

# **THE VASCULAR RESPONSE TO CONSUMPTION OF DIFFERENT SUGAR TYPES IN ADOLESCENTS**

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# Contents

<b>List of Abbreviations</b> .....	4
<b>Acknowledgements</b> .....	5
<b>List of Tables</b> .....	6
<b>List of Figures</b> .....	7
<b>Abstract</b> .....	8
<b>Chapter 1: Introduction</b> .....	10
1.1 Cardiovascular Disease.....	10
1.2 Sugar-Sweetened Beverages.....	12
<b>Chapter 2: Literature Review</b> .....	14
2.1 Paediatric Origins of Atherosclerosis and CVD.....	14
2.2 Endothelial Dysfunction .....	16
2.3.1 <i>Fructose and cardiovascular risk</i> .....	25
2.3.2 <i>Fructose and uric acid</i> .....	28
2.4 Postprandial lipaemia and cardiovascular disease .....	30
2.5 Reliability of macro- and micro- vascular measurements.....	33
<b>Chapter 3: Methodology</b> .....	37
3.1 Ethics and informed consent .....	37
3.2 Participants .....	38
3.3 Experimental overview .....	38
3.4 Preliminary visit: visit 1 .....	39
3.4.1 <i>Anthropometry</i> .....	39
3.4.2 <i>Peak oxygen uptake and gas exchange threshold</i> .....	41
3.5 Experimental visits: visits 2-5.....	42
3.5.1 <i>Baseline measurements</i> .....	42
3.5.2 <i>Assessment of macrovascular function</i> .....	45
3.5.3 <i>Assessment of microvascular function</i> .....	48
3.6 Bloods analyses .....	49
3.7 Statistical analyses.....	50
<b>Chapter 4: Reliability Chapter</b> .....	52

4.1 Introduction .....	52
4.2 Methods .....	54
4.3 Results .....	56
4.4 Discussion.....	60
<b>Chapter 5: The Vascular and Metabolic Responses to Consumption of Fructose, Sucrose and Glucose in the Acute and Postprandial Phase .....</b>	<b>64</b>
5.1 Introduction .....	64
5.2 Methods .....	68
5.3 Results .....	70
5.4 Discussion.....	83
<b>Chapter 6: Conclusions.....</b>	<b>95</b>
<b>Chapter 8: References.....</b>	<b>97</b>
<b>Chapter 9: Appendix.....</b>	<b>131</b>

## List of Abbreviations

[ ]	concentration
95% CI	95% confidence interval for the true difference
ANOVA	analysis of variance
BMI	body mass index
cIMT	carotid intima-media thickness
CON	control
CVD	cardiovascular disease
DBP	diastolic blood pressure
ECG	electrocardiogram
ES	effect size
FMD	flow-mediated dilation
FRU	fructose
GET	gas exchange threshold
GLU	glucose
iAUC	incremental area under the curve
LDF	laser Doppler flowmetry
LDL	low-density lipoproteins
mRNA	messenger RNA
NO	nitric oxide
PRH	peak reactive hyperaemia
SBP	systolic blood pressure
SR <sub>AUC</sub>	area under the curve for shear versus time
SSB	sugar sweetened beverage
SUC	sucrose
TAG	triacylglycerol
tAUC	total area under the curve
TRH	total reactive hyperaemia
VLDL	very-low density lipoproteins
VO <sub>2 peak</sub>	peak oxygen uptake

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## **List of Tables**

4.3.1 Within-day reproducibility of novel CVD outcomes

4.3.2 Between-day reproducibility of novel CVD outcomes

5.3 Participant characteristics

5.3.3 Post drink + meal incremental (iAUC) and total area under the curve (tAUC) data for plasma glucose, lactate and uric acid for each trial

## List of Figures

- 3.5.1.2 Protocol schematic
- 3.5.2 FMD scan of brachial artery
- 5.3.1 Percentage change in flow-mediated dilation and  $SR_{AUC}$  data at baseline, 1 h post drink (1 hour post drink consumption) and 3 h post meal (3 hour post meal consumption)
- 5.3.2 Peak reactive hyperaemia (A; PRH%) and total reactive hyperaemia (B; TRH) data at baseline, 1 h post (1 hour post drink consumption) and 3 h post (3 hour post meal consumption)
- 5.3.3 Data for plasma [glucose] (A), [lactate] (B), [uric acid] (C) and [triacylglycerol] (D; [TAG]) for each trial

## Abstract

Cardiovascular disease (CVD) is the leading global cause of mortality and the underlying atherosclerotic disease process which precedes overt CVD originates in youth. Consumption of sugar-sweetened beverages (SSB) are associated with CVD risk factors in youth and SSB intake is a key contributor to added dietary sugar intake in teenagers in the United Kingdom. Endothelial dysfunction is a prerequisite for atherosclerosis and previous research demonstrates that SSB consumption acutely impairs endothelial function. However, these studies have predominantly focused on adult populations, or have used a glucose bolus which is not representative of the sugar found in commercially available SSBs. The purpose of this thesis was to investigate the influence of different dietary sugars found in SSBs on macrovascular and microvascular outcomes and then observe whether the sugar type influences the cardiometabolic responses to a subsequent test meal challenge. This thesis also determined the reliability of measures for the assessment of macrovascular (flow-mediated dilation; FMD) and microvascular (laser Doppler flowmetry) function. **Chapter 4:** Assessed the within-day and between day reliability of the simultaneous assessment of macrovascular and microvascular function. The within-day and between-day typical error expressed as a coefficient of variation were as follows: FMD, 7.2% and 8.0%; peak reactive hyperaemia (PRH), 24.5% and 26.1%. These results determined that the reliability of outcomes in this study were consistent with existing paediatric data. **Chapter 5:** There was a large ( $\eta_p^2=0.27$ ) but not significant ( $P=0.06$ ) interaction effect for FMD. There was a non-significant difference between the sucrose and control trials for FMD at 1 hour post drink consumption ( $P=0.06$ ) and 3 hours post test meal consumption ( $P=0.09$ ) but there was a medium effect at both times with effect



sizes of 0.77 and 0.65, respectively, where FMD increased following sucrose consumption. PRH increased 1 hour post drink consumption in the fructose ( $P=0.01$ ), glucose ( $P=0.04$ ) and sucrose trials ( $P=0.04$ ) when compared to control. PRH was greater in the fructose trial when compared to the control ( $P=0.03$ ), glucose ( $P=0.01$ ) and sucrose ( $P=0.02$ ) trials. These findings suggest that SSB intake acutely improves macro- and micro-vascular function in adolescents. Overall, these findings provide important information in regards to the effects of SSBs on macrovascular and microvascular function in adolescents.

## **Chapter 1: Introduction**

### **1.1 Cardiovascular Disease**

In 2012, out of the 56 million deaths which occurred globally, 38 million of these were due to noncommunicable diseases (cardiovascular disease [CVD], cancer, respiratory diseases and diabetes) (WHO, 2014). Cardiovascular diseases were responsible for 46% (17.5 million) of these deaths making them the leading cause of mortality globally (WHO, 2016a). Eighty percent of CVD deaths are caused by stroke or heart attack and a third of these deaths are in people under the age of 70 years (WHO, 2016a). Furthermore, CVD accounted for 125,000 of the 491,000 deaths which occurred in the UK in 2016 (British Heart Foundation, 2018a). More specifically, CVDs were responsible for 25% of deaths in men and 17% of deaths in women under the age of 75 years (British Heart Foundation, 2018a). As the average life expectancy in the UK is 79.2 years for males and 82.9 years for females (Office for National Statistics, 2018) these statistics highlight the significant role CVD plays in early mortality in the UK.

Over the past 20 years significant reductions in CVD mortality have been observed in high-income countries as a result of government policies which facilitate adoptions of healthier lifestyles and improvements in available healthcare (WHO, 2014). However, data from the British Heart Foundation (2018b) found that people in the UK living with CVD had increased from 7.3% in 1988 to 11.1% in 2011 in men and from 7.7% to 9.1% in women, respectively. This could be explained by advances in CVD healthcare which has resulted in people living with CVD for longer. However, this in turn causes an increase in healthcare costs and a recent European report estimated the total annual healthcare cost of CVD in the UK was £24 billion in 2015 (European Heart Network, 2017) and with the associated decline in CVD mortality and increase

in CVD morbidity the impact of CVD on the economy will continue to rise (Public Health England, 2018). The increase of CVD morbidity in the UK makes tackling CVD a primary issue and further work needs to be done to reduce healthcare costs and to improve standard of life.

The underlying disease process which causes CVD is atherosclerosis, an inflammatory disease which progresses throughout life (Ross, 1999; McGill & McMahan, 2003). The primary stage of atherosclerosis is endothelial dysfunction, an impairment in functioning of the endothelium, which occurs prior to structural changes to the artery (Tounian et al., 2001; Meyer et al., 2006).

This is followed by the deposition of cholesterol and its entry into the subendothelial space to form fatty streaks (McGill & McMahan, 1998). This process has been determined to originate during childhood (McGill et al., 2000a). The probability of an individual developing CVD can be predicted by individual characteristics known as risk factors which include age, male gender, family history of precocious heart disease, high total serum cholesterol concentration, high low-density lipoprotein (LDL) cholesterol, low high-density lipoprotein cholesterol level, smoking hypertension, obesity and diabetes (McGill & McMahan 1998). Over many years, in the presence of continued CVD risk factor exposure, the proliferation of smooth muscle and connective tissue causes foam cell formation alongside the sequestering of lipid rich macrophages into the vessel wall which results in the development of a fibromuscular cap (McGill & McMahan, 1998). Progression in size continues until the lesion has imposed itself on the lumen of the artery which causes it to become vulnerable to rupture causing the release of lipid-rich substances into the bloodstream (McGill & McMahan, 1998). This leads to the development of a

thrombus, promoting occlusion and subsequently leading to a cardiovascular event such as myocardial infarction or stroke.

## **1.2 Sugar-Sweetened Beverages**

SSBs are made up of equal parts of the sugars fructose and glucose (which form the sugar sucrose) and the consumption of fructose has been positively associated with CVD risk factors such as obesity, elevated lipid production in the liver and high blood pressure independent of glucose (Johnson et al., 2009; Bray, 2012; Vartanian et al., 2007; Bray, 2010). Fructose consumption has also been associated with an increase in the fractional rate of *de novo* lipogenesis which is implicated in the progression of atherosclerosis (Stanhope et al., 2009; Faeh et al., 2005). Adolescents in the UK aged 11 to 18 years consume 191 g of SSBs per day which is equal to 22-28 g of sugar (Public Health England, 2018; Malik et al., 2006). The Scientific Advisory Committee on Nutrition recommend that no more than 5% of daily energy intake comes from added sugar and current average intake for adolescents is three times this value (Scientific Advisory Committee, 2015; Public Health England, 2015). Current guidelines recommend a limit of <25 g per day of added sugar for young people meaning adolescents in the UK are consuming the recommended maximum amount of sugar from SSBs alone (Vos et al., 2017). SSB consumption has been linked to increased risk of CVD, coronary heart disease, diabetes and CVD risk factors including increased systolic blood pressure, plasma [triglyceride] ([TAG]) and adiposity in children and adolescents (Vos et al., 2017; Huang et al., 2014; Basu et al., 2013; Chan et al., 2014). With the early subclinical manifestations of atherosclerosis and subsequent CVD originating in the earlier years it is important to determine whether SSB consumption contributes to this process in order to determine CVD risk in this population.

This thesis aims to identify the effects of fructose, glucose and sucrose on vascular and metabolic function in adolescents to determine whether there is a link between SSB consumption and CVD related outcomes.

## Chapter 2: Literature Review

### 2.1 Paediatric Origins of Atherosclerosis and CVD

The atherosclerotic disease process originates in childhood and in the continued presence of CVD risk factors, can precede overt CVD (Bridger, 2009). Importantly, it is understood that the atherosclerotic process can be expedited by exposure to known CVD risk factors in childhood (Berenson et al., 1998). We know that the severity of atherosclerotic lesions in the aorta and coronary arteries of young people (2 to 39 years) is proportional to known CVD risk factors including body mass, elevated blood pressure, serum cholesterol, triglycerides, LDL and high-density lipoprotein cholesterol collectively (Berenson et al., 1998).

Holman et al. (1958) initially determined that in the first two decades of life there was already a prevalence of fatty streaks in the coronary arteries of New Orleans residents. Subsequent research in ~3,000 autopsied young persons aged 15 to 34 years who died from noncommunicable deaths (not due to communicable or chronic disease), found that very-low density lipoproteins (VLDL), LDL, smoking, hypertension, body mass index (BMI) and hyperglycaemia were associated with atherosclerotic lesions (McGill et al., 2000b; McGill et al., 1995; McGill et al., 1997; McGill et al., 1998; McGill et al., 2002). The Pathobiological Determinants of Atherosclerosis in Youth (PDAY) Study developed risk scores to predict the prevalence of advanced lesions in adolescents and young adults (McMahan et al., 2005). By using these risk scores it was determined that subclinical lesions seen in the early years are associated with advanced clinical lesions in later life (McMahan et al., 2006). Based on these findings it is suggested that the prevention of CVD needs to begin in the early years (McMahan et al., 2005).

Newer noninvasive ultrasound techniques have made it possible to observe vascular alterations associated with CVD in living populations (Groner et al., 2006). Thickening of the intima and media in the carotid artery (carotid intima-media thickening; cIMT) is associated with known CVD risk factors and coronary artery disease (Heiss et al. 1991; Chambless et al., 1997) and is used as a marker for atherosclerotic progression in adults and as a predictor of cardiovascular events (Bridger, 2009). A recent meta-analysis conducted by the International Childhood Cardiovascular Cohort (i3C) Consortium used data from the Cardiovascular Risk in Young Finns (Finland), the Childhood Determinants of Adults Health (Australia), the Bogalusa Heart (US) and the Muscatine (US) studies (Juonala et al., 2010). These studies all measured childhood risk factor exposure and followed participants into adulthood and have evidenced that atherosclerotic lesions and cIMT at death were associated with CVD risk factor exposure during childhood (Juonala et al., 2010). Research has shown that LDL level and BMI in childhood were independent predictors of elevated cIMT in adulthood (Shengxu et al., 2003). This was supported by further research which evidenced that, as well as LDL level and BMI, systolic blood pressure and smoking measured during the ages of 12-18 years were independently associated with cIMT in adulthood (Raitakari et al., 2003) determining that risk factor status in the adolescent years is a strong predictor of cIMT in adulthood. Thus, limiting exposure to CVD risk factors in youth is an important consideration in the battle against CVD.

Research regarding the reversibility of cIMT in children and adults is conflicting. Studies using exercise interventions have demonstrated that exercise alone does not reduce cIMT in patients with diabetes or in obese or overweight children and adolescents (Byrkjeland et al., 2016; Ngai & Cheung, 2015).

However, cIMT has been shown to reduce following routine care or a controlled diet plan (Ngai & Cheung, 2015) and carotid vessel wall volume was also attenuated following a 2 year diet intervention (Shai et al., 2010). Further research has found that cIMT can be reduced with insulin and oral hypoglycemic treatments (Tenjin et al., 2016) and by bariatric surgery in obese participants (Garcia et al., 2013). The evidence indicates that non-pharmacological interventions to reverse cIMT have limited success. Thus, the focus of interventions should be on prevention of structural changes through either improvement or preservation of vascular function rather than treatment.

The research discussed provides evidence for the early beginnings of the atherosclerotic process and how there is a potential association with cardiovascular risk factors. It has been consistently observed that when these risk factors are measured during the younger years they predict the extent of the atherosclerosis in later life. The evidence provided shows that early risk factor exposure can result in clinically significant atherosclerosis in later life. Interventions need to occur in youth and should focus on preservation or improvement of vascular function.

## **2.2 Endothelial Dysfunction**

The endothelium plays a key role in the atherosclerotic process and is implicated from the beginning to the clinically advanced stages (Thijssen et al., 2019). It regulates the tone, growth, adhesion and coagulation of the vasculature (Vanhouste et al., 2009) and is also sensitive to haemodynamic stimuli including shear stress and circumferential wall strain (Green et al., 2017; Thijssen et al., 2019). Endothelial dysfunction is defined as an impairment in the endothelium's ability to respond to haemodynamic stimuli and is the earliest detectable change of the atherosclerotic process preceding any structural



change to the vasculature (Ross, 1999). Therefore, function of the endothelium is an important outcome to focus on in regards to CVD prevention.

(Dys)function of the endothelium can be non-invasively assessed using the ultrasound based technique of flow-mediated dilation (FMD) of the brachial artery (for details please see section 3.5.2) (Celermajer et al., 1992; Corretti et al., 2002).

In studies where FMD was measured on peripheral arterial disease patients, those which later suffered a cardiovascular event had a significantly lower FMD than those that did not (Brevetti et al., 2003; Gokce et al., 2003). Additionally, there was nearly a 5-fold increase in cardiovascular event risk in those with an FMD below the median (6.7%) than in those with an FMD above the median (Brevetti et al., 2003). Research has also shown that participants with an FMD below the median (4.7%) suffered from cardiovascular events more than those with an FMD above the median (Muiesan et al., 2008). A meta-analysis of 14 studies determined that for every 1% increase in FMD the likelihood of a cardiovascular event occurring reduced by 13% (Inaba et al., 2010). Another meta-analysis of 23 studies found that the pooled overall CVD risk was 0.92 for every 1% increase in FMD (Ras et al., 2013). More recently, Matsuzawa et al. (2015) systematically reviewed 35 FMD studies and evidenced a relative risk for CVD of 0.88 for every 1% increase in FMD.

In the first study to use FMD, it was found that endothelial dysfunction occurs in children present with CVD risk factors (Celermajer et al., 1992). Use of the FMD technique has also demonstrated that endothelial dysfunction is present in children who are overweight (Woo et al., 2004), obese (Aggoun et al., 2008; Tounian et al., 2001), have type 1 diabetes (Jarvisalo et al., 2004) and present with hypercholesterolemia (Sorensen et al., 1994). The Cardiovascular Risk in

Young Finns cohort demonstrated that in young adults the number of CVD risk factors were only predictive of an elevated cIMT in those with an FMD in the tenth percentile (FMD of  $1.1 \pm 1.4\%$ ) (Juonala et al., 2004). This association was no longer present in participants when endothelial function was not impaired proposing that even in the presence of risk factors, if endothelial function is maintained then the likelihood of developing atherosclerosis is reduced (Juonala et al., 2004). This suggests that by preventing endothelial dysfunction this can protect against atherosclerotic related change even when exposed to risk factors. These findings have been supported by Hopkins et al. (2013) where it was determined in children aged 10 years that there was no association between changes in FMD occurring over a 30 month period and cIMT measured at 30 months. This suggests that endothelial dysfunction needs to occur over a longer period of time before any atherosclerotic changes take place. Preventing an impairment in endothelial function even in early life is therefore important (Hopkins et al., 2013).

It has been shown that impaired macrovascular endothelial function occurs prior to any atherosclerotic change in children, adolescents and adults. It is also known that that microvascular function can be impaired in the presence of CVD risk factors. Evidence has shown an inverse association between microvascular function and CVD risk factors in 11 to 14 year old children (Khan et al., 2003). Further research has demonstrated that microvascular function is impaired in the presence elevated blood pressure and insulin resistance in adults (Wascher, 1997; Serne et al., 1999). More recently, a study of 1500 vascular disease free men found that microvascular function and cIMT predicted later cardiovascular events (Anderson et al., 2011). Interestingly, in the same study FMD was not associated with subsequent cardiovascular events which

suggests that microvascular function provides important prognostic information which cannot be captured using FMD.

The literature behind the mechanisms of microvascular function are currently limited however, it has been proposed that there are four major factors which are involved in the hyperaemic response (Cracowski et al., 2006). These four factors include metabolic vasodilators, endothelial vasodilators, the myogenic response and sensory nerves (Cracowski et al., 2006). Nitric oxide (NO), an endothelial vasodilator, has been reported not to be crucial in the response. Wong et al. (2003) demonstrated that NO synthase inhibition by infusion of NG-nitro-L-arginine methyl ester into the forearm did not influence the hyperaemic response. Another study, which assessed [NO] by using a NO selective amperometric electrode inserted into the skin of the forearm, found that [NO] did not increase during reactive hyperaemia in the skin of the forearm (Zhao et al., 2004). Engelke et al. (1996) also demonstrated that total hyperaemic response remained unchanged before and after administration of the NO synthase inhibitor NG-monomethyl-L-arginine. However, this study also demonstrated a 33% reduction in the total hyperaemic response when ibuprofen (which inhibits prostaglandins) was administered before the NO synthase inhibitor. This suggests that prostaglandin inhibition might determine a role for NO in the reactive hyperaemic response. It has also been demonstrated that combined sensory nerve and prostaglandin inhibition caused a 72% reduction in the post-occlusive response (Larkin & Williams, 1993). Thus, the mechanisms behind the microvasculature are most likely attributed to a series of interactions of endothelium dependent and independent mechanisms.

