in [TAG] between the conditions indicates that adolescents may be protected from fructose-induced exacerbations in lipaemia over the course of the day (Malik and Hu, 2015, Chong et al., 2007). Following the MMTT, the control (water) condition resulted in the greatest elevations in plasma [glucose] compared to any of the sugar conditions. This was contrary to the hypotheses of the MMTT exacerbating the metabolic effects of the sugar conditions. Similarly, the sucrose condition also failed to elevate [uric acid] after the MMTT, which the current data indicate are acutely possible in this population following a high fructose dose. These findings suggest that following acute SSB consumption (sucrose), vascular function in healthy adolescents may be unaffected

from increases in glucose, uric acid and TAG. However, further research is warranted on the chronic effects of SSB consumption in adolescents.

Study considerations

This study is the first to investigate the acute metabolic, peripheral and cerebral vascular responses to a sucrose load reflective of a SSB, and isolate the relative contributions of glucose and fructose. A further novelty is the inclusion of the MMTT, providing further insights into the short-term implications of different dietary sugars. Despite the strengths of the present study, which include an overnight fast, the counterbalanced order of conditions and the use of different vascular outcomes, it is important to consider study limitations. Firstly, the metabolic time course of glucose, fructose and sucrose differ, which could not be accounted for in the timing of the vascular measures, which were standardised. The time course of any changes in vascular function after the MMTT may also be different following the test drinks, and vary between individuals, so our inferences from a single postprandial assessment are made with caution. In addition, simultaneous acquisition of peripheral and cerebral vascular function was not possible, therefore these measurements were separated by ~20 minutes. However, this study remains the only investigation to present the acute macro-, micro- and cerebro-vascular responses to different sugars within the same study. An additional consideration is that measurement of intracranial cerebral blood flow relies on the assumption that the diameter of the MCA remains constant, such that increases in blood flow velocity are proportional to increases in cerebral blood flow. In the current study this is a reasonable assumption given that changes in CO_2 identified following the breath hold challenge were relatively small (<6 mmHg), and dilation of the MCA has been shown to be constant until marked elevations (>15 mmHg) in end-tidal CO_2 concentrations (Verbree et al., 2014). The main limitation of the current study was that we were unable to determine [insulin] or markers of oxidative stress, so the suggested influence of pubertal development on insulin-mediated arterial dilation and redox state remains speculative. Lastly, we cannot extrapolate our findings beyond our healthy adolescent population. It therefore remains to be seen whether the responses presented here

are observed in other adolescent populations with increased CVD risk, or in pre-pubertal children.

The current data do not demonstrate that SSB consumption acutely impairs macro-, micro- or cerebro-vascular function in adolescents. Additionally, these sugars did not alter the cardiometabolic responses to a test meal. However, habitual SSB consumption in youth is associated with cardiometabolic risk (Ambrosini et al., 2013, Basu et al., 2013, Vos et al., 2017, Nguyen et al., 2009), and remains a contemporary health concern. Therefore, our data do not endorse SSB consumption as being entirely "risk free", and efforts are needed to understand whether these acute responses are replicated in adolescents who identify as habitual SSB drinkers, or following several days of intervention.

Conclusion

This study investigated the acute and postprandial effects of glucose, sucrose and fructose consumption on a range of important cardiometabolic outcomes in healthy adolescents. The findings of this study demonstrate acute glucose and sucrose consumption induce elevations in peripheral macrovascular function, with no changes in micro- and cerebro-vascular function, despite the disparate metabolic responses of the sugar moieties.

Declarations

Conflicts of interest The authors have no conflicts of interest

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Tables

Table 1. Participant characteristics

	Male (n = 11) *Mean (SD)*	*Female (n = 10)* *Mean (SD)*
Age (years)	14.4 (0.4)	14.2 (0.2)
Stature (cm)	166.7 (9.8)	162.2 (6.2)
Body mass (kg)	51.8 (12.0)	58.8 (10.2)
BMI (kg/m^2)	18.3 (2.1)	22.2 (2.7)
Body fat (%)	16.9 (7.7)	25.5 (6.1)
$\dot{V}O_{2\ peak}$ ($L.min^{-1}$)	2.10 (0.83)	2.18 (0.45)
$\dot{V}O_{2\ peak}$ ($mL.min^{-1}.kg^{-1}$)	43.2 (13.0)	37.2 (5.5)
Fasting plasma TAG ($mmol.L^{-1}$)	0.66 (0.34)	0.62 (0.15)
Fasting plasma glucose ($mmol.L^{-1}$)	4.75 (0.37)	4.81 (0.33)
Fasting blood uric acid ($\mu mol.L^{-1}$)	5.19 (1.70)	4.94 (1.68)

Data expressed as mean ± SD. BMI, body mass index; $\dot{V}O_{2\ peak}$, peak oxygen uptake; TAG, triglyceride.