FMD assesses the function of the brachial artery which is a NO dependent process when the pneumatic cuff is placed distal to the ultrasound probe and

inflated for a period of 5 min at a pressure of 220mmHg (Thijssen et al., 2019). Post-occlusive reactive hyperaemia of the cutaneous circulation assesses the function of the small capillaries located in the forearm and is not dependent on NO (Wong et al., 2003). We also know that FMD and post-occlusive reactive hyperaemia do not significantly correlate (Dhindsa et al., 2008). Therefore, microvascular function cannot be inferred by FMD and must also be measured. Additionally, the earliest detectable manifestations of the metabolic syndrome occur at the microvascular level (Pinkney et al., 1997).

It was shown that little association existed between macro- and micro-vascular function in a study which assessed FMD, reactive hyperaemic flow, hyperaemic shear stress and reactive hyperaemia index in 40 healthy normotensive adults (Dhindsa et al., 2008). Although FMD was positively associated with reactive hyperaemic index, it was not associated with hyperaemic flow or hyperaemic shear stress. However, this study assessed microvascular function using finger plethysmography. A study which assessed macro- and micro-vascular function showed there was no association between FMD and laser Doppler imaging in coronary artery disease patients (Shamim-Uzzaman et al., 2002). This study also suggested that microvascular function was a greater predictor of coronary artery disease than macrovascular function. More recent research has supported the notion that macro- and micro-vascular function are unrelated where it was shown that FMD had no association with microvascular cutaneous reactive hyperaemia and acetylcholine-induced microvascular vasodilation (Gori et al., 2006). Given the contrasting views in the literature it would be prudent for research to measure both macro- and micro-vascular function, as both are important and one cannot be inferred from the other. Prior research has used laser Doppler imaging to assess microvascular function by post-occlusive

reactive hyperaemia (Schlager et al., 2011). Furthermore, Bond et al. (2016) has demonstrated that microvascular function can be assessed simultaneously and noninvasively alongside the FMD protocol to reliably determine vascular function in adolescents.

Research which has assessed the predictability of microvascular function for cardiovascular events in ~300 cardiac patients found that in patients in the lower 2 tertiles (tertiles were divided according to the  $\Delta$ CVR [coronary vascular resistance] with intracoronary acetylcholine) events occurred in 14.1% of patients and in the highest tertile this percentage was 5.8% (Halcox et al., 2002). Another study prospectively assessed FMD of the brachial artery in ~430 asymptomatic adults with an average age of 54 years (Shechter et al., 2009). Here they divided subjects into two groups, those with an FMD either above or below the median of 10.7%. These groups also factored in how comparable subjects were for coronary artery disease risk factors including age, sex, smoking, diabetes, hypercholesterolemia, hypertension and BMI as well as lipoproteins, fasting glucose, C-reactive protein and concomitant medications. After a 32 month follow up it was determined that cardiovascular events were significantly more common in subjects with an FMD below the median. Additionally, it was found that median FMD significantly predicted cardiovascular events with an odds ratio of 2.78 (95% CI=1.35 to 5.71). A multivariate analysis which included conventional risk factors for coronary artery disease also found that median FMD was the best significant independent predictor of long term cardiovascular adverse events with an odds ratio of 2.70 (95% CI=1.16 to 6.32). These findings indicate that the functioning of the endothelium plays an important role in CVD risk and therefore warrants measurement.

### **2.3 Sugar and cardiovascular risk**

Adolescents in the UK aged 11 to 18 years consume 191 g of SSBs per day which is equal to 22-28 g of sugar (Public Health England, 2018; Malik et al., 2006). Current guidelines recommend a limit of <25 g per day of added sugar for young people meaning adolescents in the UK are consuming the recommended maximum amount of sugar from SSBs alone (Vos et al., 2017). Additionally, the Scientific Advisory Committee on Nutrition recommend that added sugars should represent no more than 5% of daily energy intake however current average intake for adolescents is three times this value (Scientific Advisory Committee, 2015; Public Health England, 2015).

The health concerns associated with SSB consumption in the UK has been highlighted by a recent sugar tax on SSBs. This tax was introduced to tackle childhood obesity, type 2 diabetes and other health issues caused from sugar consumption. As a result, over 50% of SSB manufacturers have reduced the sugar content of their drinks equivalent to 45 million kg of sugar ever year (GOV.UK, 2018). This evidences the relevance of the current project and the importance of understanding the effects overconsumption of sugar has on youth.

A recent meta-analysis of four studies which used participants aged 9 to 28 years determined risk of coronary heart disease is increased by 16% by a one-serving per day increase in SSBs (Huang et al., 2014). The American Heart Association provided a scientific statement that determined that added sugars in the diet increase CVD risk in children due to increased adiposity and dyslipidemia (Vos et al., 2017). Importantly, these outcomes were present at levels far below current levels of consumption (Vos et al. 2017). A study which

used econometric models of repeated cross-sectional data on diabetes and nutritional components of food from 175 countries it was found that ~1 can of SSB per day increased diabetes prevalence by 1.1% (Basu et al., 2013). A cross-sectional study by Chan et al. (2014) assessed the effects of dietary SSB intake in ~2700 Vietnamese 12 to 16 year olds. It was found that increased consumption of SSB per week was associated with greater waist circumference in both sexes and systolic blood pressure in boys. High and moderate SSB consumption was also associated with a significantly higher level of plasma [TAG] in boys (8.0 and 8.2 mg/dL) than non-SSB consumers. Furthermore, consumption of >500 mL/day of SSB was associated with a 10-fold increase in metabolic syndrome risk in boys. A prospective study assessed SSB intake and CVD risk factors in adolescent girls from the ages of 14 to 17 years and found that those in the top tertile of SSB consumption (>1.3 servings per day) demonstrated an increase in BMI, body weight and obesity risk and a greater overall cardiometabolic risk (Ambrosini et al., 2013). Another prospective study assessed added sugars and CVD risk factors in children aged 8 to 10 years and followed them up 2 years later and determined that a higher consumption of added sugar from liquid sources (10 grams per day) was associated with higher plasma [glucose] and [insulin] and an increase in insulin resistance and these were elevated in overweight or obese children (Wang et al., 2014).

A recent meta-analysis of 39 studies which assessed the influence of acute hyperglycaemia from SSB consumption on macro- and micro-vascular function concluded that SSB consumption negatively influences vascular function in healthy and diseased adults (Loader et al., 2015). However, this conclusion is not consistent with the limited paediatric data available. Dengel et al. (2007) assessed the effects of an oral glucose tolerance test (OGTT) (1.75 g of

glucose per kg in body weight) on FMD before and 30, 60 and 120 minutes after ingestion in healthy and obese adolescents. Here it was found that the OGTT increased FMD by ~2% in the healthy group but there was no change in FMD in the obese group. Another paediatric study assessed the effects of acute hyperglycaemia compared to euglycaemia on forearm vascular resistance in adolescents with type 1 diabetes (Dye et al., 2012). In this study glucose and dextrose was infused into an antecubital vein to raise plasma [glucose] to 200 mg/dL for 60 minutes for the hyperglycaemic state. It was determined that acute hyperglycaemia caused a significant decrease in preocclusion forearm vascular resistance and the postocclusion fall in forearm vascular resistance was reduced when compared to euglycaemia. This resulted in an increase in vascular function as opposed to the adult data reported in Loader et al., (2015) which reported attenuations in FMD following SSB ingestion.

The literature currently states that SSB consumption does not lower vascular function in youth. However, these studies have predominantly focused on findings from adult populations. Only 2 of the articles cited in the meta-analysis by Loader et al. (2015) were conducted on youth meaning the conclusions drawn are unrepresentative of the paediatric population. Furthermore, these studies have concentrated on OGTT which are not representative of commercially available SSBs. 27 of the 39 articles cited by Loader et al. (2015) used glucose as the sugar when assessing the effects on vascular function and only 2 reported using the sugar known as sucrose. However, the main sugars in commercially available SSBs are sucrose or high-fructose corn syrup. Sucrose is a disaccharide equally composed of the monosaccharides glucose and fructose and high-fructose corn syrup is predominantly made up of the monosaccharide fructose. These sugars are metabolised differently and the



effects these have on the vasculature and CVD risk may differ (Lustig, 2012). It is understood that the ratio of fructose to glucose in popular SSBs is 50:50 (sucrose) but Walker et al. (2014) have reported this to be 60:40. Therefore, the previous studies are unrepresentative of the biggest contributor of habitual sugar intake in adolescents (Loader et al., 2017), and fail to identify the acute effects of SSB consumption on vascular function. Recent research which has assessed the effects of 600 mL of commercial SSB consumption on macro- and micro-vascular function compared to a control trial (600 mL of water) in healthy, sedentary adult males (Loader et al., 2017). FMD and the microvascular response to acetylcholine iontophoresis were significantly lowered following consumption of the SSB when compared to the control trial. These findings provide an insight into the consequences of SSB consumption on vascular function. However, there is currently no data on the effects of commercially available SSB on vascular function in youth.

### **2.3.1 Fructose and cardiovascular risk**

Fructose, high-fructose corn syrup and sucrose have been introduced to the diet and sucrose accounts for >90% of nutritive sweetener consumed worldwide (Rippe & Angelopoulos, 2015; White, 2008). Epidemiological studies have demonstrated that these sugars, independent of glucose, are associated with CVD risk factors including obesity, elevated lipid production and high blood pressure in youth and adulthood (Johnson et al., 2009; Bray, 2012; Vartanian et al., 2007; Bray, 2010). Fructose is known to increase hepatic VLDL output due to the conversion of fructose to triacylglycerol (TAG) by a process known as *de novo* lipogenesis (Stanhope et al., 2009). VLDL are lipoproteins which contain TAG and cholesterol (Goldstein & Brown, 2015) and are hydrolysed into LDL, which play a key role in the atherosclerotic process (Brown & Goldstein, 1986).

Research has shown that the fractional rate of *de novo* lipogenesis in humans for glucose is 2% but for fructose this increases to 10% following 6 days of high-fructose feeding (Schwarz et al., 2009; Faeh et al., 2005). Stanhope et al. (2009) found that *de novo* lipogenesis was increased to 17% following fructose feeding in adults. When glucose and fructose are consumed simultaneously, more fructose undergoes the hepatic lipogenic pathway, which results in the rate of *de novo* lipogenesis being tripled as opposed to when fructose is consumed independently (Hudgins et al., 2011). Therefore, it is by this mechanism that smaller, but daily, intakes of fructose might be bad for health.

The only study in the meta-analysis by Loader et al. (2015) which included a combined sucrose and glucose challenge (39.1 g total) found that microvascular function was increased in adults (Grasser et al., 2014). The meta-analysis provided no study which examined the effects of a sucrose drink on macrovascular function but did report one study which demonstrated a fall in FMD in type 2 diabetic adults following consumption of a sucrose test meal (Shige et al., 1999). Thus, further research is required to explore the effects of sucrose and fructose on the vasculature to provide an accurate understanding of the effects of habitual SSB consumption on vascular and metabolic outcomes.

There is limited research examining the effects of fructose on vascular function however, a study which examined the influence of consumption of a sucrose load representative of that found in commercially available SSBs found that there was no change in microvascular reactivity in healthy young adults (West et al., 2019). This is not reflective of previous research where Loader et al. (2017) used an SSB which contained equal parts of fructose and glucose to

determine that SSB consumption also reduced FMD and microvascular function in adults.

Despite the limited and contrasting research exploring the effects of fructose consumption on vascular function in adulthood and the lack of research into the effects of fructose in youth, rodent data has provided an insight into the effects of fructose on metabolic and vascular responses. A systematic review of 26 studies into the effects of fructose consumption on rats determined that consumption of 10-21 mL of fructose per 100 mL of SSB increased rodent body weight, systolic blood pressure and blood [glucose], [insulin] and [TAG] (Toop & Gentili, 2016). A study which compared the effects of fructose consumption to glucose consumption in rats found that fructose consumption increased [TAG] and impaired endothelial function but glucose consumption did not (Sanguesa et al, 2017). In the same study both fructose and glucose impaired insulin signalling which resulted in glucose not being metabolised and converted into fat via *de novo* lipogenesis however, the impairment was greater in the fructose group.

Further research has demonstrated that high-fructose corn syrup feeding attenuated expression of insulin receptor substrate-1, endothelial NO synthase mRNA and protein, aortic NO and endothelial relaxation (Babacanoglu et al., 2013; Akar et al., 2012) which results in an increase in synthesis of glucose in the liver and vascular dysfunction. Fructose consumption has also been determined to increase insulin, total cholesterol and blood pressure as well as the expression levels of inducible NO synthase mRNA and protein (Malakul et al., 2018; Babacanoglu et al., 2013; Akar et al., 2012) which has been associated with impaired insulin signalling in the livers of diabetic mice (Fujimoto et al., 2005) and endothelial dysfunction in diabetic rats (Nagareddy

et al., 2005). More recently, a study determined that 2 months of high-fat and high-fructose feeding in rats increased body weight, leptin, oxidative stress in plasma and in the liver and after 8 months induced endothelial dysfunction (Lozano et al., 2016).

Given the findings shown in rodent data and that existing OGTT data is not representative of habitual sugar consumption it is important to explore whether fructose consumption induces similar vascular and metabolic responses in youth.

### **2.3.2 Fructose and uric acid**

Fructose-rich beverage consumption is known to increase [uric acid] when compared to a control trial (Choi et al., 2008). As the body breaks down fructose, chemical compounds called purines are released and uric acid is produced during purine metabolism which also generates oxidants and in humans this is the final end product of purine metabolism (Johnson et al., 2013). The normal range for [uric acid] in humans is usually 3 to 7 mg/dL in the blood and this is increased by consumption of purine rich foods or fructose (Johnson et al., 2013). [Uric acid] has been associated with CVD in high risk groups and healthy populations. A five year follow up study assessed [uric acid] and cardiovascular events in healthy men and women (Kivity et al., 2013). In this study, [uric acid] was found to be an independent predictor of cardiovascular events in both men and women. This study also found that fructose consumption was positively associated with an increase in [uric acid] when they compared those that consumed more than 75 mg per day to those that consumed less than 10 mg per day. Furthermore, [uric acid] did not increase following “diet” SSB consumption (no sugar) demonstrating that the fructose element of these drinks are what causes the increase in [uric acid]. An

in vivo study which fed rats a 60% fructose diet for 10 weeks found that [uric acid] was elevated when compared to a control group (Nakagawa et al., 2006). A more recent study using rats administered a single dose of fructose (5 g per kg) where it was determined that fructose consumption inhibited functioning of ileac uric acid excretion which resulted in hyperuricemia (Kaneko et al., 2017). Another study fed overweight and obese adults fructose-sweetened beverages for 10 weeks (25% of energy requirements) and compared this to a glucose trial (Cox et al., 2012). Fasting plasma [uric acid] increased in both groups however, the increase was greater in the fructose trial. The findings from these studies demonstrate that fructose consumption causes hyperuricemia and that this may be due to an inhibition in uric acid excretion.

A study which assessed CVD risk factors in ~1100 men and women with diabetes aged 45 to 64 years calculated hazard ratios for [uric acid] (Lehto et al., 1998). It was found that for every 1.01 mg/dL increase in [uric acid] the hazard ratio for all-cause mortality was 1.09 for men and 1.26 for women and for cardiovascular mortality the hazard ratio was 1.19 for men and 1.3 for women, respectively. Further research which followed ~13000 participants from the Multiple Risk Factor Intervention Trial (MRFIT) for 6.5 years determined that [uric acid] was an independent risk factor for myocardial infarction (Krishnan et al., 2006).

Further research has contradicted the role uric acid plays in CVD risk and CVD mortality. The Framingham Heart Study followed ~6700 men for 23 years for [uric acid] and incidence of CVD events, CVD deaths and all other deaths (Culleton et al., 1999). This study concluded that uric acid does not cause CVD death but is associated with other CVD risk factors. The Bogalusa Heart Study assessed [uric acid] in ~570 children aged 5 to 17 years and reassessed these

participants during adulthood ~12 years later and found that childhood [uric acid] was predictive of diastolic and systolic blood pressure in adulthood (Alper Jr et al., 2005). A recent study demonstrated that elevated [uric acid] in asymptomatic, prepubertal children was associated with several CVD risk factors including elevated insulin resistance, C-reactive protein, [TAG], BMI, waist circumference, systolic blood pressure and cIMT (Bassols et al., 2016). Another study found that for every 1 mg/dL increase in [uric acid] the risk of prehypertension or hypertension increased by at least 50% for 6 to 18 year olds (Viazzi et al., 2013).

Research has demonstrated that increased fructose consumption causes an increase in [uric acid]. Although it is suggested that uric acid is not a direct cause of CVD it is independently associated with CVD in adults and has been associated with CVD risk factors in youth. Thus, it is important to explore the effects of fructose consumption on [uric acid] in adolescents to provide a clearer insight into CVD risk in this population.

#### **2.4 Postprandial lipaemia and cardiovascular disease**

Previous research has demonstrated that elevated plasma [TAG] is an independent risk factor for CVD (Hokanson & Austin, 1996; Patel et al., 2004; Sarwar et al., 2007). However, these studies have assessed plasma [TAG] in the fasted state when approximately 18 hours of the day is spent in the postprandial state (Jackson et al., 2012) making these observations unreflective of daily living. Elevated postprandial [TAG] impairs the endothelium which makes the arterial wall vulnerable to penetration by the remnants of TAG carrier chylomicrons which results in subsequent endothelial dysfunction (Proctor & Mamo, 2003). This subsequently leads to the progression of atherosclerotic lesions with the lipid constituents to which they're associated (Jackson et al.,

2012). Furthermore, atherosclerosis is considered to be a postprandial phenomenon (Zilvermit, 1979; Kolovou et al., 2005). Postprandial lipaemia is the term given to the consequence of consuming a high amount of fat and this is associated with CVD (Boquist et al., 1999). Previous research has demonstrated a relationship between postprandial lipaemia and atherosclerosis. One study which assessed cIMT 2 hours following an oral fat tolerance test demonstrated a significant relationship between postprandial [TAG] and an elevated cIMT (Boquist et al., 1999). Another study found that cIMT and postprandial [TAG] following consumption of a controlled breakfast were significantly correlated (Ahmad et al., 2005). It is suggested that plasma [TAG] following consumption of a high-fat meal is a better marker of metabolic health and CVD risk than fasting [TAG]. A previous study compared postprandial plasma [TAG] between the healthy sons of men with coronary artery disease and the healthy sons of healthy men (Uiterwaal et al., 1994). Although fasting [TAG] between the groups were similar, postprandial [TAG] was elevated significantly more in the sons of coronary artery disease patients suggesting that postprandial [TAG] provides a better reflection of metabolic health and future CVD risk than does fasting [TAG]. Importantly, data has also demonstrated that non-fasting [TAG] determined in the early years can independently predict CVD related events in later life. The Copenhagen City Heart Study conducted a ~30 year follow up of ~14000 men and women aged 20 to 93 years and assessed baseline levels of non-fasting [TAG] and other CVD risk factors and the incidence of ischemic stroke (Freiberg et al., 2008). Of the 14000 participants, ~1600 participants developed ischemic stroke and the findings determined that the cumulative incidence of ischemic stroke was higher with increasing non-fasting [TAG]. Morrison et al. (2009) conducted 22 to 31

year follow up studies in schoolchildren to assess paediatric and adult body mass and lipids in those that did and did not suffer a CVD event. This paper found that childhood plasma [TAG] was consistently and independently associated with CVD in early adulthood. Those which suffered from a CVD event also had higher levels of plasma [TAG] during childhood and in early adulthood. These findings display the association between [TAG] in the earlier years and CVD in later life.

Previous investigations in adolescents have used a high-fat meal to induce postprandial lipaemia and as this occurred a fall in FMD was observed (Sedgwick et al., 2013a; Sedgwick et al., 2013b; Sedgwick et al., 2015) and microvascular function (Bond et al., 2015a). This attenuation in vascular function has been supported by adult data which demonstrated a fall in endothelial function in men following consumption of a high-fat meal (Tyldum et al., 2009). A previous study assessed the effects of glucose, sucrose and fructose consumption on lipaemia following the consumption of a meal containing 40 g of fat (Cohen & Schall, 1988). It was found that although there was no difference in postprandial lipaemia between glucose consumption with the meal ( $2.11 \text{ mmol.L}^{-1} \cdot 7\text{h}^{-1}$ ) and the meal alone ( $2.42 \text{ mmol.L}^{-1} \cdot 7\text{h}^{-1}$ ), consumption of sucrose ( $3.77 \text{ mmol.L}^{-1} \cdot 7\text{h}^{-1}$ ) and fructose ( $4.23 \text{ mmol.L}^{-1} \cdot 7\text{h}^{-1}$ ) prior to the meal increased postprandial lipaemia compared to the meal alone. Thus prior feeding of fructose augments postprandial lipaemia and this may be due to an increased rate of *de novo* lipogenesis as a result of prior fructose feeding. It is important to understand the effect of prior sugar feeding from SSBs has on postprandial lipaemia and vascular function for results to be reflective of the dietary lifestyle of adolescents in the UK.



## **2.5 Reliability of macro- and micro- vascular measurements**

There have been several studies which have examined the reproducibility of the FMD technique but many of these use a wide variation in adherence to FMD guidelines (Thijssen et al., 2019). Ghiadoni et al. (2012) pooled the results of studies which strictly followed FMD guidelines and determined that the FMD assessment was highly reproducible with a coefficient of variation of 11.6 to 16.1%. However, adherence to FMD guidelines is not enough to eliminate for all sources of error and the FMD technique is known to be operator dependent (Thijssen et al., 2019). Therefore, it is necessary for the reliability of the operator performing the FMD protocol to be assessed in this thesis. There is only one known study which has assessed the between-day and within-day reliability of the FMD assessment in adolescents (Bond et al., 2016). This study reported a coefficient of variation (CV) of 10.6 and 5.1% for between-day and within-day reproducibility, respectively. Recent guidelines for the FMD assessment have recommended that a CV for consecutive FMD scans should be <15% (Thijssen et al., 2019). Based on the data from previous paediatric and adult studies and recent guidelines this thesis will assess reliability of the FMD technique and expect a CV of <15%.

Previous work by our research group has used laser Doppler imaging for the assessment of microvascular function (Bond et al., 2016) which uses a laser scanner. The laser Doppler flowmetry technique however, uses probes rather than a laser scanner which is likely to be associated with smaller error.

Therefore, there is a possible advantage to using this technique as opposed to other assessments of microvascular function and this thesis aims to determine the reliability of this method to inform future investigations. However, the laser Doppler flowmetry technique has been considered poorly reproducible but this

is most likely attributed to changes in the positioning of the probe (Cracowski et al., 2006). To account for the heterogeneity of the microvascular circulation the reliability of 2 laser probes rather than 1 will be assessed and the probe location on the first scan of each visit will be marked. When the recording site has been standardised the reproducibility of post-occlusive hyperaemia has compared well with FMD with a coefficient of variation of <10% (Kubli et al., 2000; Boignard et al., 2005). A study which assessed the reliability of the laser Doppler flowmetry technique to assess post-occlusive reactive hyperaemia reported a CV of 38% (Roustit et al., 2010). The only previous paediatric study to assess reliability of peak reactive hyperaemia reported a within-day CV of 11.0% and a between-day CV of 13.3% (Bond et al., 2016). This study also reported a within-day CV of 29.9% and a between-day CV of 23.1% for total reactive hyperaemia which was consistent with previous research which has evidenced a CV of 25.0% for total reactive hyperaemia (Wong et al., 2003). Therefore, this thesis aims to report a CV for peak reactive hyperaemia and total reactive hyperaemia which is consistent with the literature.

### **Thesis Aims**

The work in this thesis aims to determine the effects of sugars found in commercially available SSBs on vascular and metabolic outcomes in adolescents. In order for the findings to be reliable and valid the reproducibility of the macro- and micro-vascular methods of assessment are needed to be examined. Therefore, Chapter 4 will assess the reproducibility of the FMD and laser Doppler flowmetry techniques for the assessment of macro- and micro-vascular function, respectively. Following this, Chapter 5 will focus on the effects of sugars found in commercially available SSBs on the macro- and micro-vasculature and on plasma [glucose], [lactate], [uric acid] and [TAG] in

the acute phase following drink consumption and in the postprandial phase following consumption of a high-fat, high-sugar test meal. Due to time constraints and the fine detail required for FMD assessment this thesis will only explore the effects of sucrose and water (control) on FMD. Sucrose is the main sugar constituent of commercially available SSBs (Loader et al., 2017) thus this sugar was chosen as findings for FMD would be applicable to the diets of adolescents in the UK. Furthermore, only 10 of the 22 subjects will be assessed for FMD due to these reasons. However, assessment of microvascular function will be performed for all sugar trials and in all subjects.

1. To determine the reliability of the FMD and laser Doppler flowmetry techniques to determine macro- and micro-vascular function, respectively (Chapter 4).
  - It is hypothesised that the reliability of these outcomes will be consistent with findings from previous reliability studies for the assessment of macro- and micro-vascular function.
2. To determine the effects of glucose, fructose and sucrose consumption on acute microvascular function and the effects of sucrose on macrovascular function post-drink consumption in adolescents (Chapter 5).
  - It is hypothesised that glucose, fructose and sucrose will impair microvascular function compared to water with the greatest impairment being from fructose consumption.
  - It is hypothesised that sucrose consumption will impair macrovascular function compared to water 1 hour post drink consumption.

3. To establish the effects of glucose, fructose and sucrose consumption on metabolic outcomes (plasma [glucose], [lactate], [TAG] and [uric acid]).
  - It is hypothesised that consumption of all these sugars will increase plasma concentrations of plasma [glucose], [lactate], [TAG] and [uric acid].
  - It is hypothesised that there will be a dose response increase in uric acid which is proportional to the fructose content of the drinks.
4. To distinguish the effects of sucrose, fructose and glucose on postprandial microvascular function and metabolic outcomes following consumption of a high-fat, high-sugar test meal challenge and the effects of sucrose consumption on postprandial macrovascular function.
  - It is hypothesised that the high-fat, high sugar test meal will attenuate macrovascular and microvascular function.
  - It is hypothesised that the high-fat, high sugar test meal will increase postprandial TAG.
  - It is hypothesised that the prior feeding of fructose and sucrose will elevate postprandial [TAG] more than the glucose and control trials and this will manifest into a proportionately greater reduction in vascular function.

## **Chapter 3: Methodology**

### **3.1 Ethics and informed consent**

Ethical approval for the work contained in this thesis was granted by the University of Exeter's Sport and Health Sciences Ethics Committee (171206/B/07). Participants were recruited from a local school in Exmouth, Devon with the consent of the Head Teacher and the Physical Education department. Details of the study were presented during an assembly to the pupils and those which were interested were provided with a participant information pack which included a detailed explanation of the study aims, experimental procedures, the potential benefits and risks of taking part and the participants' right to withdraw from the study at any point without any adverse consequence. The pack also contained a participant assent form, a parental consent form and a standard health screen for child volunteers. Participants and their parents were given a 7 day period to ask any questions regarding the study and ensure they had a thorough understanding of what the study entailed. Parents and participants confirmed their desire to be involved in the study by signing and returning these forms to the school contact. A follow up telephone call was also made to the parents to clear up any uncertainties and for them to ask any questions they had regarding the study. Health and safety guidelines established by the Sport and Health Sciences department for all testing procedures were followed at all times by the research team. All members of the research team held valid and in date Disclosure and Baring Service (DBS) certificates. Two DBS checked members of the research team were with participants at all times from the moment they were picked up from school up until they were returned to school. Three members of the research team were

first aid trained and emergency contact details for all participants were also available at all times.

### **3.2 Participants**

Following discussion and authorisation with the teachers, participants were recruited via a presentation during a school assembly to ~400 children aged 13 to 14 years old in the Exmouth, Devon area. Using G\* Power (3.9.1.2) calculation, based upon a power of 0.8, alpha level of 0.05, between-day repeatability of our main outcome (FMD) of  $R=0.78$  (Bond et al., 2016) and a partial eta squared effect size of 0.05, a sample size of 32 participants was the objective for this study. Children were allowed to ask any questions and address any queries they had regarding the study following the assembly. Approximately 100 pupils were interested in the study and were provided with an information pack to take home to their parents/guardians to discuss their involvement in the study. Thirty-one packs were returned with participant assent, parental consent and health screening forms completed and signed. Three of these participants withdrew themselves and a further four were not given authorisation to take part in the study due to school behavioural issues. A further three were withdrawn from the study due to availability or no longer wishing to take part in the study leaving a final sample size of 22. Exclusion criteria included any known cardiometabolic diseases, contraindications to exercise, food allergies or the use of any supplement or medication which can influence blood vessel function or plasma [glucose] or [TAG].

### **3.3 Experimental overview**

Participants visited the laboratory on five separate occasions. One of these visits was a preliminary visit to familiarise participants to the testing equipment and procedures and to obtain anthropometrical and cardiorespiratory fitness

data. The following four visits were experimental visits and all visits were separated by approximately one week, with study completion achieved within six weeks. The study followed a repeated measures design, with counterbalanced trial and the drinks were delivered to the subjects in a double blind manner. Prior to every visit participants were transported by car to the laboratory by two DBS checked members the research team in a 12 hour fasted state and having not completed any structured exercise in the 24 hours prior to arriving at the laboratory. The effects of glucose, fructose and sucrose on vascular function compared to a control trial (water) before and after consumption of a high-fat, high-sugar challenge meal were assessed. A double-blinded repeated measures design was used. The amount of sugar provided was not scaled to body mass, as performed elsewhere (Dengel et al., 2007), but rather made reflective of the sugar load present in commercially available sugar sweetened beverages (60 g of sugar). The sugar was mixed with 300 mL of water. For the control trial participants were given 300 mL of water without any sugar.

### **3.4 Preliminary visit: visit 1**

#### *3.4.1 Anthropometry*

Participants were collected from school and driven to the laboratory for 8:00 am. Body mass (Hampel XWM-150K, Hampel Electronics Co. Taiwan) and stature (Seca stadiometer SEC-225, Seca, Hamburg, Germany) were measured to the nearest 0.1 kg and 0.1 cm, respectively, using standard procedures. From these values BMI was then calculated. Age- and sex-appropriate BMI cut off values (Cole et al., 2000) were used to define whether participants were normal weight, overweight or obese.

Body fat percentage was estimated with BodPod (Body Composition System, Life Measurement Instruments, Concord, California, USA). The air displacement plethysmography technique was used as it a valid measure of adipose tissue in children and adolescents (Winsley et al., 2005). Two measurements of air displacement were performed and if the difference between measurements was more than 150 mL in body volume, a third measurement was taken. An average of the closest two measurements was taken. Equipment was calibrated in accordance with the manufacturer's guidelines with the use of a cylinder of specific volume (49.887 L). Participants wore a swimsuit and swim cap to minimise error of air trapped in clothes and hair and were weighed on the BodPod calibrated digital scale before entering the BodPod chamber. They were then instructed to remain seated at all times and to breathe normally. The body volume mean was obtained using the manufacturer's recommendations (McCrary et al., 1995) and this value was integrated into the calculation of lung volume. Total body fat mass was calculated using the appropriate Siri equation (Siri, 1993). The validity of the air displacement plethysmography technique has been determined in a study which assessed the method against dual energy x-ray absorptiometry in tracking changes in body composition before and after a 16 month weight loss program in adult women (Minderico et al., 2006). The findings determined a significant correlation between the methods of assessment for change in body fat percentage with a  $R$  of 0.76.

Tanner stage measurement of maturity (Tanner, 1962) was determined by the self-assessment of secondary sex characteristics (pubertal hair). This required participants to look at scientific drawings depicting 5 stages of pubertal development (Morris & Udry, 1980) and to identify which stage best describes their own development before sealing this information into an envelope. This



method was used due to its validity in paediatric research ( $R=0.60$ ) (Morris & Udry, 1980) and the simplistic and practical nature of the method.

#### *3.4.2 Peak oxygen uptake and gas exchange threshold*

Peak oxygen uptake ( $\dot{V}O_{2\text{ peak}}$ ) was determined by an incremental ramp test using an electronically braked cycle ergometer (Lode Excalibur Sport, Groningen, the Netherlands).  $\dot{V}O_{2\text{ peak}}$  was used to characterise the sample population to reflect the health status of the participants. Participants maintained a cadence of 70-80 revolutions per minute (rpm) throughout the warm up (unloaded) and during the incremental ramp test. The ramp rate was set at  $25\text{ W min}^{-1}$  in order to elicit fatigue within 8-12 minutes (Buchfuhrer et al., 1983). Exhaustion was characterised as a drop below 60 rpm in cadence for 5 consecutive seconds despite strong verbal encouragement. This was followed by a 5 minute cool down at 20 W.

Pulmonary oxygen uptake ( $\dot{V}O_2$ ) and carbon dioxide production ( $\dot{V}CO_2$ ) were assessed throughout the incremental ramp test using a Medgraphics metabolic system (MedGraphics, UK, Ltd), which was calibrated using standard calibration gas (15.1%  $O_2$ , 5%  $CO_2$ ) and a 3.0 L calibration syringe (Hans Rudolph, USA) prior to each test. Peak power output was defined as the highest work rate achieved during the ramp test. Peak  $\dot{V}O_2$  ( $\dot{V}O_{2\text{ peak}}$ ) was determined as the highest 10 s average in  $\dot{V}O_2$  elicited during the ramp test (Barker et al., 2011). Although the current thesis did not use a supramaximal verification test, the ramp test to exhaustion is shown to provide a 'true' measurement of oxygen uptake in ~90% of adolescents in our laboratory (Sansum et al., 2019; Barker et al., 2011). Due to unavailability, one of the 22 participants was unable to carry out the ramp test. Age- and sex- appropriate  $\dot{V}O_{2\text{ max}}$  cut off points were used to

define low fitness as this is an accurate tool for screening children for clustering of CVD risk factors (Adegboye, 2011). The cut off points were 33.0 and 46.0ml/min/kg for 15-year-old girls and boys, respectively.

### **3.5 Experimental visits: visits 2-5**

#### *3.5.1 Baseline measurements*

A protocol schematic for the experimental visits is provided in Figure 3.5.1.2. Upon arrival to the laboratory at 8:00 am, participants rested for 30 minutes in the supine position in a temperature controlled room (24°C). Following the 30 minutes of rest FMD and microvascular function were simultaneously assessed and a baseline capillary blood sample for plasma [glucose], [lactate], [uric acid] and TAG was taken.

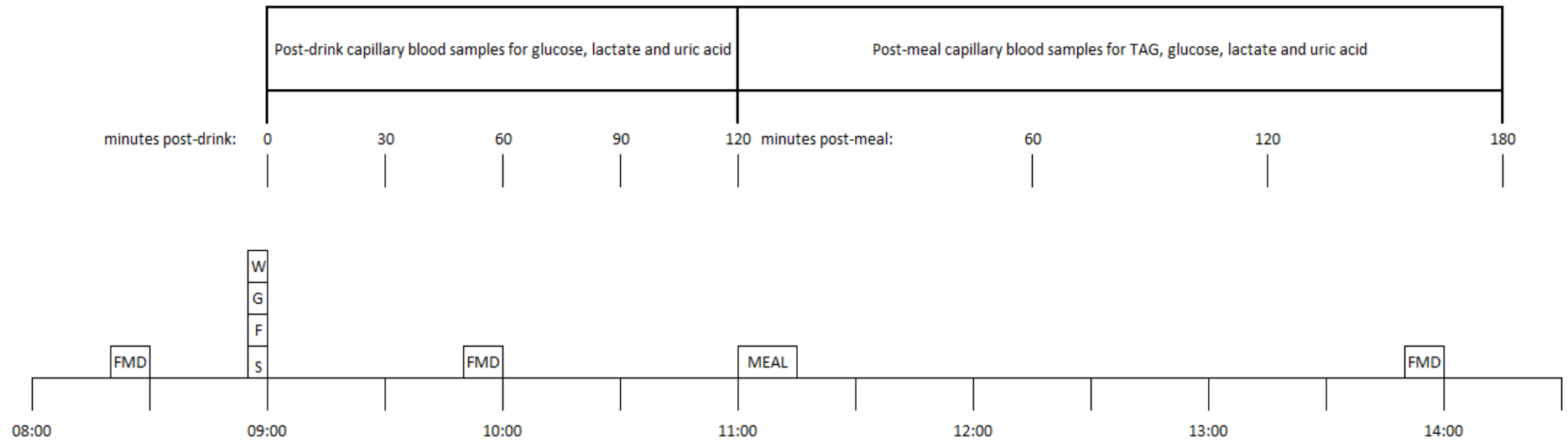
##### *3.5.1.1 Sugary drink consumption*

Participants were then given a sugary drink (glucose, fructose or sucrose) or control (water) to consume in 10 minutes. For the following two hour period blood samples for plasma [glucose], [lactate] and [uric acid] were taken every 30 minutes. The final sample was also assessed for plasma [TAG] to align with the known peak in postprandial lipaemia (Bond et al., 2015b). The simultaneous assessment of FMD and microvascular function was repeated one hour following drink consumption to coincide with the expected peak in hyperglycaemia and uric acid concentrations (Bidwell et al., 2010).

##### *3.5.1.2 High-fat meal challenge*

Participants then consumed a standardised high-fat, high-sugar test meal (60 g fat + 45 g sugar, 1316 kcal) two hours following sugary drink consumption. The meal included pizza (Chicago Town® four cheese pizza, 310 g), ice cream (Essential Waitrose® soft scoop vanilla ice cream, 125 g) and a chocolate

pudding (Cadbury® milk chocolate sticky puds, 95 g). The macronutrient composition of this meal was selected as it has previously been shown to cause an impairment in vascular function in adolescents (Bond et al., 2015d). Following consumption of the meal, capillary blood samples for plasma [glucose], [lactate] and [uric acid] were taken every 60 minutes for the following three hours with the final sample also being assessed for [TAG]. The simultaneous assessment of FMD and microvascular function was performed three hours after consumption of the test meal to coincide with the elevation in plasma [TAG] in the postprandial period (Bond et al., 2015d). FMD assessment was performed for the control and sucrose trials only but microvascular assessment was performed for all trials as sucrose represents the sugar found in commercially available SSBs (Loader et al., 2017) and due to time constraints on the assessor.



**Figure 3.5.1.2.** Protocol schematic. FMD: flow mediated dilation (for control & sucrose trials only) and microvascular ultrasound assessment, W: water, G: glucose, F: fructose, S: sucrose. TAG: triacylglycerol.

### *3.5.2 Assessment of macrovascular function*

Macrovascular function of the brachial artery was assessed via FMD in line with contemporary guidelines (Thijssen et al., 2019; Thijssen et al., 2011) The FMD technique is a widely used, noninvasive ultrasound technique which examines the change in brachial artery diameter to an increase in blood flow (Meyer et al., 2006). The increased blood flow is stimulated following temporary limb occlusion for a period of ~5 minutes (Ras et al., 2013). To ensure that FMD is NO-dependent, to reflect NO-mediated vascular function, the cuff is positioned distal to the ultrasound probe and inflated for a period of 5 minutes (Thijssen et al., 2019; Thijssen et al., 2011). A strong body of evidence indicates that FMD independently predicts future cardiovascular events in populations at risk of CVD (Inaba et al., 2010; Ras et al., 2013; Matsuzawa et al., 2015).

Furthermore, a study which assessed FMD in coronary artery disease patients found that FMD explained 62% of the variance in coronary artery function (Takase et al., 1998). It was also determined that the vasodilator response of the coronary and brachial arteries when administered with acetylcholine were closely related in individuals with and without coronary artery disease (Takase et al., 2005). These findings suggest that FMD is an important marker of endothelial function, indicative of vascular health beyond the brachial artery, and can be readily applied for paediatric use as it is non-invasive.