Figure Captions

Figure 1 Protocol schematic for the four experimental visits. The single arrows represent collection of capillary blood samples for whole blood uric acid and plasma glucose and lactate. The double arrows represent additional blood samples for plasma triglyceride. FMD, flow-mediated dilation; LDF, laser Doppler flowmetry; CVR, cerebrovascular reactivity; MMTT, mixed meal tolerance test; GLU, glucose; SUC, sucrose; FRU, fructose; CON, control (water).

Figure 2 Allometrically-adjusted flow mediated dilation (FMD) and shear stimulus (SR_{AUC}) pre- and post-drink, and post meal. A time by condition interaction effect was present for FMD (*P*=0.03). There was a main effect of time following the MMTT. For SR_{AUC} there was no time by condition interaction effect for SR_{AUC} (*P*=0.77, η_p^2=0.02; Figure 2B), however there was a main effect of time (*P*<0.001, η_p^2=0.42).

a, P<0.05 glucose v fructose. *b, P*<0.05 glucose vs water. *c, P*<0.05 sucrose vs water.

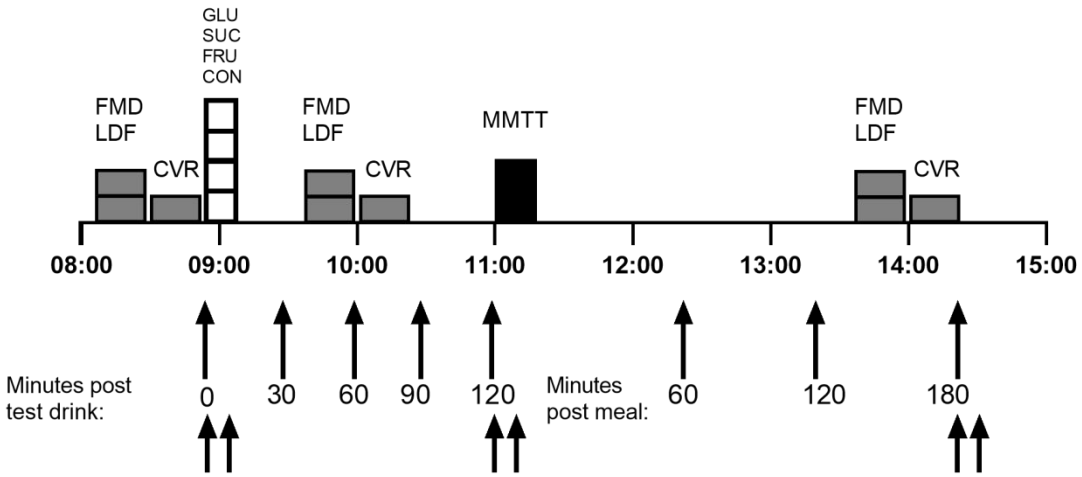
Figure 3 Peak reactive hyperaemia (PRH) and total reactive hyperaemia (TRH) pre- and post-drink, and post meal. There was a time by condition interaction for PRH (*P*=0.01, η_p^2=0.13; Figure 3A). There was no time by condition interaction for TRH (*P*=0.57, η_p^2=0.04; Figure 3B), however there was a main effect of time (*P*<0.001, η_p^2=0.38).

a, P<0.05 glucose v sucrose. *b, P*<0.05 glucose v fructose. *c, P*<0.05 glucose vs water. *d, P*<0.05 sucrose vs fructose. *e, P*<0.05 sucrose vs water. *f*, fructose vs water.

Figure 4 Baseline (A) and peak (B) $MCAv_{mean}$ and cerebrovascular reactivity (CVR) (C) data pre-drink, post drink, and post meal. There was no significant condition by time interaction *(P*=0.06, η_p^2=0.11, Figure 4A), or main effect of condition for resting MCAv (*P*=0.71, η_p^2=0.025). There was a main effect of time (*P*=0.005, η_p^2=0.25). There was no significant condition by time interaction for CVR (*P*=0.87, η_p^2=0.02, Figure 4C), nor was there a significant main effect between conditions (*P*=0.85, η_p^2=0.01) or time (*P*=0.40, η_p^2=0.05).