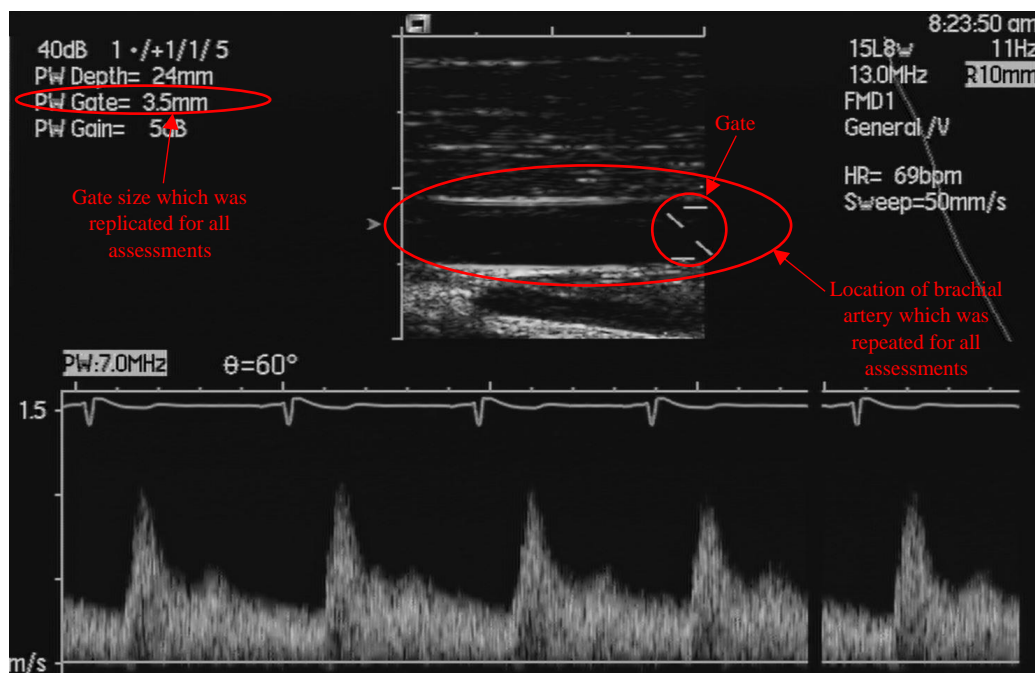
FMD was expressed as the percentage increase from baseline diameter to peak diameter. This method of assessment of brachial artery vasodilation has come under scrutiny due the dependency of this outcome on baseline diameter (Atkinson & Batterham, 2012; Atkinson & Batterham, 2013; Atkinson et al., 2009). It has been shown that participants with a wider baseline arterial

diameter will have a lower FMD statistic than those with a lower baseline diameter as FMD is inversely correlated with baseline diameter (Thijssen et al., 2008; Celermajer et al., 1992). Although, this ratio-scaling method of assessment for FMD has been questioned due to the influence of baseline diameter not being adjusted for (Packard & Boardman, 1999) it has been shown to independently predict future CVD events (Brevetti et al., 2003; Gokce et al., 2002; Gokce et al., 2003; Meyer et al., 2005) whereas the allometric-scaling method of assessment for FMD does not (Atkinson & Batterham, 2015).

The imposed shear stimulus also effects the magnitude of change in artery diameter (Pyke et al., 2004). This is influenced by many factors and has a high variability between individuals (Joannides et al., 2002; Mitchell et al., 2004; Pyke et al., 2004). If shear and FMD are related FMD is required to be normalised for shear in order to determine whether low FMD values are due to an unhealthy endothelium or due to low shear (Harris et al., 2010; Pyke & Tschakovsky, 2007; Thijssen et al., 2011). It has been reported that there is no relationship between FMD and shear in paediatric populations where it was found that shear explained only 2% of the variance in FMD (Thijssen et al., 2009). FMD and  $SR_{AUC}$  data obtained for this thesis determined that there was no relationship between these two outcomes ( $R=0.12$ ,  $P=0.48$ ). Despite this, it is recommended by current guidelines to report the shear stimulus although it is inappropriate to normalise FMD for shear if the variables are not related (Thijssen et al., 2011).

The diameter of the brachial artery was measured using high resolution ultrasonography (Sequoia 512, Acuson, Siemens Corp, Aspen, USA) in duplex mode with a 13 MHz linear array transducer. Once the image of the luminal-arterial wall was optimised the transducer was clamped in place. B mode

images of the brachial artery in longitudinal section were captured at a reproducible point in the upper arm. Doppler velocity was assessed using an insonation angle of  $60^\circ$  with the gate as wide as possible without making contact with the vessel walls (Thijssen et al., 2019; Thijssen et al., 2011). The gate size was noted and scanning location was marked using a pen for each participant and replicated for all assessments as demonstrated in Figure 3.5.2.



**Figure 3.5.2.** Flow-mediated dilation ultrasound scan of brachial artery. PW Gate: size of gate in mm which was repeated for all assessments for that participant.

Baseline diameter of the artery was assessed for 60 s followed by the rapid inflation of a pneumatic cuff (Hokanson, Bellevue, USA) to 220 mmHg. The cuff remained inflated for 5 minutes before rapid ( $<0.3$  sec) deflation and during this period arterial diameter and blood flow were not assessed. Arterial diameter and blood flow was measured for a further 3 minutes following occlusion. During cuff occlusion arterial diameter and blood flow was not measured. The primary investigator performed all FMD analyses and was blinded to the trial. Arterial

diameter was determined during end diastole using validated ECG-gating software ( $R=1.0$ ) (Medical Imaging Applications LLC, Coralville, USA) (Mancini et al., 2002). Diameter change was determined using the equation as described in Equation 1 and shear rate using the equation as described in Equation 2 for the period between cuff deflation and peak arterial diameter (Harris et al., 2010). The total shear stimulus ( $SR_{AUC}$ ) was determined as the area under the shear curve versus time from the moment the cuff was released until diameter peak was reached (Pyke and Tschakovsky, 2007).

$$\text{Diameter change (\%)} = ((\text{Peak diameter}/\text{Baseline diameter}) \times 100) - 100$$

**Equation 1.** Calculation of diameter change

$$\text{Shear rate} = (8 \times \text{mean blood velocity})/\text{artery diameter}$$

**Equation 2.** Calculation of shear rate

### *3.5.3 Assessment of microvascular function*

Microvascular function of the cutaneous circulation was assessed throughout the FMD protocol in both chapters via the Doppler flowmetry (LDF) technique (MoorVMS-LDF, moor instruments Ltd, UK). Two low power laser probes were attached onto a reproducible point of the distal third of the left forearm using moulded flexible sockets and secured adhesive discs. When placing the probes freckles, scars and hairs were avoided and the area of placement was marked with a pen for reproducibility. The assessor drew around each of these probes after the first scan to best replicate their location to minimise error. A red laser beam (785 nm) was used at a power of 2.5 mW for the assessment of cutaneous blood flow using the Doppler principle. Red blood cell velocity and concentration was then used to calculate flux in perfusion units (PU) by the Moor Instruments Vascular Monitoring System. Raw microvascular data for both



probes were visually inspected and the data across the 2 probes were averaged prior to further handling. Data was then converted into 1 second averages and baseline microvascular data was assessed and averaged for the first 60 s of the FMD protocol prior to 5 min of cuff occlusion. The peak value was taken as the highest value obtained following cuff deflation. Peak reactive hyperaemia (PRH) was determined using the highest value following cuff deflation and expressing this as a percentage of baseline perfusion. The total hyperaemic response (TRH) was expressed as described in Equation 2. When determined in this manner, the TRH outcome does not reflect an NO-dependent pathway (Wong et al., 2003).

Total hyperaemic response = Area under the post-occlusive curve – baseline  
blood flow (% maximal cutaneous vascular conductance) x time taken for  
reactive hyperaemia to return to baseline

**Equation 2.** Total hyperaemic response (TRH) (Wong et al., 2003).

### **3.6 Bloods analyses**

During the experimental visits fingertip capillary blood samples were taken before and 30, 60, 90 and 120 minutes after consumption of test drinks using a safety lancet (Sarstedt Ltd., Leicester, UK) for analysis of plasma [glucose], [lactate] and [uric acid]. The first and final blood sample was also analysed for [TAG]. This was repeated at 60, 120 and 180 minutes following consumption of the standardised test meal for assessment of [glucose], [lactate] and [uric acid]. The final blood sample (180 minutes post-meal) was assessed for [glucose], [lactate], [uric acid] and [TAG]. Each blood sample (<1 mL) was collected into heparin coated microvette tubes (CB 300 tubes, Sarstedt Ltd., Leicester, UK)

and immediately centrifuged at 13000 g for ~15 minutes at 4°C. Plasma was then stored at -80°C or analysed immediately for [glucose] and [lactate] simultaneously (YSI 2300 Stat Plus Glucose analyser, Yellow Springs, OH, USA). [TAG] was assessed in capillary whole blood in line with the manufacturer's instructions (CardioChek; Polymer Technology Systems). [Uric acid] was assessed using whole blood from a single sample using a portable uric acid meter for which the manufacturer's reported a correlation coefficient of 0.97 for the reliability of the testing meter against an accepted laboratory analyser (UASure, Apex Biotechnology Corp., Hsinchu, Taiwan).

### **3.7 Statistical analyses**

All statistical analyses for the current thesis were performed using SPSS (version 25.0, Chicago, USA) other than area under the curve (AUC) analysis which was performed on GraphPad and calculated area using the trapezium rule (Prism 8, GraphPad software, San Diego, California, USA). The pre-drink concentration was used as baseline and AUC was determined as the total area. All data are presented as mean  $\pm$  standard deviation. Comparisons between means for the experimental chapter were also expressed as 95% confidence intervals (95% CI) in accordance with current recommendations (Hopkins et al., 2009). Standardised effect sizes (ES) were determined as the differences in means divided by the pooled standard deviation. This is due to recent criticisms that the null hypothesis is always false as all effects are statistically significant if there is a large enough sample size and that the null hypothesis approach inadequately deals with the real-world importance of an effect (Hopkins et al., 2009). This was used to assess whether the mean change in a variable was meaningful using the thresholds recommended by Cohen (1988): small (0.2), moderate (0.5) and large (0.8). Effect sizes for the repeated measures ANOVAs

used in the experimental chapter were presented as partial eta squared ( $\eta_p^2$ ), and interpreted as small ( $<0.06$ ), moderate (0.06 to 0.14) and large ( $\leq 0.14$ ).

## Chapter 4: Reliability Chapter

### 4.1 Introduction

Atherosclerosis is the underlying disease process which precedes subsequent CVD and progresses throughout life (Ross, 1999; McGill & McMahan, 2003).

The early atherosclerotic manifestations which precede overt CVD occur during childhood (Stary, 1989), thus the ability to non-invasively detect the originating manifestations of the atherosclerotic process during the earlier years is a matter of importance for the prevention of CVD (Celermajer et al., 1992). It is important to quantify the reliability of these outcomes to determine whether they are suitable for use in future interventions with this population and can also inform future power calculations.

The primary stage of the atherosclerotic process is endothelial dysfunction which precedes subsequent arterial damage (Tounian et al., 2001; Meyer et al., 2006). The FMD technique has become the most widely used method of assessment for endothelial function in the macrovasculature. This technique uses high resolution ultrasound imaging during a protocol in which brachial artery vasodilation is determined before and after 5 minutes of forearm cuff occlusion to quantify brachial artery function (Bond et al., 2016). Celermajer et al. (1992) developed the FMD technique and with it identified that children and adolescents present with CVD risk factors demonstrated a lower FMD than healthy controls. Paediatric research has since used this technique to measure endothelial function in adolescents and has also used this method to assess within- and between-day endothelial function in adolescents in interventional and observational studies (Bond et al., 2015b; Hopkins et al., 2012).

The earliest changes in the atherosclerotic process may occur in the capillary and arteriole beds and not at the macrovascular level (Pinkney et al., 1997).

Furthermore, microvascular function has been shown to predict future cardiovascular events when FMD has not (Anderson et al., 2011). There has been little association between FMD and post-occlusive reactive hyperaemia (microvascular function) in adults in the literature ( $R \leq 0.09$ ,  $P > 0.05$ ) (Shamim-Uzzaman et al., 2002; Dhindsa et al., 2008). Therefore, microvascular function cannot be inferred from FMD status and the two can be assessed simultaneously. A method for which this is done is by measuring the hyperaemic response of the peripheral vascular endothelium (cutaneous circulation of the microvasculature in the forearm) during the FMD protocol following cuff occlusion (Bond et al., 2016). Microvascular function has been determined by measuring cutaneous perfusion in response to forearm cuff occlusion in adolescents (Bond et al., 2016). However, this study used laser Doppler imaging whereas the current study aims to determine the reliability of the laser Doppler flowmetry technique which has been used to assess microvascular function in the forearm of adults (Ozbebit et al., 2004; Farkas et al., 2004). The reliability of this technique has been assessed in adults (Herron et al., 2015) but has not been assessed in adolescents. Therefore, this study aims to determine the reliability of this technique in adolescents to distinguish whether this technique is suitable for use in Chapter 5.

These assessments are usually performed separately but the between- and within-day reliability of simultaneously performing assessments of FMD and microvascular function in adolescents has been examined (Bond et al., 2016). The researchers of this recent study concluded that a single protocol can be used to reliably and non-invasively assess these outcomes in line with current guidelines. However, adherence to guidelines for these techniques does not determine reproducibility and operation of the FMD technique is operator

dependent (Thijssen et al., 2019). Additionally, the laser Doppler flowmetry technique has been considered poorly reproducible when guidelines are not strictly followed (Cracowski et al., 2006) (see section 2.5). This study aimed to determine the reliability of the laser Doppler flowmetry technique for assessment of microvascular function using two laser probes. Therefore, the current study aims to compare the reliability of the data collected as part of this thesis against known reliability data to determine whether the operator is reliable in assessing FMD and whether the laser Doppler flowmetry technique is reliable for assessment of microvascular function in adolescents.

## **4.2 Methods**

### ***4.2.1 Participants***

The data in this study was collected from the experimental study in Chapter 5. Data was collected from twenty-two healthy adolescents (age:  $14.3 \pm 0.4$  years, height:  $1.64 \pm 0.08$  m, mass:  $55.1 \pm 11.0$  kg).

### ***4.2.2 Study protocol***

Participants visited the laboratory on 4 separate occasions to perform the experimental trial on both occasions as outlined in Chapter 5. These visits occurred over the course of a 6 week period. Participants arrived at the laboratory following a 12 hour overnight fast ready to begin at 8:00 am. Participants were rested in a darkened, temperature-controlled ( $24^{\circ}\text{C}$ ) room for 5-10 minutes before the assessment of macrovascular (FMD) and microvascular function which were performed simultaneously. For the assessment of within-day reliability participants consumed 300 mL of water and the test was repeated 1 hour after consumption. During the 1 hour period participants remained sedentary in the laboratory.

### **4.2.3 Assessment of vascular function**

For FMD and microvascular protocol see 3.5.2 and 3.5.3. Ten of the twenty-two participants were assessed for FMD due to time constraints and all twenty-two were assessed for microvascular function.

### **4.2.4 Statistical analyses**

A mixed model analysis of variance (ANOVA) was used to analyse FMD and microvascular function with visit (between-day) or assessment (within-day) as the main effects. The reproducibility of these outcomes were expressed by using the typical error, typical error expressed as a coefficient of variation (CV) and Pearson's correlation coefficient ( $r$ ) for within-day assessment and intraclass correlation coefficient for between-day assessment (Hopkins, 2000). The typical error accounts for the random variation in an individual's values on repeated testing and the coefficient of variation is used to express typical error as a percentage of its respective mean to account for the large variability of values (Hopkins, 2000). The Pearson's correlation is appropriate for assessing the relationship between 2 trials and the intraclass correlation coefficient is unbiased for any sample size and is the most sensible approach to determine the correlation for more than two trials (Hopkins, 2000). FMD was not normalised for  $SR_{AUC}$  as they were not related ( $R=0.12$ ,  $P=0.48$ ) which is consistent with previous paediatric data (Bond et al., 2016; Hopkins et al., 2015). Many researchers publish FMD/ $SR_{AUC}$  data, so this aids comparison across the literature (Bond et al., 2016; Hopkins et al., 2015). Statistical significance was inferred at  $P<0.05$ .

## **4.3 Results**

### **4.3.1 Within-day reliability**

No significant differences were apparent between the means for assessments 1 and 2 for all outcomes (Table 4.3.1). The within-day typical error was 1.07 for percentage change in FMD (FMD), 1.25 for PRH and 1.34 for TRH. The within-day CV was 7.2% for FMD, 24.5% for PRH and 33.7% for TRH. The Pearson's correlation was 0.93 for FMD, 0.18 for PRH and 0.37 for TRH.



**Table 4.3.1. Within-day reproducibility of novel CVD outcomes**

	Assessment 1	Assessment 2	Change in mean	<i>P</i> value	Typical error	Typical error as CV (%)	<i>R</i>
Baseline FMD (mm)	3.03±0.33	2.97±0.36	-0.06	0.13	1.03	2.9	0.95
Peak FMD (mm)	3.32±0.36	3.25±0.41	-0.07	0.10	1.03	2.8	0.96
Ratio-scaled FMD (%)	9.7±2.3	9.5±2.4	-0.2	0.47	1.07	7.2	0.93
SR <sub>AUC</sub>	88054±36912	71069±18442	-16984	0.12	1.24	24.4	0.68
Baseline PRH (AU)	26±5	24±8	-2	0.37	1.32	32.5	0.20
Peak PRH (AU)	121±21	115±39	-6	0.46	1.27	27.3	0.44
PRH (%)	477±82	497±141	20	0.55	1.25	24.5	0.18
PRH <sub>t</sub> (s)	27±13	26±10	-1	0.86	1.77	77.5	-0.01
TRH (AU)	11512±2924	11043±4319	-469	0.63	1.34	33.7	0.37

FMD, flow-mediated dilation. SR<sub>AUC</sub>, area under the postocclusive curve until peak dilation for shear rate. PRH, peak reactive hyperaemia. PRH<sub>t</sub>, time taken to achieve peak reactive hyperaemia. TRH, total reactive hyperaemia. CV, coefficient of variation. *R*, Pearson's correlation.

### **4.3.2 Between-day reliability**

No significant differences were apparent between the means for assessments 1, 2, 3 and 4 for all outcomes (Table 4.3.2). The between-day typical error was 1.08 for FMD, 1.26 for PRH and 1.33 for TRH. The between-day CV was 8.0% for FMD, 26.1% for PRH and 32.7% for TRH. The between-day ICC was 0.96 for FMD, 0.03 for PRH and 0.49 for TRH.

**Table 4.3.2. Between-day reproducibility of novel CVD outcomes.**

	Assessment 1	Assessment 2	Assessment 3	Assessment 4	<i>P</i> value	Typical error	Typical error as CV (%)	Intraclass correlation coefficient (ICC)
Baseline FMD (mm)	3.03±0.33	3.04±0.29	2.99±0.30	3.06±0.33	0.41	1.04	3.7	0.88
Peak FMD (mm)	3.32±0.36	3.34±0.30	3.27±0.32	3.35±0.38	0.44	1.04	3.8	0.87
Ratio-scaled FMD (%)	9.7±2.3	9.7±2.7	9.6±3.0	9.8±2.3	0.73	1.08	8.0	0.96
SR <sub>AUC</sub>	82858±38850	77268±29947	87156±44435	91555±36298	0.82	1.43	42.6	0.47
Baseline PRH (AU)	26±5	26±10	24±9	26±9	0.83	1.31	31.2	0.14
Peak PRH (AU)	121±21	117±29	119±40	115±24	0.92	1.28	27.9	0.40
PRH (%)	476.50±82.13	477.45±105.61	502.53±137.98	467.55±143.27	0.83	1.26	26.1	0.03
PRH <sub>t</sub> (s)	26.68±12.77	28.18±13.33	25.36±13.62	23.68±12.12	0.60	1.70	69.6	-0.07
TRH (AU)	11512±2924	11514±3714	11215±4563	111137±2848	0.96	1.33	32.7	0.49

FMD, flow-mediated dilation. SR<sub>AUC</sub>, area under the postocclusive curve until peak dilation for shear rate. PRH, peak reactive hyperaemia. PRH<sub>t</sub>, time taken to achieve peak reactive hyperaemia. TRH, total reactive hyperaemia. CV, coefficient of variation. ICC, Intraclass correlation coefficient.

#### 4.4 Discussion

The key findings of this study were that the within-day CV for the FMD outcome was 7.2% and was 24.5 and 33.7% for PRH and TRH, respectively. The between-day CV for FMD was 8.0% and 26.1 and 32.7% for PRH and TRH, respectively. Early guidelines suggested that a CV of 20-30% was appropriate for the reproducibility of the FMD outcome (Corretti et al., 2002). FMD analysis software has developed since this publication, resulting in an almost 4-fold improvement of the reproducibility of the FMD technique (Woodman et al., 2001). Accordingly, dedicated analysis software is now recommended in current guidelines for the FMD protocol (Thijssen et al., 2019; Thijssen et al., 2011; Harris et al., 2010) after the initial findings by Corretti et al. (2002).

There is only one known paediatric study which has assessed both the within-day and between-day reproducibility of the FMD protocol (Bond et al., 2016). The between-day variability of the FMD outcome in this study replicates early findings from our lab (Bond et al., 2016) which used an identical setup and reported a CV of 10.6%. The within-day variability of the FMD outcome in this study (8.0%) is also consistent with the CV of 5.1% reported in Bond et al. (2016) and compares favourably to adult data which reports a CV of 5.8-14.0% (Meirelles et al., 2007; Donald et al., 2008; Ghiadoni et al., 2012; Onkelinx et al., 2012; Craiem et al., 2007; Beux et al., 2001).

The between-day CV of 8% for the FMD outcome reported in this study is also consistent with the ranges presented by previous studies in adults (9.6-16.1%) (Meirelles et al., 2007; Donald et al., 2008; Ghiadoni et al., 2012; Onkelinx et al., 2012; Craiem et al., 2007).

By following standardised protocols (Thijssen et al., 2019; Thijssen et al., 2011), known sources of error were minimised in the current study to minimise known protocol- and environmental-based confounders of the FMD outcome, and best isolate the physiological variability of macro- and micro-vascular reactivity. The findings represent good reproducibility for the FMD outcome and it is thought that this is due to following the standardised protocols. All participants were free from overt cardiometabolic disease and all scans were assessed by the same operator. The CV of 7-8% in the current study could be as a result of measurement error as the operator in this study was newly trained at performing the method. However, it is more likely that this could be due to the small sample size of 10 participants in the current study whereas the previous paediatric reproducibility study for the FMD technique used a sample size of 40 and reported lower CVs for within-day and between-day reproducibility (Bond et al., 2016).

The  $SR_{AUC}$  outcome demonstrated poorer reproducibility in this study with both a within- and between-day CV of 24.4 and 42.6%, respectively, exceeding the values reported by Bond et al. (2016) of 12.0 and 29.6%, respectively. These differences are most likely attributed to the difference in sample sizes used in the current study and Bond et al. (2016). The current study and previous paediatric research has failed to demonstrate a meaningful relationship between FMD and  $SR_{AUC}$  (Bond et al., 2016; Hopkins et al., 2015; Thijssen et al., 2009). This could be as a result of poor variability of the  $SR_{AUC}$  outcome which renders such a relationship difficult to observe. Normalising FMD for  $SR_{AUC}$  would therefore be inappropriate and would result in poorer reliability within the data for within-day and between-day variation which suggests there is not a strong argument for expressing FMD in this manner in a paediatric

population. FMD/SR<sub>AUC</sub> in the current study demonstrated poor reproducibility with a CV of 23.3 and 38.6% for within-day and between-day, respectively. These exceed the values reported by Bond et al. (2016) of 12.0 and 25.7% for within-day and between-day CV, respectively. Unless a meaningful relationship is observed between these two outcomes, the FMD/SR<sub>AUC</sub> outcome should not be included in analysis in paediatric population due to the poor reproducibility of this outcome in both these studies.

This study also assessed microvascular function during the FMD protocol, which has been adopted previously (Bond et al., 2016). To minimise for error for the microvascular assessment all participants were acclimatised to a temperature controlled room as temperature has been demonstrated to affect endothelial function (Cracowski et al., 2006). The CV for both within-day and between-day TRH in the current study was consistent with the CV of 25.0% in Wong et al. (2003) and the within-day CV of 29.9% and between-day CV of 23.1% reported by Bond et al. (2016). Although the within-day and between-day CV for PRH in the current study exceeds the values of 11.0 and 13.3% reported in Bond et al. (2016), the between-day CV of the current study was able to replicate the findings of another study which assessed post-occlusive reactive hyperaemia using laser Doppler flowmetry to determine microvascular function (CV of 38.0%) (Roustit et al., 2010). Thus, the variability in the microvascular data between the current study and others could be due to the method of assessment. Bond et al. (2016) used a laser Doppler perfusion imager whereas the current study and Roustit et al. (2010) used laser Doppler flowmetry. As laser Doppler imaging uses a laser scanner rather than the probe used in the laser Doppler flowmetry technique thus the difference between these methods of assessment could explain the differences in reproducibility. The variability for

between-day PRH and TRH could also be attributed to the placement of the laser probes. Probe location was marked prior to the first scan of each visit however was not repeated between-days. It has been determined that the poor reproducibility often seen for the laser Doppler flowmetry technique is attributed to non-standardisation of the placement site (Cracowski et al., 2006). The current study however, does show that the simultaneous assessment of PRH and TRH during the FMD protocol is possible using laser Doppler flowmetry. Researchers may wish to consider this increased variability when performing power calculations for sample sizes. If a sample size for a future study using this technique is too small it may struggle to report a significant effect when in reality there is one. Further research is required to assess the reproducibility of the laser Doppler flowmetry technique in assessing cutaneous perfusion during post-occlusive reactive hyperaemia in order to determine boundaries of reproducibility for PRH and TRH when assessed in this manner.

A limitation of this study was that 22 participants were used as opposed to the 32 recommended by the G\*Power calculation. Additionally, only 10 participants were assessed for FMD. This could potentially affect our ability to detect true change in FMD, PRH, TRH and microvascular function in the experimental study in Chapter 5 due to wider variation in the data.

The current study aimed to distinguish the within-day and between-day reproducibility of FMD and laser Doppler flowmetry for the assessment of macro- and micro-vascular function, respectively. The findings for FMD are consistent with the literature and are reliable. Our findings for microvascular reproducibility are consistent with previous research which has assessed the reproducibility of laser Doppler flowmetry.

## **Chapter 5: The Vascular and Metabolic Responses to Consumption of Fructose, Sucrose and Glucose in the Acute and Postprandial Phase**

### **5.1 Introduction**

The atherosclerotic process which precedes subsequent CVD originates in childhood and progresses throughout life (Ross, 1999; McGill & McMahan, 2003). Paediatric research has shown that the progression of atherosclerosis in childhood and into later life is associated with the clustering of CVD risk factors (Farah et al., 2014) and the clustering of CVD risk factors tracks into adulthood (Bao et al., 1994; Katzmarzyk et al., 2001). It has been suggested that introducing interventions in the first two decades of life to modify CVD risk factors are important for the prevention of CVD in later life (McGill et al., 2000a). As a result, it is important to tackle CVD risk in these early years as it may delay the onset of atherosclerosis and CVD.

The primary stage of atherosclerosis is endothelial dysfunction (Tounian et al., 2001; Meyer et al., 2006). It has been shown that asymptomatic children and adolescents with CVD risk factors display endothelial dysfunction when compared to healthy controls (Celermajer et al., 1992). As endothelial dysfunction is prerequisite for subsequent atherosclerosis (Juonala et al., 2004; Tounian et al., 2001; Meyer et al., 2006), it is of importance to prevent endothelial dysfunction in the early years to delay later structural changes to the vessel wall. The FMD ultrasound technique has become the adopted non-invasive method of assessment for endothelial function in vivo (Thijssen et al., 2019), and has an inverse association with CVD risk in adults and children (Ras et al., 2013; Green et al., 2011; Chan et al., 2003; Tounian et al., 2001; Sorensen et al., 1994; Woo et al., 2004).



It has also been shown that microvascular function has predicted future cardiovascular events when FMD has not (Anderson et al., 2011) and that the post-occlusive response in these two vascular beds are unrelated (Shamim-Uzzaman et al., 2002). Unlike FMD, the post-occlusive microvascular response is not NO-mediated (Wong et al., 2003). Thus, assessing microvascular as well as macrovascular function may provide important prognostic information which cannot be obtained from FMD assessment alone.

Current guidelines recommend a limit of <25 g per day of added sugar for young people (Vos et al., 2017) and sugar-sweetened beverage (SSB) consumption is a key contributor to sugar intake. In fact, adolescents in the UK aged 11 to 18 years consume 191 g of SSB per day, equating to 22-28 g of sugar, which is more than any other group (Public Health England, 2018; Malik et al., 2006).

These statistics are worrying as the consumption of SSBs has been associated with an increase in CVD risk factors in youth including dyslipidaemia, insulin resistance, future weight gain and hypertension (Vos et al., 2017; Basu et al., 2013; Ludwig et al., 2001; Chan et al., 2014).

A recent meta-analysis of 39 studies which assessed the influence of acute hyperglycaemia on macro- and micro-vascular function concluded that SSB consumption negatively influences vascular function (Loader et al., 2015). However, studies which investigated the effects of sugar consumption in adolescents have not supported this. These studies used an oral glucose tolerance test (OGTT) to assess the vascular response in adolescents which were either healthy or obese (Dengel et al., 2007) and with type 1 diabetes (Dye et al., 2012) and found no reduction in vascular function and Dengel et al.

(2007) reported a ~2% increase in FMD. However, these studies used an OGTT which is not reflective of commercially available SSBs which contain sucrose (Loader et al., 2017). Sucrose is made up of equal parts of glucose and fructose and these sugars are metabolised differently (Lustig, 2012). Although glucose can be metabolised at multiple sites, fructose can only be metabolised by the liver and this process is insulin independent (Lustig, 2012). Epidemiological studies have demonstrated that consumption of fructose and sucrose is associated with cardiovascular risk factors including obesity, elevated lipid production, high blood pressure and uric acid concentrations (Johnson et al., 2009; Bray, 2012; Vartanian, 2007; Bray, 2010; Malik & Hu, 2015). However, the influence of glucose, fructose and sucrose on CVD risk factors have also not been assessed and/or compared in adolescents.

Understanding the acute effects of SSB consumption on vascular and metabolic responses in the fasted state is useful but limited. It has been shown that prior fructose, but not glucose, consumption augments the postprandial lipaemic response to high-fat, high-sugar test meal (Cohen & Schall, 1988) and postprandial lipaemia has been implicated in the progression of CVD (Boquist et al., 1999). Also, endothelial dysfunction has been shown to occur in the presence of elevated [TAG] in the postprandial stage (Bond et al., 2015a). It is suggested that plasma TAG following consumption of a high-fat meal is a better marker of metabolic health and CVD risk than fasting TAG and research suggests non-fasting lipid concentrations in the early years is predictive of CVD related events later in life (Freiberg et al., 2008; Morrison et al., 2009).

Therefore, it is also important to determine whether consumption of the different types of sugars found in SSBs influence postprandial vascular function and lipaemia following a test meal.

Fructose consumption has also been associated with elevated uric acid production (Nakagawa et al., 2006). This may cause a reduction in endothelial function as a result of lowered NO bioavailability and elevated inflammation (Nakagawa et al., 2006; Roglans et al., 2007). Elevated [Uric acid] has been associated with an increased risk in cardiovascular mortality (Lehto et al., 1998) and is also an independent risk factor for myocardial infarction (Krishnan et al., 2006). Data from the Bogalusa Heart Study has also demonstrated that childhood [uric acid] is predictive of diastolic and systolic blood pressure in adulthood (Alper Jr et al., 2005). Recent data has also demonstrated that high concentrations of uric acid causes a reduction in FMD (Zhen & Gui, 2017) and induces endothelial dysfunction in human umbilical vein endothelial cells (Cai et al., 2017).

A primary aim of this study was to identify the effect of consuming different types of sugar found in commercially available SSBs on FMD and microvascular function and compare this to a control trial. This study also aimed to determine the effect of sucrose on macrovascular function. The effects of consumption of these sugars on metabolic function will also be assessed. The secondary aim of this study was to identify whether consumption of these sugars affected these outcomes in the postprandial stage following the consumption of a high-fat, high-sugar test meal. It is hypothesised that consumption of these sugars will impair macro- and micro-vascular function post-drink consumption and will increase plasma concentrations of [glucose], [lactate], [TAG] and [uric acid]. It is also hypothesised that consumption of these sugars will impair macro- and micro-vascular function in the postprandial phase and also increase plasma [TAG] in the postprandial phase.

## **5.2 Methods**

### **5.2.1 Participants**

Twenty two healthy adolescents (Table 5.3) volunteered to participate in this study.

### **5.2.2 Preliminary visit: participant characteristics**

For participant characteristics data see Table 5.3.

#### **5.2.2.1 Anthropometrical measurements**

For methodology regarding body fat percentage, body mass index, maturation and pubertal status see 3.4.1.

#### **5.2.2.4 Assessment of cardiorespiratory fitness**

For assessment of cardiorespiratory fitness see 3.4.2.

### **5.2.3 Visit 2, 3, 4 & 5: Experimental trial**

For experimental trial protocol see 3.5.

#### **5.2.3.1 Assessment of vascular function**

For FMD and microvascular assessment methodology see 3.5.2 and 3.5.3.

FMD was assessed for the same 10 participants in Chapter 4 and assessed influence of CON and SUC only due to time constraints. FMD was not normalised for  $SR_{AUC}$  as they were not related ( $R=0.12$ ,  $P=0.48$ ).

#### **5.2.3.2 Blood sampling and analyses**

For blood sampling and analyses protocol see section 3.6.

### **5.2.4 Statistical analyses**

A repeated measures analysis of variance (ANOVA) was used to determine the main and interaction effects of time and trial for all outcomes.

A one-way ANOVA was used to assess the differences between trials for plasma [glucose], [lactate] and [uric acid] for combined post drink + meal incremental (iAUC) (iAUC-[Glucose], iAUC-[Lactate], iAUC-[Uric Acid] & iAUC-[TAG]) and total (tAUC) (tAUC-[Glucose], tAUC-[Lactate], tAUC-[Uric Acid] & tAUC-[TAG]) area under the curve.

Mauchly's test of sphericity was used to assess homogeneity of variance and the degrees of freedom were adjusted using the Greenhouse-Geisser correction when necessary. Statistical significance was inferred at  $P < 0.05$ .

95% confidence intervals (CI) for the mean difference and effect sizes (ES) were also presented. Effect sizes were determined as the difference in means divided by the pooled standard deviation (i.e. Cohen's  $d$ ) and were included in order to determine the meaningfulness of the mean change in a variable using these thresholds: small (0.2), moderate (0.5) and large (0.8) (Cohen, 1988).

Effect sizes for the ANOVA were presented as partial eta squared ( $\eta_p^2$ ), and interpreted as small ( $< 0.06$ ), moderate (0.06 to 0.14) and large ( $\geq 0.14$ ). Effect sizes were used to provide information about the magnitude of an effect in the absence of any clinically relevant thresholds for endothelial function in adolescents being available. There was no interaction or main effect of sex on any of the outcomes and data was subsequently always pooled for sex (male,  $n=12$ ; female,  $n=10$ ).

### 5.3 Results

**Table 5.3. Participant characteristics.**

Age, y	14.3 ± 0.4
Body mass, kg	55.09 ± 10.96
Stature, m	1.64 ± 0.08
Body fat, %	22.0 ± 9.0
BMI (kg/m <sup>2</sup> )	20.36 ± 3.00
Overweight (n)	2
Obese (n)	0
VO <sub>2peak</sub> , L/min <sup>-1</sup>	2.13 ± 0.60
VO <sub>2peak</sub> , mL/kg <sup>-1</sup> /min <sup>-1</sup>	40.13±9.57
Low fit (n)	7
GET, L/min <sup>-1</sup>	1.20 ± 0.37
Pubertal stage	2 (n=3), 3 (n=2), 4 (n=15), 5 (n=2)

BMI, body mass index. VO<sub>2peak</sub>, maximal oxygen uptake. GET, gas exchange threshold. Data are expressed as mean ± standard deviation or as n=number of participants.

### 5.3.1 FMD

#### ***Baseline diameter of the brachial artery.***

There was a time by trial interaction effect ( $P=0.02$ ) for baseline diameter of the brachial artery. There were no significant differences between any of the trials at baseline ( $P>0.08$ ) and 1 h post ( $P>0.18$ ) for baseline diameter of the brachial artery. Baseline diameter of the brachial artery 3 h post was significantly higher in CON when compared to GLU and FRU ( $P\leq 0.05$ ,  $ES\leq 0.50$ ). There were no significant differences between any of the other trials at 3 h post.

#### ***Peak diameter of the brachial artery.***

There was no effect of trial ( $P=0.90$ ,  $\eta_p^2=0.02$ ) or a time by trial interaction effect ( $P=0.16$ ,  $\eta_p^2=0.15$ ) for peak diameter of the brachial artery. There was an effect of time ( $P=0.02$ ) for peak diameter of the brachial artery. Peak diameter of the brachial artery was significantly greater 3 h post when compared to baseline and 1 h post ( $P=0.02$ ,  $ES\leq 0.22$ ).

#### ***Percentage change in diameter of brachial artery from baseline (FMD).***

There was no effect of trial ( $P=0.07$ ,  $\eta_p^2=0.33$ ) but there was an effect of time ( $P=0.03$ ) for percentage change in FMD. FMD was significantly lower 3 h post when compared to 1 h post ( $P=0.03$ , 95% CI=0.2 to 3.3,  $ES=0.25$ ) (Figure 5.3.1). There were no significant differences for FMD between baseline and 1 h post and between baseline and 3 h post. The time by trial interaction effect approached, but did not achieve, statistical significance ( $P=0.06$ ), however post hoc tests were performed due to the large effect size of this interaction ( $\eta_p^2=0.27$ ).

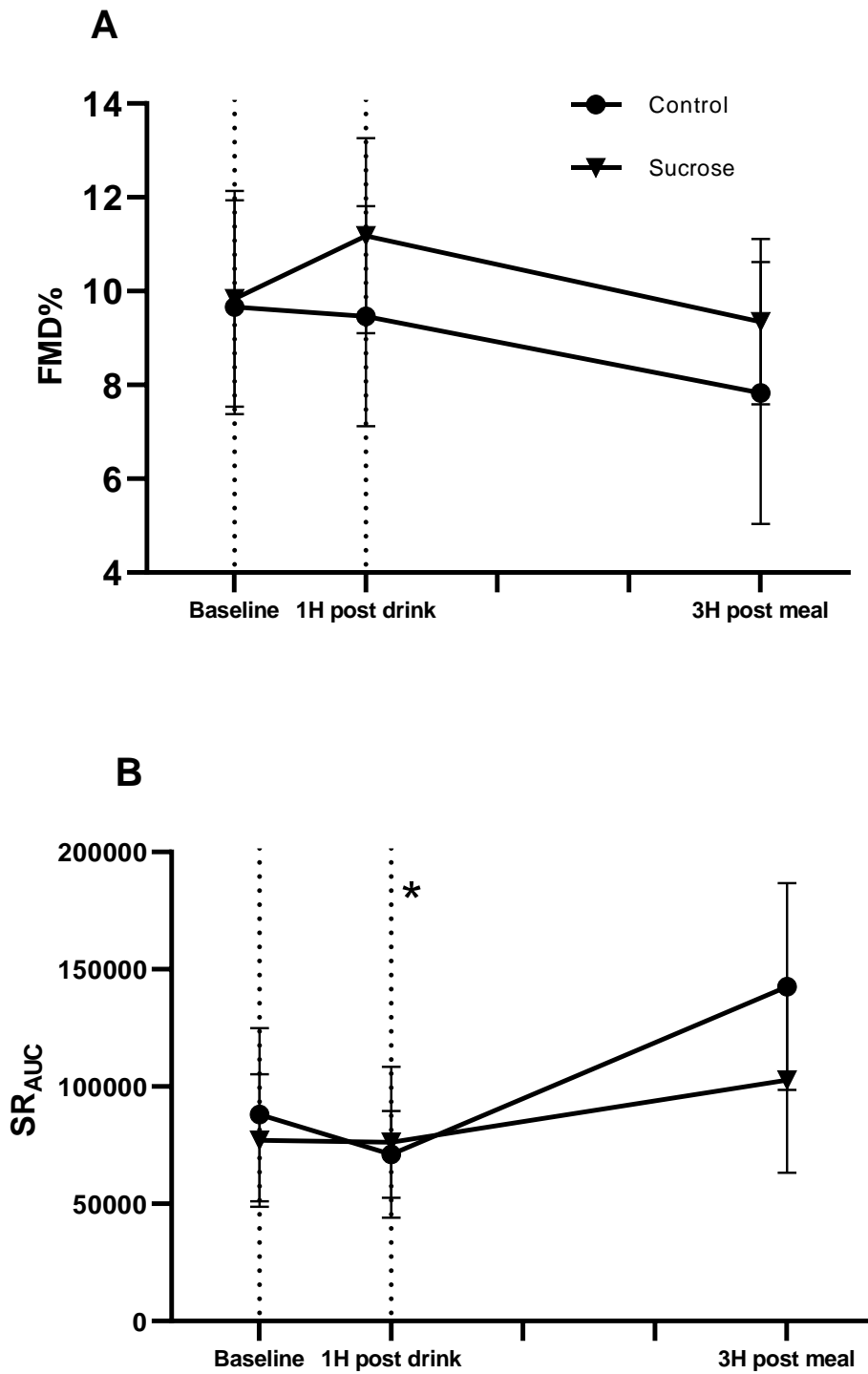
There was no difference between SUC and CON for FMD at baseline ( $P=0.44$ , 95% CI=-0.3 to 0.7,  $ES=0.08$ ). There was a non-significant difference between

SUC and CON 1 h post but there was a medium effect between the trials ( $P=0.06$ , 95% CI=-0.7 to 3.5, ES=0.77). There was a non-significant difference between SUC and CON 3 h post but there was a medium effect between the trials ( $P=0.09$ , 95% CI=-0.3 to 3.3, ES=0.65).