Figure 5 Uric acid (A), plasma glucose (B) plasma triglyceride (TAG) (C) and plasma lactate (D) responses for each condition following drink consumption (0 min). Dashed line represents consumption of mixed meal tolerance test. Data are shown as mean ± SD. There was a significant condition by time interaction for whole blood [uric acid] following drink and MMTT consumption (Figure 5A P=0.03, η_p^2=0.10). There was a significant condition by time interaction for plasma [glucose] following drink and MMTT consumption (P=, η_p^2=). There was no significant condition by time interaction effect for plasma [TAG] (P=0.24 η_p^2=0.07), nor was there a main effect for condition (Figure 5C P=0.14 η_p^2=0.09). However, there was a significant effect of time (P<0.001 η_p^2=0.87). There was a significant condition by time interaction for plasma [lactate] (Figure 5D, P<0.001, η_p^2=0.50).

a, P<0.05 glucose v sucrose. *b, P*<0.05 glucose v fructose. *c, P*<0.05 glucose vs water. *d, P*<0.05 sucrose vs fructose. *e, P*<0.05 sucrose vs water. *f*, fructose vs water.



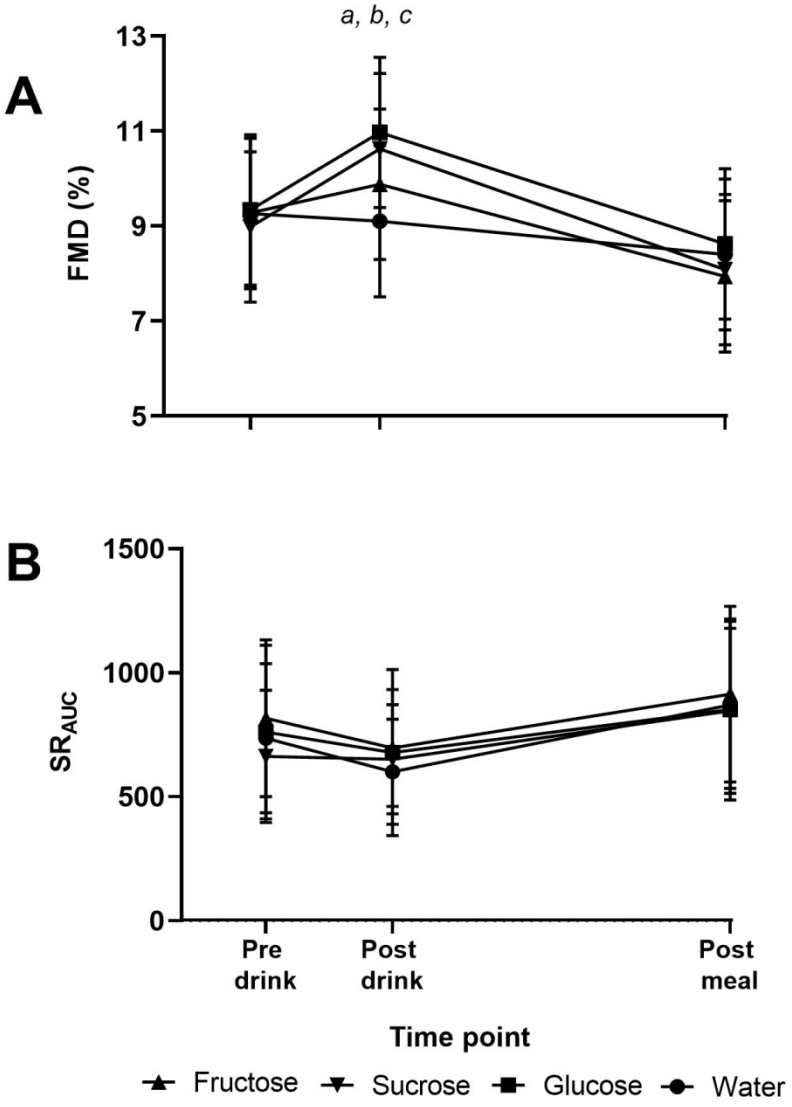


Figure 2 Allometrically-adjusted flow mediated dilation (FMD) and shear stimulus (SRAUC) pre- and post-drink, and post meal. A time by condition interaction effect was present for FMD (P=0.03). There was a main effect of time following the MMTT. For SRAUC there was no time by condition interaction effect for SRAUC (P=0.77, ηp2=0.02; Figure 2B), however there was a main effect of time (P<0.001, ηp2=0.42). a, P<0.05 glucose v fructose. b, P<0.05 glucose vs water. c, P<0.05 sucrose vs water.