***Shear rate.***

There was a time by trial interaction effect for shear rate ( $P=0.01$ ). There were no significant differences between SUC and CON for shear rate at baseline and 1 h post ( $P\geq 0.47$ ,  $ES\leq 0.32$ ). Shear rate was significantly higher in CON when compared to SUC 3 h post ( $P=0.01$ , 95% CI=15979.3 to 61929.5, ES=0.94).





**Figure 5.3.1.** Percentage change in flow-mediated dilation (FMD%) (A) and SR<sub>AUC</sub> (B) data at baseline, 1 h post drink (1 hour post drink consumption) and 3 h post meal (3 hour post meal consumption). Dashed line represents consumption of drink and meal, respectively. Data are displayed as mean  $\pm$  SD. \* indicates interaction effect.

### 5.3.2 Microvascular function.

**Peak reactive hyperaemia (PRH).** Microvascular outcomes are presented in Figure 5.3.2. There was a time by trial interaction effect ( $P=0.01$ ) for PRH. There were no significant differences between any of the trials for PRH% at baseline ( $P>0.40$ ).

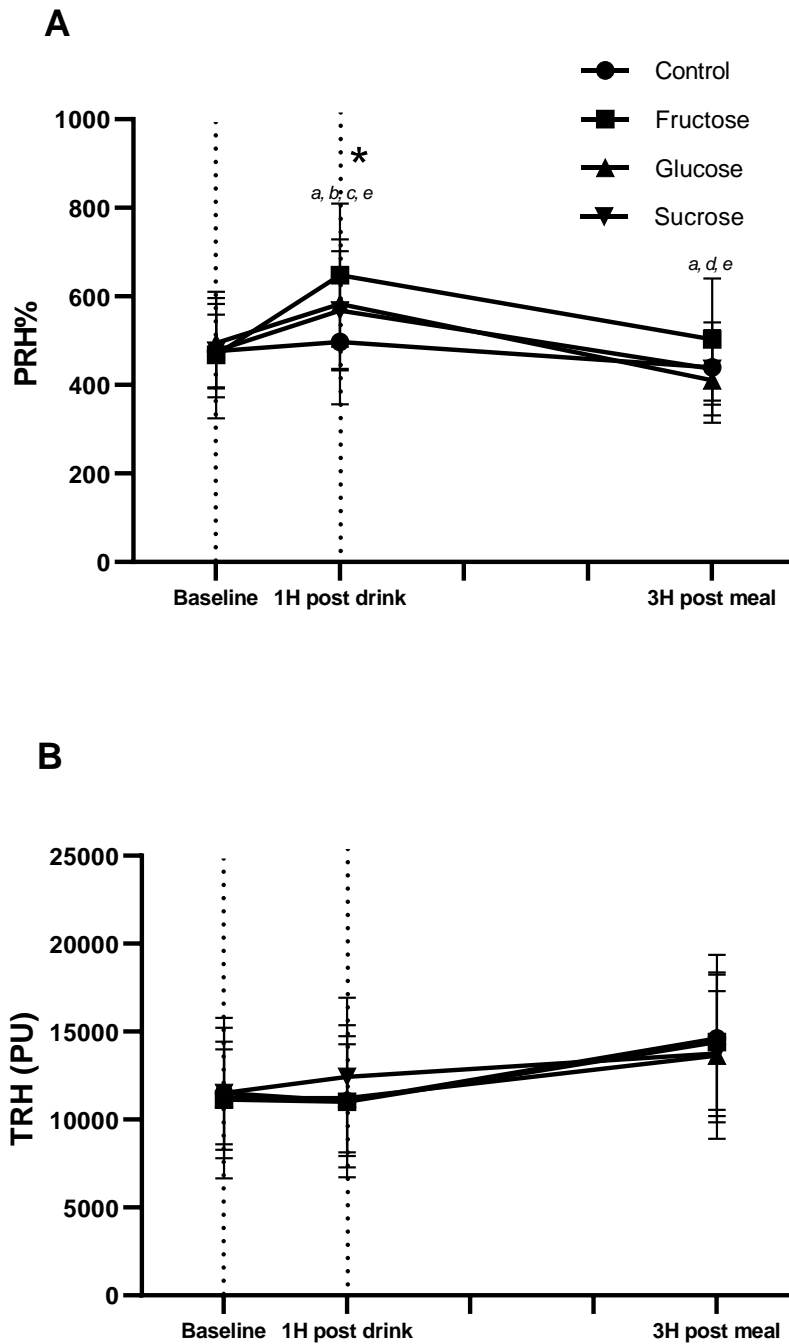
PRH 1 hour post drink consumption (1 h post) was significantly greater in FRU, GLU and SUC ( $P\leq 0.04$ ,  $ES\geq 0.51$ ) when compared to CON. PRH 1 h post was significantly greater in FRU compared to SUC ( $P=0.03$ , 95% CI=7.0 to 153.3,  $ES=0.54$ ). There were no significant differences 1 h post for PRH between FRU and GLU and between GLU and SUC ( $P>0.10$ ).

PRH 3 hour post meal consumption (3 h post) was significantly greater in FRU compared to CON, GLU and SUC ( $P\leq 0.03$ ,  $ES\geq 0.54$ ). There no significant differences 3 h post for PRH between CON, GLU and SUC ( $P>0.20$ ).

PRH was never different from baseline throughout the CON trial ( $P>0.06$ ). PRH for FRU 1 h post was significantly higher when compared to baseline and 3 h post ( $P\leq 0.001$ ,  $ES\geq 0.97$ ) and there was no significant difference between baseline and 3 h post ( $P>0.27$ ). PRH for GLU was significantly higher 1 h post when compared to baseline and 3 h post ( $P\leq 0.03$ ,  $ES\geq 0.70$ ) and was significantly higher at baseline when compared to 3 h post ( $P=0.01$ , 95% CI=25.3 to 142.7,  $ES=0.85$ ). PRH for SUC was significantly higher 1 h post when compared to baseline and 3 h post ( $P\leq 0.01$ ,  $ES\geq 0.74$ ). There was no significant difference between baseline and 3 h post for SUC ( $P>0.16$ ).

**Total reactive hyperaemia (TRH).** There was no interaction effect ( $P=0.57$ ,  $\eta_p^2=0.04$ ), or main effect of trial ( $P=0.84$ ,  $\eta_p^2=0.01$ ) for the TRH (Figure 5.3.2). There was a main effect of time ( $P<0.001$ ). TRH was always greater after the

meal compared to baseline and compared to post-drink consumption ( $P < 0.001$ ,  $ES \geq 0.57$ ).



**Figure 5.3.2.** Percentage change in peak reactive hyperaemia (PRH%) (A) and total reactive hyperaemia (TRH) (B) data at baseline, 1 h post (1 hour post drink consumption) and 3 h post (3 hour post meal consumption). Dotted lines represents consumption of drink and meal, respectively. Data are displayed as mean  $\pm$  SD. \* indicates interaction effect.

P<0.05: a, CON v FRU. b, CON v GLU. c, CON v SUC. d, FRU v GLU. e, FRU v SUC. f, GLU v SUC.

### 5.3.3 Bloods analyses.

#### **Plasma [glucose].**

There was an interaction effect ( $P < 0.001$ ) and a main effect of trial ( $P < 0.001$ ) and time ( $P < 0.001$ ) for [glucose]. Plasma [glucose] was significantly higher at baseline for CON when compared to FRU and SUC ( $P \leq 0.02$ ,  $ES \geq 0.60$ ) (Figure 5.3.3).

Plasma [glucose] 1 h post was significantly higher in GLU when compared to CON, FRU and SUC ( $P < 0.001$ ,  $ES \geq 1.11$ ). Plasma [glucose] 1 h post was significantly higher in SUC when compared to CON ( $P = 0.03$ , 95% CI = 0.1 to 1.0,  $ES = 0.72$ ). There were no significant differences for plasma [glucose] 1 h post between CON v FRU and FRU v SUC ( $P > 0.09$ ).

There were no significant differences in plasma [glucose] 3 h post between any of the trials ( $P > 0.15$ ).

Post drink + meal iAUC-[Glucose] was significantly greater for GLU when compared to CON ( $P < 0.005$ , 95% CI = 50.1 to 258.8,  $ES = 1.12$ ) and FRU ( $P = 0.05$ , 95% CI = 0.6 to 209.4,  $ES = 0.61$ ). Post drink + meal iAUC-[Glucose] was significantly greater in SUC when compared to CON ( $P = 0.02$ , 95% CI = 16.6 to 225.3,  $ES = 0.69$ ). Post drink + meal tAUC-[Glucose] was significantly greater for GLU when compared to CON, FRU and SUC ( $P \leq 0.05$ ,  $ES \geq 0.92$ ). For post drink + meal iAUC-[Glucose] and tAUC-[Glucose] data see Table 5.3.3.

**Plasma [lactate].** There was an interaction effect ( $P < 0.001$ ) and a main effect of trial ( $P < 0.001$ ) and time ( $P < 0.001$ ). There were no significant differences between trials at baseline (Figure 5.3.3).

Plasma [lactate] 1 h post was significantly higher in FRU when compared to CON ( $P < 0.001$ , 95% CI = 1.5 to 1.9,  $ES = 4.27$ ) and GLU ( $P < 0.001$ , 95% CI = 0.8

to 1.3, ES=2.29). Plasma [lactate] was significantly higher 1 h post in SUC when compared to CON and GLU ( $P<0.001$ ,  $ES\geq 1.21$ ). Plasma [lactate] was significantly higher in GLU when compared to CON ( $P<0.001$ , 95% CI=0.5 to 0.9, ES=1.52). There was no significant difference between FRU and SUC ( $P>0.05$ ).

Plasma [lactate] 3 h post was significantly higher in SUC when compared to GLU ( $P=0.01$ , 95% CI=0.0 to 0.3, ES=0.19). There were no significant differences for plasma [lactate] between any of the other trials 3 h post ( $P>0.06$ ,  $ES\leq 0.45$ ).

Post drink + meal iAUC-[Lactate] was significantly greater in FRU when compared to CON ( $P<0.001$ , 95% CI=103.3 to 200.4, ES=2.08), GLU ( $P<0.001$ , 95% CI=82.6 to 179.6, ES=1.66) and SUC ( $P=0.02$ , 95% CI=8.9 to 105.9, ES=0.60). Post drink + meal iAUC-[Lactate] was greater in SUC when compared to CON and GLU ( $P\leq 0.005$ , 95%  $ES\geq 0.84$ ). Post drink + meal tAUC-Lactate was significantly higher in FRU when compared to CON, GLU and SUC ( $P\leq 0.05$ ,  $ES\geq 0.79$ ). Post drink + meal tAUC-Lactate was significantly higher for SUC when compared to CON and GLU ( $P\leq 0.01$ ,  $ES\geq 1.10$ ). For post drink + meal iAUC-[Lactate] and tAUC-[Lactate] data see Table 5.3.3.

**[Uric Acid].** There was an interaction effect ( $P=0.03$ ) and a main effect of trial ( $P=0.01$ ) and time ( $P=0.01$ ) for [uric acid]. There were no significant differences between trials for baseline [uric acid].

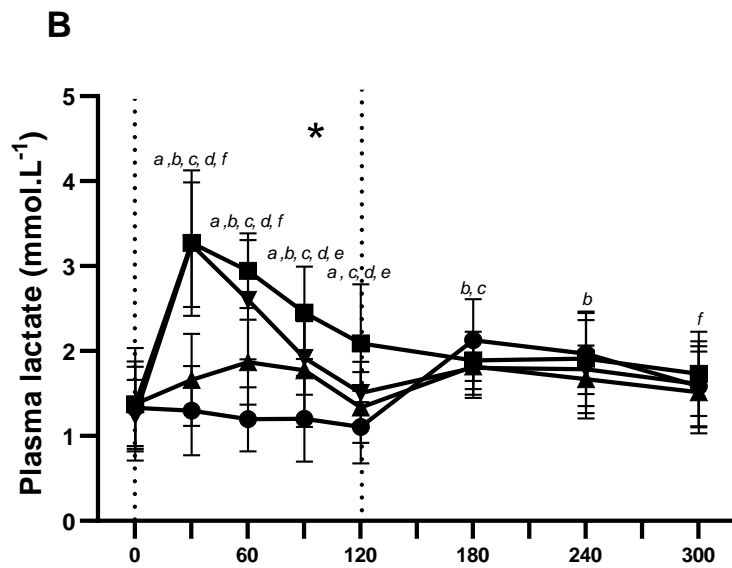
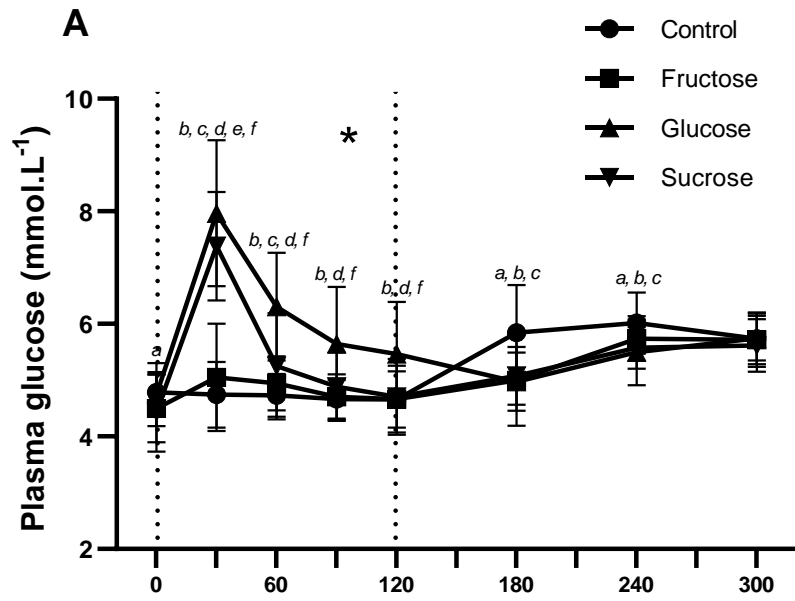
[Uric acid] 1 h post was significantly higher in FRU when compared to CON, GLU and SUC ( $P\leq 0.03$ ,  $ES\geq 0.56$ ) (Figure 5.3.3). There were no significant differences for [uric acid] between any of the other trials 1 h post ( $P>0.30$ ,  $ES\leq 0.22$ ).

There were no significant differences between trials for [uric acid] 3 h post ( $P>0.07$ ,  $ES\leq 0.40$ ).

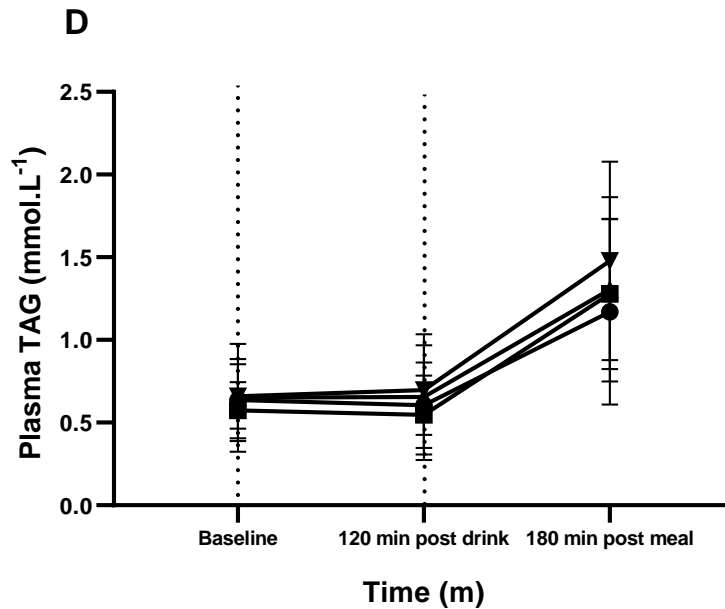
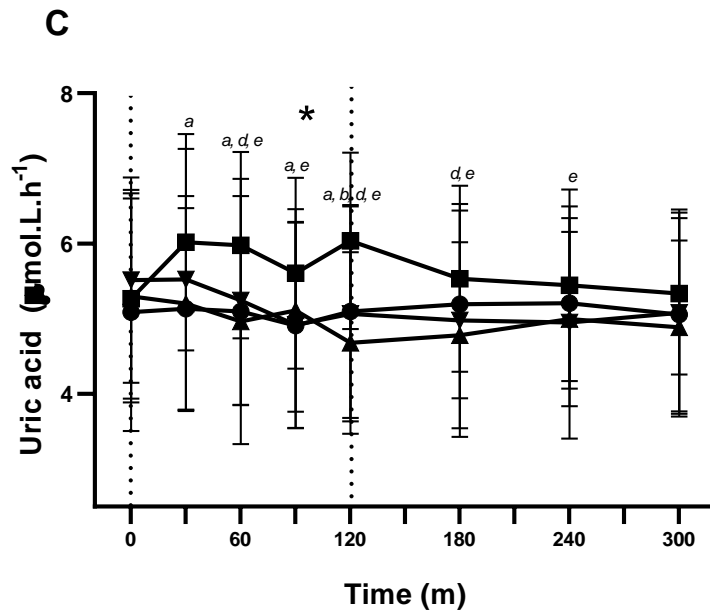
There were no significant differences for post drink + meal iAUC-[Uric Acid] ( $P<0.40$ ) and tAUC-[Uric Acid] ( $P>0.10$ ). For post drink + meal iAUC-[Uric Acid] and tAUC-[Uric Acid] data see Table 5.3.3.

**TAG.** There was no interaction effect ( $P=0.16$ ,  $\eta_p^2=0.07$ ) or main effect of trial ( $P=0.12$ ,  $\eta_p^2=0.09$ ) for plasma [TAG] concentration. There was a main effect of time ( $P<0.001$ ). Plasma [TAG] was always significantly higher 3 h post when compared to baseline and 2 hours post drink consumption ( $P<0.001$ ,  $ES\geq 1.55$ ) (Figure 5.3.3).

There were no significant differences for post drink + meal iAUC-[TAG] ( $P<0.07$ ) and tAUC-[TAG] ( $P<0.10$ ). For post drink + meal iAUC-[TAG] and tAUC-[TAG] data see Table 5.3.3.







**Figure 5.3.3.** Data for plasma [glucose] (A), [lactate] (B), [uric acid] (C) and [triacylglycerol] (D; [TAG]) for each trial. Dotted lines represents consumption of drink and meal, respectively. Data are displayed as mean  $\pm$  SD. \* indicates interaction effect.

$P < 0.05$ : a, CON v FRU. b, CON v GLU. c, CON v SUC. d, FRU v GLU. e, FRU v SUC. f, GLU v SUC.

**Table 5.3.3.** Combined post-drink and meal incremental area under the curve (iAUC) and total area under the curve (tAUC) data for plasma glucose, lactate and uric acid for each trial.

	Control	Fructose	Glucose	Sucrose	Significant differences between trials
iAUC- [Glucose] (mmol.L.5h <sup>-1</sup> )	190.36±83.78 (0.63±0.28 mmol.L.h <sup>-1</sup> )	239.79±170.78 (0.80±0.57 mmol.L.h <sup>-1</sup> )	344.81±175.48 (1.15±0.59 mmol.L.h <sup>-1</sup> )	311.30±232.91 (1.04±0.78 mmol.L.h <sup>-1</sup> )	<i>b, c, d</i>
tAUC- [Glucose] (mmol.L.5h <sup>-1</sup> )	1588.73±92.15 (5.30±0.31 mmol.L.h <sup>-1</sup> )	1533.45±118.75 (5.11±0.40 mmol.L.h <sup>-1</sup> )	1714.82±115.80 (5.72±0.39 mmol.L.h <sup>-1</sup> )	1611.05±110.02 5.37±0.37 mmol.L.h <sup>-1</sup> )	<i>b, d, e, f</i>
iAUC- [Lactate] (mmol.L.5h <sup>-1</sup> )	130.63±56.72 (0.44±0.19 mmol.L.h <sup>-1</sup> )	282.50±86.27 (0.94±0.29 mmol.L.h <sup>-1</sup> )	151.41±71.44 (0.50±0.24 mmol.L.h <sup>-1</sup> )	225.10±102.10 (0.75±0.34 mmol.L.h <sup>-1</sup> )	<i>a, c, d, e, f</i>
tAUC- [Lactate] (mmol.L.5h <sup>-1</sup> )	475.56±98.25 (2.59±0.33 mmol.L.h <sup>-1</sup> )	654.55±94.92 (2.18±0.32 mmol.L.h <sup>-1</sup> )	494.24±73.75 (1.65±0.25 mmol.L.h <sup>-1</sup> )	582.86±87.50 (1.94±0.29 mmol.L.h <sup>-1</sup> )	<i>a, c, d, e, f</i>
iAUC-[Uric Acid] (mmol.L.5h <sup>-1</sup> )	212.04±113.34 (0.71±0.38 mmol.L.h <sup>-1</sup> )	198.07±94.60 (0.66±0.32 mmol.L.h <sup>-1</sup> )	193.44±135.36 (0.64±0.45 mmol.L.h <sup>-1</sup> )	219.88±118.65 (0.73±0.40 mmol.L.h <sup>-1</sup> )	<i>n/a</i>
tAUC-[Uric Acid] (mmol.L.5h <sup>-1</sup> )	1535.25±367.44 (5.12±1.23 mmol.L.h <sup>-1</sup> )	1697.50±328.15 (5.66±1.09 mmol.L.h <sup>-1</sup> )	1481.66±329.63 (4.96±1.10 mmol.L.h <sup>-1</sup> )	1529.20±395.50 (5.10±1.32 mmol.L.h <sup>-1</sup> )	<i>n/a</i>
iAUC-[TAG] (mmol.L.5h <sup>-1</sup> )	57.84±37.59 (0.19±0.13 mmol.L.h <sup>-1</sup> )	73.99±47.20 (0.25±0.16 mmol.L.h <sup>-1</sup> )	81.77±66.58 (0.28±0.22 mmol.L.h <sup>-1</sup> )	85.91±48.11 (0.29±0.16 mmol.L.h <sup>-1</sup> )	<i>n/a</i>
tAUC-[TAG] (mmol.L.5h <sup>-1</sup> )	234.36±94.26 (0.78±0.31 mmol.L.h <sup>-1</sup> )	231.32±72.61 (0.77±0.24 mmol.L.h <sup>-1</sup> )	254.78±110.08 (0.85±0.37 mmol.L.h <sup>-1</sup> )	277.14±94.68 (0.92±0.32 mmol.L.h <sup>-1</sup> )	<i>n/a</i>

All data are expressed as mean ± standard deviation.

P<0.05: *a*, CON v FRU. *b*, CON v GLU. *c*, CON v SUC. *d*, FRU v GLU. *e*, FRU v SUC. *f*, GLU v SUC.

## 5.4 Discussion

This study aimed to distinguish the effects of fructose, sucrose and glucose, sugars found in commercially available SSBs, on FMD, microvascular function and metabolic outcomes in healthy adolescents.

The main finding of this study was that there was no significant influence of 60 g of sucrose consumption on FMD when compared to a control trial. However, with a *P* value of 0.06 and a  $\eta_p^2$  of 0.27, a large effect size, it is suggested that the lack of significance is as a result of a small sample size for FMD ( $n=10$ ).

Therefore, the current study concludes that there was a large but non-significant difference between SUC and CON for FMD following drink consumption. These data indicate that SSB consumption may alter vascular reactivity in adolescents, and this effect can still be observed following a meal.

Our findings for FMD are consistent with Dengel et al. (2007) which evidenced a ~2% increase in FMD following an OGTT in adolescents and a study which demonstrated improved vascular function following glucose and dextrose infusion which induced hyperglycaemia in adolescents (Dye et al., 2012). Our data however contradict those from Loader et al. (2017) which reported an attenuation in FMD in adults following SSB consumption (Loader et al., 2017). As this is the first study which has assessed the effect of sucrose on FMD in adolescents, the mechanisms behind these findings are currently unknown. A more thorough assessment of [insulin] and redox state would be advantageous to provide an insight into these findings as insulin is a known vasoactive hormone and adolescence is associated with a period of insulin resistance (Bloch et al., 1987; Baron, 2002) and oxidative stress has been linked to

endothelial dysfunction by inactivating NO (Higashi et al., 2009). However, assessing these outcomes was not the purpose of the investigation. A novelty of this study compared to previous paediatric research is the use of sucrose rather than glucose. In their study Dengel et al. (2007) used a glucose load which was aligned with the body weight of the participant (1.75 g/kg of bodyweight; maximum of 75 g) and Dye et al. (2012) infused glucose and dextrose into an antecubital vein to raise plasma [glucose] to induce a hyperglycaemic state (200 mg/dL of plasma [glucose] for 60 minutes). The current study used 60 g of sucrose which is better representative of the sugar load in commercially available SSBs (Loader et al., 2017) and contains fructose which is metabolised differently to glucose (Lustig, 2012). In their study, Loader et al. (2017) used a commercially available SSB which constituted of sucrose which aligned with the current study. The differences in our data and Loader et al. (2017) is most likely attributed to the adolescent population used in the current study which is supported by paediatric data which has evidenced improved vascular function in adolescents from either an OGTT (Dengel et al., 2007) or glucose and dextrose infusion (Dye et al., 2012). These findings suggest that the vasculature respond differently to sugars in adolescents when compared to adults and future research should explore the differences between these populations to provide further insight into the effects of SSB consumption. Another important finding of this study was that microvascular function following consumption of 60 g of fructose, sucrose or glucose, measured using laser Doppler flowmetry and defined by PRH, increased 1 hour following drink consumption in all trials and the increase was greatest in the FRU trial. Consumption of the test meal attenuated PRH to baseline level in the FRU and SUC trial and below baseline in the GLU trial. There was no observed

differences between the trials for TRH. Data indicated a wide CI for the effects of FRU when compared to CON on PRH. This could potentially be due to the small sample size used in this study but can also be attributed to body composition. In the current study, there was a standard deviation of 11 kg for participant mass and previous research has investigated the influence of body composition on reactive hyperaemia in healthy men and women (Wascher et al., 1998). In this study it was determined that an increase in forearm muscle mass was associated with an increase in reactive hyperaemia. These findings demonstrate that the wide variability for the PRH outcome between FRU and SUC could be as a result of the variability in mass between our participants. The variation in body composition could be reflective of the different biological ages/stages of maturation and sex differences within our sample which is typical when working with adolescents.

The findings for PRH are consistent with Grasser et al. (2014) which demonstrated that SSB-induced hyperglycaemia also increased microvascular function in adults. The SSB used in that study contained 39.1 g of sucrose and glucose and 114 mg of caffeine. Their study failed to distinguish whether the increase in microvascular function was as a result of the sucrose or the caffeine which has been shown to improve microvascular function (Tesselaar et al., 2017; Noguchi et al., 2015). The findings from the current study support the notion that sucrose increases microvascular function in adolescents. In the current study the increase in PRH was greatest in FRU but did increase in SUC and GLU suggesting that fructose consumption increases microvascular function beyond other sugars 1 hour following consumption of the drink. The influence of consumption of fructose, sucrose and glucose on microvascular function in the current study was only observed in PRH and not TRH which is

known not to be NO-mediated (Wong et al., 2003). The current mechanisms for PRH are currently unknown which makes it difficult to determine an explanation for our findings but based on current knowledge it is a possibility that consumption of these sugars increases the availability/production of NO in the microvasculature in adolescents which causes an increase in PRH. This is contradicted in the literature where it has been reported that NO synthase inhibitors (Wong et al., 2003; Engelke et al., 1996) do not influence the post-occlusive microvascular response. However, Engelke et al. (1996) also reported a 33% fall in TRH when prostaglandins are inhibited with ibuprofen prior to the administration of the NO synthase inhibitor. This suggests that prostaglandin inhibition might determine a role for NO in PRH. Combined sensory nerve and prostaglandin inhibition has also been determined to cause a 72% reduction in the post-occlusive response (Larkin & Williams, 1993). Therefore, it is likely that the mechanisms behind the functioning of the microvasculature are controlled by a series of endothelium dependent and independent mechanisms. Further research is required to determine these mechanisms before conclusions of why consumption of dietary sugars increase microvascular function can be drawn.

The reduction in microvascular function in Loader et al. (2017) may be explained by the adult population used in their study. Dengel et al. (2007) concluded that age, which is a surrogate of vessel health, might have an interaction effect on the hyperglycaemic vascular response. The current study recruited adolescents and adolescence is consistent with a period of insulin resistance and as insulin is a vasoactive hormone, insulin-resistance causes impairments to insulin-mediated and endothelium-dependent vasodilation (Bloch et al., 1987; Baron, 2002). Our findings could be attributed to this however, a limitation of this work is that [insulin] was not assessed to determine

whether our findings could be attributed to this outcome. Further research is required to assess [insulin] following consumption of fructose, sucrose and glucose in adolescents to determine whether microvascular function is associated to [insulin].

The majority of the studies reported in Loader et al. (2015) were carried out on an adult population meaning that findings from these studies are not reflective of the age-related difference in microvascular function of adolescents (Gates et al., 2009). Adult ageing is associated with a reduction in microvascular endothelial function (Gates et al., 2009). Therefore, more paediatric research is required to be done exploring the effects of SSB consumption on vascular and metabolic outcomes in youth. The findings from this study suggest that consumption of sucrose, fructose and glucose increases microvascular function in adolescents and the increase is greatest following fructose consumption. These findings are important as they suggest there is a difference between the vascular response to SSB consumption between adults and adolescents and further research is needed to be done to explore whether these observations are consistent with findings from future studies.

An aim of this study was to assess the independent influence of fructose, sucrose and glucose consumption on metabolic responses. The findings determined that consumption of these sugars produced significantly different responses for metabolic outcomes and it is important to consider their effects on the vasculature before and after consumption of the drink as well as in the postprandial phase. Previous research has demonstrated that the postprandial change in [TAG] and [glucose] are unrelated to FMD demonstrating the need to assess metabolic outcomes in addition to vascular outcomes (Bond et al., 2015a, c).

Plasma [glucose] did not elevate in FRU and responded similarly to CON following drink and meal consumption. However, plasma [glucose] was elevated following drink consumption in GLU and to a lesser extent in SUC. The elevation in plasma [glucose] in SUC is cause for concern due to it being the main sugar constituent in commercially available SSBs (Loader et al., 2017) and the relationship between hyperglycaemia and CVD. According to the World Health Organisation, 2.2 million deaths in 2012 were linked to hyperglycaemia (WHO, 2016b). In their study, Levitanen et al. (2004) demonstrated that hyperglycaemia under the diabetic threshold is associated with an increased risk of fatal and nonfatal CVD and a similar association is apparent between CVD related events and fasting or 2 h postprandial plasma [glucose]. Additionally, treatment with acarbose, a  $\alpha$ -glucosidase inhibitor which reduces postprandial plasma [glucose], has been shown to reduce the risk of CVD events (Hanefield et al., 2004) implicating postprandial plasma [glucose] in the development of CVD. It is suggested that the association between plasma [glucose] and CVD is as a result of oxidative stress (Davidson & Parkin, 2009; Fiorentino et al., 2013) which is known to be implicated with CVD (Cervantes Gracia et al., 2017) and has been suggested to be the underlying cause of macro- and micro- vascular complications from type 2 diabetes (Khatri et al., 2004; von Harsdorf et al., 1999; Brownlee, 2001). Adolescents therefore are at risk of obtaining CVD from chronic SSB consumption and future interventions are required to reduce SSB intake in this population.

Plasma [lactate] increased in FRU, GLU and SUC and increased similarly in FRU and SUC following drink consumption and remained elevated in SUC following meal consumption. This is consistent with previous research which demonstrated that consumption of fructose-sweetened beverages increased



plasma [lactate] concentrations significantly more than consumption of glucose-sweetened beverages (Teff et al., 2009). The findings from the current study and Teff et al. (2009) suggest that fructose increases [lactate] significantly more than consumption glucose. The increase in [lactate] as a result of fructose consumption is associated with an increase in lipogenesis (Carmona & Freedland, 1989; Walli, 1978) which previous research has demonstrated is a response to fructose (not glucose) consumption in humans (Samuel, 2011). However, the findings in the current study are not consistent with this with fructose not inducing an increase in plasma [TAG]. A limitation of this study is that VLDL concentrations were not assessed which would have provided an insight into the metabolic fate of fructose and de novo synthesis of [TAG].

FRU was the only trial to induce an increase in [uric acid] following drink consumption. Early research has demonstrated similar findings where the rapid intravenous infusion of fructose resulted in an increase in [uric acid] (Fox & Kelley, 1972). Another study demonstrated that both artificial and natural fructose-rich rich beverages were associated with an increase in [uric acid] whereas diet soft drinks, which did not include fructose, did not increase [uric acid] (Choi et al., 2008). These findings support the notion that fructose induces an increase in [uric acid] and it has been proposed that this is as a result of fructose causing an increase in ATP degradation to AMP which is a known uric acid precursor (Choi et al., 2005; Gibson et al., 1983; Fox & Kelley, 1972). Uric acid has been demonstrated to be an independent predictor of CVD events in healthy men and women (Kivity et al., 2013) and an independent risk factor for myocardial infarction (Krishnan et al., 2006). Childhood [uric acid] has also been found to be predictive of diastolic and systolic blood pressure in adulthood (Alper Jr et al., 2005). Thus, an increase in [uric acid] as a result of fructose

consumption, a sugar found in commercially available SSBs, is implicated in CVD and childhood levels of [uric acid] are related to CVD risk factors in later life.

There were no significant differences between any of the trials for plasma [TAG] although [TAG] did increase following consumption of the meal but did not change following consumption of any of the drinks. It was expected that fructose consumption would cause an increase in plasma [TAG] in line with the increase in [lactate] due to the association between lipogenesis and plasma [lactate]. However, [TAG] did not increase as a result of fructose consumption and assessment of VLDL concentrations would provide insight into these findings. This is consistent with previous research which has demonstrated that consumption of  $\leq 100$  g/d of fructose had no effect on [TAG] when participants were fasted beforehand (Livesey & Taylor, 2008) and no change in AUC for [TAG] following consumption of glucose and sucrose (Jameel et al., 2014). Our findings that [TAG] did not increase following consumption of fructose but [lactate] did are consistent with findings from previous research which demonstrated no increase in [glycerol] but an increase in [lactate] following consumption of a fructose meal (Brundin & Wahren, 1993).

### ***Conclusions***

This study is the first to explore the effects of sucrose, fructose and glucose on vascular and metabolic outcomes in adolescents. As these are sugars found in commercially available SSBs and it is in this population that SSBs are consumed the most in the UK this work provides an insight into the vascular and metabolic health of adolescents in the UK. The techniques used to assess vascular function are reliable and widely used in the literature and by following

current guidelines (Thijssen et al., 2019) this study has provided an insight, which has minimised for known sources of error, into the effects of dietary sugars on vascular function in adolescents. By exploring the effects of these sugars on these outcomes following consumption of a high-fat, high-sugar test meal these findings also provide an insight into the postprandial effect of these sugars.

The current study has shown that consumption of sucrose causes a non-significant increase in macrovascular function of the brachial artery in healthy adolescents and this increase is still apparent when compared to control following consumption of a high-fat, high-sugar test meal which attenuated FMD. Alongside the increase in FMD, microvascular function of the cutaneous circulation of the forearm also increased following consumption of fructose, sucrose and glucose in adolescents. The mechanisms behind the increase in vascular function are unknown and further research is required to assess the effect of consumption of these sugars on insulin and redox state to determine whether the increase in vascular function observed in this study can be explained by these outcomes.

### ***Implications***

For future trials with a larger sample size, the amount of time available needs to be considered with regards to how many participants can be assessed within a day and over the course of the trial if all FMD analysis is to be carried out by a single assessor. Participants may need to be recruited from more than one school (or from multiple sources) for larger trials as all that volunteered for the current study were recruited with the exception of those that dropped out during the study or were excluded for behavioural concerns at school. The current

study specifically recruited healthy 14-15 year olds and it was observed that those which volunteered had an interest for the area of study. This could be considered a source of bias, but it is unavoidable that pupils which find the area interesting will be more likely to volunteer for the study. Future studies could overcome the bias of age by investigating the influence of these sugars on vascular and metabolic outcomes in other age groups to determine whether they occur differently to the age group used in this study. Additionally, future studies could investigate the influence that consumption of these sugars have in unhealthy populations such as in those with diabetes and compare findings to a healthy population.

As the current study demonstrated that single consumption of sugars found in SSBs improve acute microvascular function, future research may wish to explore the influence of chronic/daily consumption of these sugars on the vasculature through an observational study of those which regularly consume SSBs. This will provide a better reflection of habitual consumption of these sugars. Furthermore, future research may wish to explore the influence of FRU and GLU on FMD as the current study only explored the effects of SUC on FMD when compared to CON. This will provide a more detailed insight into the influence of sugars found in SSBs on the macro- vasculature.

Another limitation of this study was that the taste aspect of the drinks was not controlled for with fructose having a sweeter taste. Future studies could control for this by using a non-sugar-based sweetener in the glucose, sucrose and control drinks to ensure that all drinks taste the same.

The vascular measures in this study were also not standardised to the peak appearance time of each type of sugar consumed as this was not possible for

the current thesis. Chong et al. (2007) identified that plasma glucose concentrations peak between 30 and 60 min following independent feeding of glucose and fructose. This has been supported by Abraha et al. (1998) which found that plasma glucose peaked 30 min following fructose feeding and remained constant up to 60 min in non-diabetic subjects. It is understood that the time course of blood fructose and glucose will differ between trials but the standardised 1 hour post drink time point for vascular analysis was picked as it would not be possible to align plasma [glucose] measurement with peak [glucose] or [fructose] (which was not measured in this study) within an individual. Future research may wish to use separate groups for each trial to ensure that assessment of vascular function can be standardised to align with the peak in [glucose] which will provide a more accurate insight into the metabolic response to these sugars. Additionally, another limitation of this study was that plasma [fructose] was not assessed. As fructose consumption has been associated with an increase in cardiovascular risk it may be important to assess [fructose] following consumption of the sugary drinks. Future research could assess this to determine whether consumption of these sugars produce different responses between [glucose] and [fructose].

Although the current study demonstrated that consumption of sugars found in SSBs improves microvascular function in the acute phase, it is important that this is not used to promote consumption of SSBs. It is important to remember that the findings from the current study are based on single consumption of sugars found in SSBs and not consumption of these sugars over time.