116x160mm (300 x 300 DPI)

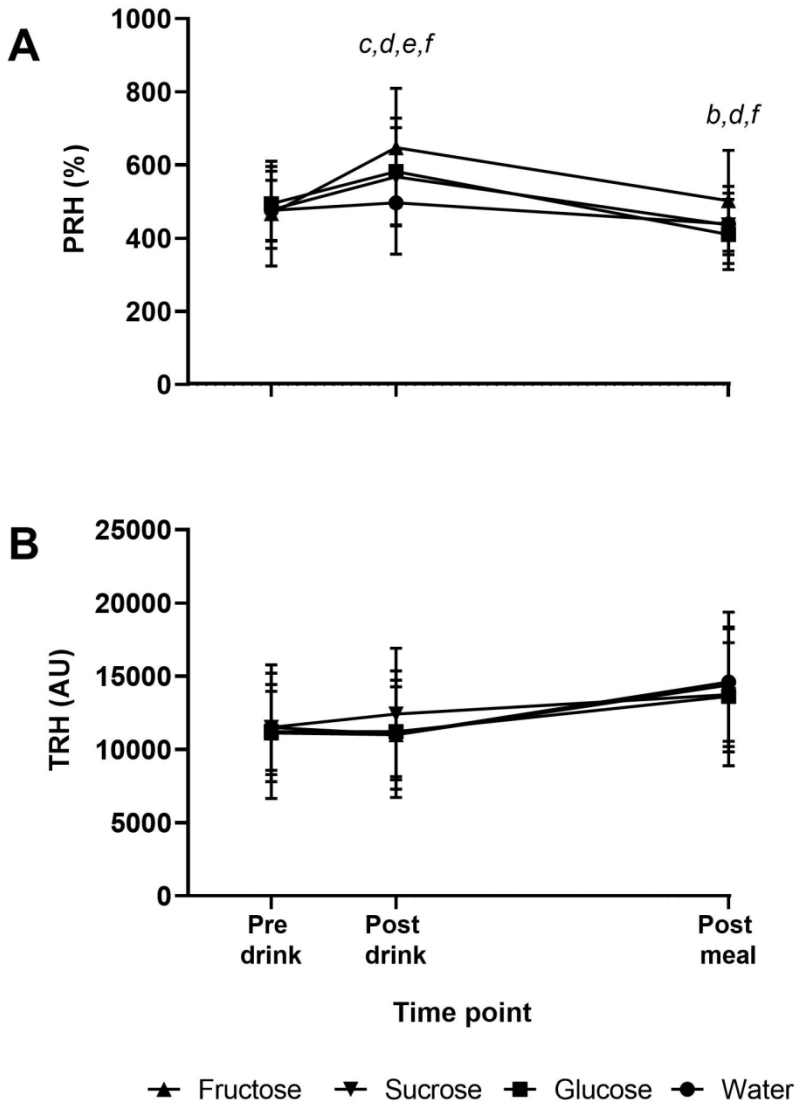


Figure 3 Peak reactive hyperaemia (PRH) and total reactive hyperaemia (TRH) pre- and post-drink, and post meal. There was a time by condition interaction for PRH (P=0.01, ηp2=0.13; Figure 3A). There was no time by condition interaction for TRH (P=0.57, ηp2=0.04; Figure 3B), however there was a main effect of time (P<0.001, ηp2=0.38).
a, P<0.05 glucose v sucrose. b, P<0.05 glucose v fructose. c, P<0.05 glucose vs water. d, P<0.05 sucrose vs fructose. e, P<0.05 sucrose vs water. f, fructose vs water.