Research has shown that increased consumption of SSBs can result in increased risk of coronary heart disease, diabetes, greater waist circumference, systolic blood pressure and metabolic syndrome (Huang et al., 2014; Basu et

al., 2013; Chan et al., 2014). Thus, before changes in advertisement and policy can be made further research is required to explore the effects of chronic SSB consumption on the vasculature and metabolic outcomes over time. Parents and schools should continue to take similar precautions to what they do now as this research will be used to inform future research rather than informing currently policy.

## **Chapter 6: Conclusions**

The aim of the current thesis was to expand our understanding of the vascular and metabolic responses to consumption of sugars found in commercially available SSBs. Two studies were completed to achieve this which addressed the following:

1. Are assessments of flow-mediated dilation and laser Doppler flowmetry reliable for the assessment of macro- and microvascular function? -  
Reliability chapter
2. What are the acute and postprandial macrovascular, microvascular and metabolic responses to consumption of fructose, sucrose and glucose? -  
Experimental chapter

### **Experimental considerations and future directions**

The current thesis is an addition to the literature regarding the effects of SSBs on vascular and metabolic function in adolescents. The work in thesis was performed in a counterbalanced order in a controlled study with the drinks being delivered in a double blind manner. Previous research has focused on the effects of glucose which is not representative of commercially available SSBs. The work in this thesis is the first to assess the individual effects of sucrose, fructose and glucose, the sugars found in commercial SSBs, on vascular and metabolic outcomes. This research adds to the literature as sucrose is the main sugar constituent of commercially available SSBs and it is made up of equal parts of glucose and fructose and these sugars are metabolised differently. The metabolism of fructose has been implicated with CVD risk factors thus making it important for the effects of these sugars to be assessed. Furthermore, adolescents are the highest consumers of SSBs in the UK making them vulnerable to the reported effects of SSB on cardiovascular health. Assessing

these outcomes following a high-fat, high-sugar test meal also provides insight into the postprandial effects of consumption of these sugars. The work here indicates that consumption of these sugars increase vascular function in adolescents which suggest that they may be good for vascular function however, the mechanisms behind this are uncertain. Further work is required to determine whether there are age-related differences in the vascular response to SSB consumption. If this is the case then research also needs to assess whether these changes are as a result of the age-related differences in insulin resistance and/or whether redox state also plays a role.

## **Conclusion**

The research regarding the effects of SSBs on vascular and metabolic function are ongoing however, the effects of sucrose, the sugar known to make up SSBs, on these outcomes is more recent. The current thesis aimed to determine the effects of glucose, sucrose and fructose on these outcomes in adolescents in the acute and postprandial phase. It can be determined that consumption of these sugars increase vascular function in the acute phase (post-drink) and although consumption of a high-fat meal does attenuate vascular function, when compared to a control trial the elevation was still apparent in the sucrose trial for FMD and the fructose trial for microvascular function. The mechanisms behind these outcomes are unknown however, it is suggested that investigations into insulin resistance and redox state may provide an understanding.



## Chapter 8: References

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## Chapter 9: Appendix

### Appendix 1: Certificate of ethical approval



College of Life and Environmental Sciences  
SPORT AND HEALTH SCIENCES

St. Luke's Campus  
University of Exeter  
Heavitree Road  
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United Kingdom

#### Certificate of Ethical Approval

Proposal Ref No: 171206/E/07

Title: To examine whether the consumption of different types of sugar (fructose, glucose and sucrose) Impairs blood vessel health in adolescents when compared to the consumption of water

Applicants: Jodie Koep, Rohit Banger MSc, Rhys Banks PhD, Dr Bert Bond, Dr Alan Barker, Kate Sansum Placement student (UG), Ricardo Oliveira PhD, Sascha Kranen PhD, Chloe Bland MSc, Dimitris Vlachopoulos, Owen Tomlinson

The proposal was reviewed by the Sport and Health Sciences Ethics Committee.

Decision: *This proposal has been approved until July 2018*

Signature:

A handwritten signature in black ink, appearing to read 'Melvyn Hillsdon'.

Date: 7/2/2018

Name/Title of Ethics Committee Reviewer: Dr Melvyn Hillsdon

*Your attention is drawn to the attached paper which reminds the researcher of information that needs to be observed when Ethics Committee approval is given.*

## Appendix 2: Participant Information Sheet



### Sport and Health Sciences College of Life and Environmental Sciences

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## Participant Information Sheet

**Study title** To examine whether the consumption of different types of sugar (fructose, glucose and sucrose) impairs blood vessel health in adolescents when compared to the consumption of water.

### Invitation and brief summary

We would like to invite your child to take part in a research study into the effects of sugar sweetened drink consumption on blood vessel health in adolescents. Taking part in the study is entirely up to you and your child so before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information and to discuss it with other people to decide whether you wish for your child to take part or not. Thank you for taking the time to read this information. Please be aware that participation in this study means that your child will be required to spend approximately five school days outside of school/the classroom.

### What's involved?

High sugar consumption in youth is linked with poor blood vessel and metabolic health and obesity. Worryingly, teenagers in the UK are known to consume eight times the recommended maximum amount of added sugar from sugary drinks alone. However, the effect of sugary drinks on blood vessel function in adolescents is poorly understood. This study will investigate the effect of different sugar types found in sugary drinks on blood vessel health. We will do this by using a range of non-invasive techniques (including the use of ultrasound) and also by taking a series of fingertip capillary blood samples (<1 mL each) to measure the response to different sugary drinks. We will also see if sugary drink consumption influences the blood vessel response to a subsequent meal (Pizza, ice cream and a chocolate pudding).

### What would taking part involve?

We have invited your child to take part because we are looking for healthy participants between the ages of 12-15 years old. We will be inviting 30-35 male and female participants to take part.

If you would like your child to take part they will be asked to attend a laboratory at the University of Exeter's St. Luke's campus on five separate occasions. You will be asked to visit our laboratory in a group of two or three for all visit.

### Preliminary testing visit (visit 1)

This will take four hours. Having completed and handed in participant assent, parental consent, health screening and contact details forms, we will collect your child from school at 7.30 am and drive them to the University for their familiarisation visit. This visit will initially provide your child with an opportunity to discuss with the investigators any questions they may have regarding any aspect of the study's objectives, procedures or results.

If your child wishes to continue to take part, the rest of this visit will involve some preliminary measurements including, height, sitting height, body weight and body fat percentage (using a machine in which you sit inside a chamber). We will also take a resting measure of your child's blood pressure. We will then familiarise your child with all testing procedures and what to expect on future visits, so that they can make sure that they would like to take part. This includes non-invasive measures of blood vessel function at three different sites; the arm, forearm and head. We will also demonstrate how we take small capillary blood samples from the fingertip as this will be done on future visits.

After we have shown all the techniques, and providing that your child is still happy to take part, we will measure blood vessel function in the arm. This involves sticking a small laser probe to your child's forearm, and scanning the large blood vessel in the bicep using ultrasound. We will then pump up a blood pressure cuff around the forearm of the same arm for 5 minutes. During this time, your child may experience pins and needles in their hand, but this is normal and short lived. We often use this technique for our research with teenagers, and it is well tolerated. Following this we will also measure blood vessel function within the head by placing a small ultrasound probe at the temple, and asking your child to hold their breath for 30 seconds. Blood vessel function at the head will also be measured during a 5 minute period where we ask your child to sit and stand repeatedly.

Your child will then be asked to perform a cycling test to maximal effort cycle test. This test will feel like cycling up a hill as it gets steeper and steeper until your child can no longer carry on. It will only feel very hard at the end. During this cycle test your child will be wearing a heart rate monitor and a face mask in order to determine their aerobic fitness. We will also monitor blood flow to the head using the ultrasound probe at the temple as used above, monitoring your child throughout this time. After the exercise test, measures of blood vessel health in the arm and head will be repeated as above using non-invasive ultrasound. Measures of blood pressure will also be routinely assessed following the exercise bout.

Before we drive your child back to school (at ~ 12.30 pm) they will be given an instruction pack for the remainder of the study. Your child will be required to;

1. Wear an accelerometer (a small activity monitor which looks like a wrist watch) for 7 days prior to the first visit and then 2 days prior to each subsequent visit. They will hand this in to our research team during the subsequent visits.
2. Record their food intake on the two days prior to each subsequent visit.
3. Avoid any structured moderate or vigorous intensity physical activity (other than day to day tasks) for 48 hrs prior to their next visit.
4. Refrain from eating or drinking anything apart from water after 8 pm the night before all laboratory visits.
5. Take home a set of scientific drawings showing five stages of pubic hair development and circle the picture that best describes them. They will seal this in an envelope and return it to us on their next visit.

## **Trials 1-4 (visits 2, 3, 4, 5)**

Each trial day will last from 8.00 am to 2.40 pm. As for visit 1, we will collect your child from school at 7.30 am having not had any breakfast. The following procedures will be included in these visits:

- 8 am - We will assess the health of your child's blood vessels by scanning the artery in the arm and side of the head as described in visit 1.
- 9 am – Your child will then be given one of three sugary drinks or water (to act as a comparison), receiving a different one on each visit.
- A small sample of blood (less than 1mL, or about the size of a pin head) will be taken from your child's fingertip every 30 mins for the first 2 hours and then every hour following for the next 3 hours.
- Hunger levels will be assessed requiring your child to report their hunger on a visual scale, 60 and 120 minutes after sugary drink consumption, and 180 minutes after test meal consumption.
- 10 am - Blood vessel health will then be reassessed after 35-75 minutes. As well as measures of blood pressure.
- 11am – Your child will then be given a meal of a pizza, ice cream and a chocolate pudding.
- During this time, we will discuss with your child aspects of sports science, medicine and nutrition. Your child will also be able to watch a film, play on the Playstation® or do some school work.
- 2 pm - Three hours after the breakfast we will measure your child's blood vessel health and blood pressure. After this measurement, we will drive you child back to school for 3.30 pm.

### [What are the possible benefits of taking part?](#)

The main benefits of the proposed research are educational and there will be limited personal benefit to your child. However, the results will increase our understanding of the risks associated with sugar intake and the different types of sugars found in sugary drinks. The study will hopefully be enjoyable and interesting for your child and allow them to learn about exercise physiology and nutrition in a fun and interactive way. By taking part your child will get to spend time in a University Laboratory, and we will give you access to full fitness and nutrition assessment from our report. This study will give your child first-hand experience about what it's like to be involved in science at a higher level.

### [What are the possible disadvantages and risks of taking part?](#)

Blood sampling can cause some temporary discomfort if participants are not comfortable with blood. However, this technique is used extensively in physiological testing. The investigators are trained and experienced in all aspects of these procedures to ensure that they are performed safely and with the minimum possible discomfort.

### [Does my child have to take part?](#)

Please remember that participation in this study is entirely voluntary. It is up to you and your child to discuss and decide whether you would like them to take part or not. If you decide for them to take part they free to leave the study at any time without giving a reason as to why they wish to do so.

If you and your child do agree for them to participate in this study then please complete the following:

- Sign the parent/guardian consent form
- Sign the child assent form
- Complete the contact details form
- Complete the health questionnaire

Please return these documents to your child's school in the brown envelope provided, so we can collect them. A member of the research team will then be in contact to arrange your child's involvement in the study. You will also be given a copy of the forms and this information sheet for your own records.

### Are my results confidential?

If you consent to take part in this study you have a right to privacy. Your child's name will be linked to an ID number on a password protected database and only these IDs will be used as labels during blood and data analysis.

### What will happen to the results of this study?

The results will increase our understanding of the risks associated with different types of sugars found in sugary drinks. We will aim to publish the findings as a masters by research project, in research journals and to present them at conferences in the UK or abroad. Your data will always remain anonymous and your name will not appear on any results. However, we will explain all your results of the study to you at the end if you would like to know them.

### Who has reviewed this study?

All research activity at the University of Exeter is examined and approved by an ethics committee to protect your interests. This study has been approved by the Ethics Committee of Sport and Health Sciences, College of Life and Environmental Sciences, University of Exeter.

### Who is funding/sponsoring this study?

This study will be funded by the University of Exeter.

### Contacts for further information

If you would like more information or if you have any further questions about the study please contact the investigators using the details below:

Ms Jodie Koep	Dr Alan Barker	Dr Bert Bond
Sport and Health Sciences	Sport and Health Sciences	Sport and Health Sciences
Baring Court	Baring Court	Baring Court
St. Lukes Campus	St. Lukes Campus	St Lukes Campus
Exeter University	Exeter University	Exeter University
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**Study:** To examine whether the consumption of different types of sugar (fructose, glucose and sucrose) impairs blood vessel health in adolescents when compared to the consumption of water.

**Researcher:** Jodie Koep (MSc)

**Organisation:** The University of Exeter

**Version:** #2 15/01/18: reviewed by The University of Exeter ethics committee

Participant Identification Number: ID no.

**Informed consent form for parent/guardian**

**Please  
initial  
box**

I confirm that I have read and understand the information sheet version #2 15/01/2018 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my child's participation is voluntary and that I am free to withdraw them at any time, without giving any reason.

I understand that any information given by me may be used in future reports, articles or presentations by the research team.

I understand that my child's name will not appear in any reports, articles or presentations.

I understand that my child will perform an incremental cycle tests to exhaustion on their first visit.

I understand that my child will be required to drink three high sugar drinks or water on four separate occasions.

Ultrasound will be used to determine changes in the width of the arm artery. A laser will also be placed onto the forearm to quantify skin blood flow. All of these techniques are routinely used with children for research purposes and are considered to be non-invasive.



I can confirm the absence of any food allergies related to this study.

I understand that ultrasound will be used to measure the speed of blood flow at the side of the head during a 30 second breath hold and when performing 5 minutes of repetitive sitting and standing.

I understand that my child will be asked to record dietary information and wear an accelerometer to measure their physical activity. My child will consume the same meal no later than 8:00 pm before each visit to the laboratory.

I understand that my child will be required to assess their pubertal status according to five drawings of secondary sex characteristics. The purpose of this has been made clear to me.

I understand that on each of the four test visits five capillary blood samples will then be taken from the fingertip (<1 mL each time) in order to measure fat, sugar, insulin in the blood.

I understand that on each of the four test visits my child will have to consume a meal provided consisting of a pizza, ice cream and chocolate pudding. My child will not have eaten beforehand

I understand that on each visit my child will have their blood pressure measured by placing a cuff around their bicep which will be inflated during measurement.

I agree for my child to take part in the above study.

Name of Parent/Guardian                      Date                      Signature  
\_\_\_\_\_

Name of Researcher                      Date                      Signature  
\_\_\_\_\_

## Appendix 4: Participant Assent Form



**Sport and Health Sciences  
College of Life and Environmental  
Sciences**

St Luke's Campus, Heavitree Road, Exeter, EX1  
2LU  
Telephone: +44 (0)1392 26  
Email: [sshs-school-office@ex.ac.uk](mailto:sshs-school-office@ex.ac.uk)  
Web: [www.ex.a.uk](http://www.ex.a.uk)

**Study:** To examine whether the consumption of different types of sugar (fructose, glucose and sucrose) impairs blood vessel health in adolescents when compared to the consumption of water.

**Researcher:** Jodie Koep

**Organisation:** The University of Exeter

**Version:** #2 15/01/18 reviewed by The University of Exeter ethics committee

Participant Identification Number: ID no.

### **Informed assent form for participants**

**Please  
initial  
box**

I confirm that I have read and understood the information sheet version #2 15/01/2018 for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily.

I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason.

I understand that any information given by me may be used in future reports, articles or presentations by the research team.

I understand that my name will not appear in any reports, articles or presentations.

I understand that I will have to perform an incremental cycle test to exhaustion on my first visit.

I will be required to drink three high sugar drinks or water on four separate visits.

Ultrasound will be used to measure changes in the width of the artery in my arm, and a laser will be placed on my forearm to measure changes in skin blood flow. This will happen before and after a blood pressure cuff is inflated below my elbow for 5 minutes. During this time I may experience “pins and needles” in my hand. Ultrasound will be used to measure the speed of blood flow at the side of the head during a 30 second breath hold and when performing 5 minutes of repeatedly sitting and standing.

I understand that 5 small (<1 mL) of blood will be taken from the fingertip to measure the amount of fat, sugar and insulin in my blood.

On all test visits, I will have to consume a meal which will be provided, consisting of a pizza, ice cream and chocolate pudding. I will not have eaten beforehand.

I will be required to assess my pubertal status according to five drawings of secondary sex characteristics. The purpose of this has been made clear to me.

I will be required to record any dietary information and wear an accelerometer to measure physical activity for seven days prior to the first experimental visit and two days prior to each subsequent experimental visit.

I will have my arterial blood pressure measured during each visit by placing a cuff around my bicep which will be inflated during measurement.

I agree to take part in the above study.

Name of Participant	Date	Signature
_____	_____	_____

Name of Researcher	Date	Signature
_____	_____	_____

Appendix 5: Health Screen for Child Volunteers (Parental Form)



**HEALTH SCREEN FOR CHILD VOLUNTEERS (PARENTAL FORM)**

**Name:** .....

It is important that volunteers participating in research studies are currently in good health and have had no significant medical problems in the past. This is:

- i) To ensure their own continuing well-being
- ii) To avoid the possibility of individual health issues confounding study outcomes

Your answers to the questions in this questionnaire, on behalf of your child, are strictly **confidential**.

**Please complete this brief questionnaire to confirm your child's fitness to participate:**

1. **At present**, does your child have any health problem for which they are:
  - (a) On medication, prescribed or otherwise ..... YES  NO
  - (b) Attending a general practitioner ..... YES  NO
  - (c) On a hospital waiting list ..... YES  NO
  
2. **In the past two years**, has your child had any illness that required them to:
  - (a) Consult your family GP..... YES  NO
  - (b) Attend a hospital outpatient department ..... YES  NO
  - (c) Be admitted to hospital..... YES  NO

3. **Has your child ever** had any of the following:

- |   |     |                          |    |
|---|-----|--------------------------|----|
| (a) Convulsions/epilepsy .....                | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (b) Asthma .....                              | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (c) Eczema .....                              | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (d) Diabetes .....                            | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (e) A blood disorder .....                    | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (f) Head injury .....                         | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (g) Digestive problems .....                  | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (h) Heart problems .....                      | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (i) Lung problems .....                       | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (j) Problems with bones or joints .....       | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (k) Disturbance of balance/coordination ..... | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (l) Numbness in hands or feet .....           | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (m)Disturbance of vision .....                | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (n) Ear/hearing problems .....                | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |
| (o) Thyroid problems .....                    | YES | <input type="checkbox"/> | NO |
| <input type="checkbox"/>                      |     |                          |    |

- (p) Kidney or liver problems ..... YES  NO
- (q) Allergy to nuts ..... YES  NO
- (r) Eating disorder ..... YES  NO

4. Do you know of any other reason why your child should not engage in physical activity?  
YES  NO

If **YES** to any question, please describe briefly (for example, to confirm the problem was/is short-lived, insignificant or well controlled).

A member of our research team may contact you if we have any further questions.

Thank you for your cooperation



**Primary investigator:**  
 Bert Bond MSc  
[bb266@exeter.ac.uk](mailto:bb266@exeter.ac.uk)  
 01392 264889

**Project coordinator:**  
 Dr. Alan Barker  
[A.R.Barker@exeter.ac.uk](mailto:A.R.Barker@exeter.ac.uk)  
 01392 722766

## Appendix 6: Emergency Contact Detail Form



**Children's Health and Exercise Research  
Centre  
Sport and Health Sciences  
College of Life and Environmental Sciences  
St. Luke's Campus  
University of Exeter  
Heavitree Road  
Exeter  
EX1 2LU  
United Kingdom**

Telephone +44 (0)1392 722807  
Fax +44 (0)1392 264726  
Email [sshs-school-office@exeter.ac.uk](mailto:sshs-school-office@exeter.ac.uk)  
Web [sshs.exeter.ac.uk](http://sshs.exeter.ac.uk)

The influence of participant characteristics on blood vessel function following consumption of a sugary drink in adolescent boys and girls

### Contact details Form

Please provide as up-to-date information as possible. This information will be used in case we need to contact you due to a change in plans, or in the unlikely event of an emergency.

We will maintain the confidentiality of this information at all times, with access available to the researchers only.

Participant name	
Date of birth	
Parents'/guardians' name	
Home address	
Telephone number (please provide a direct contact number in case of emergency)	Home:  Mobile:  Work:
Email address	