134x188mm (300 x 300 DPI)

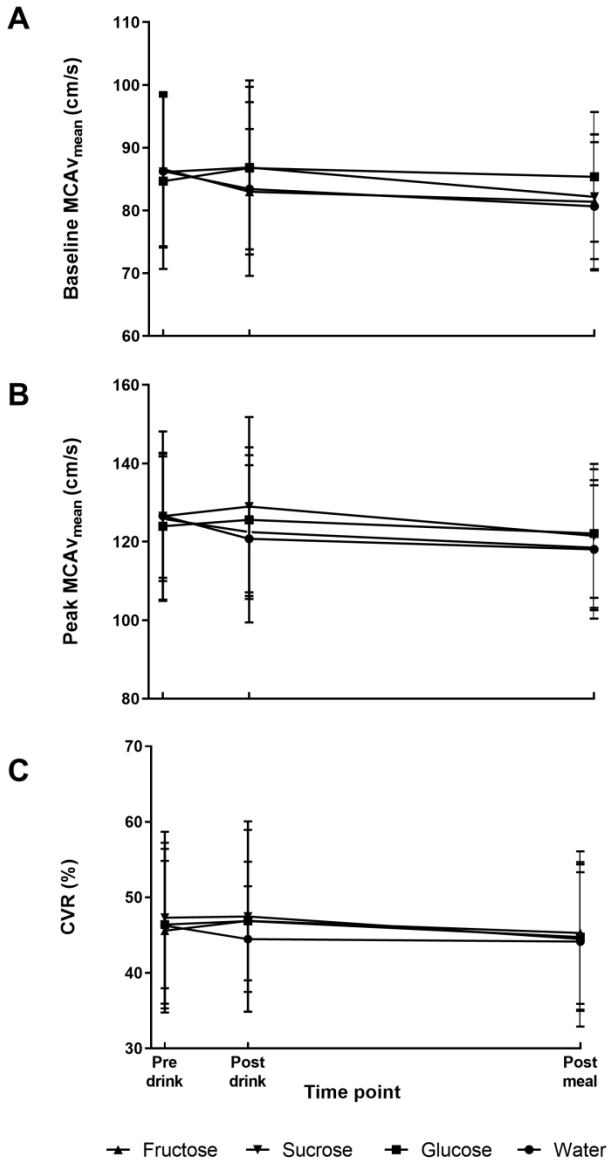


Figure 4 Baseline (A) and peak (B) MCAvmean and cerebrovascular reactivity (CVR) (C) data pre-drink, post drink, and post meal. There was no significant condition by time interaction (P=0.06, ηp2=0.11, Figure 4A), or main effect of condition for resting MCAv (P=0.71, ηp2=0.025). There was a main effect of time (P=0.005, ηp2=0.25). There was no significant condition by time interaction for CVR (P=0.87, ηp2=0.02, Figure 4C), nor was there a significant main effect between conditions (P=0.85, ηp2=0.01) or time (P=0.40, ηp2=0.05).

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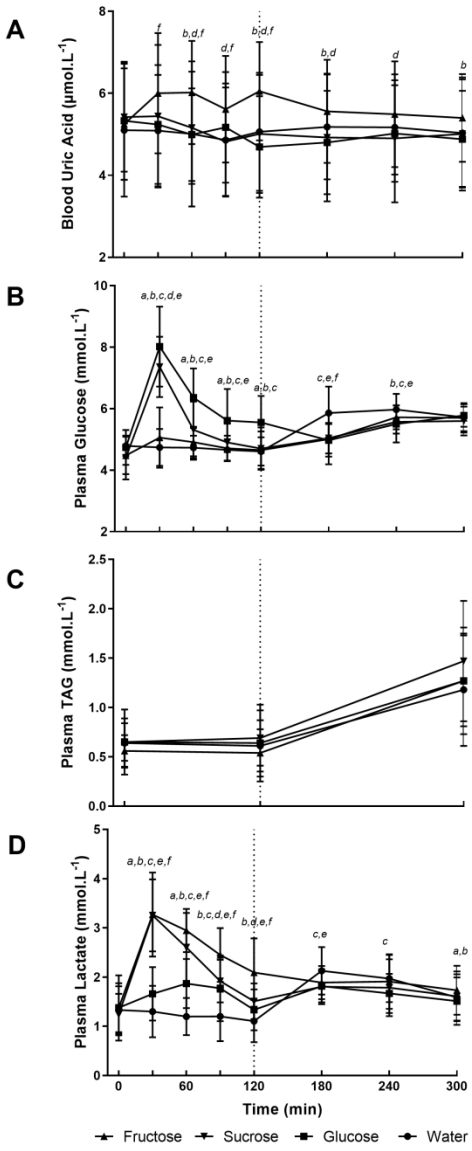

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a, P<0.05 glucose v sucrose. b, P<0.05 glucose v fructose. c, P<0.05 glucose vs water. d, P<0.05 sucrose vs fructose. e, P<0.05 sucrose vs water. f, fructose vs water.

123x284mm (300 x 300 DPI)