Diet cola and glycaemia: The acute effects of a preload containing the non-nutritive sweeteners aspartame and acesulfame-K on the glycaemic response to a glucose load

Solomi, L.

http://hdl.handle.net/10026.1/16507
Diet cola and glycaemia: The acute effects of a preload containing the non-nutritive sweeteners aspartame and acesulfame-K on the glycaemic response to a glucose load

Luke Solomi

Project Advisor: Dr Gail A. Rees, School of Biomedical Sciences, University of Plymouth, Drake Circus, Plymouth, PL4 8AA.

Abstract
Epidemiological evidence has linked non-nutritive sweeteners (NNS) with potential adverse health outcomes such as obesity, metabolic dysfunction, and poor blood glucose control. This study aims to ascertain the acute glycaemic effects of the NNS aspartame and acesulfame-K in diet cola when consumed prior to a glucose load. Ten participants were recruited to take part and instructed to attend the laboratory in a fasted state on two occasions. Participants drank a preload containing either 250 mL carbonated water (CW) or 250 mL caffeine-free diet cola (DC) 10 minutes prior to consumption of a 25 g glucose beverage in 125 mL water. Using portable glucometers, blood glucose was measured both before and 10 minutes after preload consumption, and every 15 minutes thereafter for a 120-minute period. A paired t test showed no significant difference in incremental area under the curve (AUC) for blood glucose between preload conditions (117.8 ± 16.1 versus 115.1 ± 13.4 mmol·L⁻¹·120 min⁻¹ for CW and DC respectively, \( P > 0.05 \)). Similarly, the decremental AUC was not significantly different between conditions (-22.1 ± 5.3 compared with -40.7 ± 9.9 mmol·L⁻¹·120 min⁻¹ for CW and DC respectively, \( P > 0.05 \)). Blood glucose increments were significantly lower in the DC compared with the CW preload condition at 120 minutes only. Incremental blood glucose values were not significantly different between conditions at any other time point. Findings demonstrate the glycaemic inactivity of the NNS aspartame and acesulfame-K in UK diet cola, though the discrepancy between conditions at 120 minutes warrants further investigation.
Introduction
Overweight and obesity are widespread, having doubled worldwide since 1980 (Withrow and Alter, 2011). Prevalence is continuing to increase both considerably and consistently. Figures from the most recent Health Survey for England show that 64% of UK adults aged 16 and over are overweight or obese, a rise of 2.5% from the previous year (Office for National Statistics, 2018). Obesity and consequent comorbidities place an ever-increasing burden on state healthcare systems. For example, the annual cost to the UK National Health Service of type 2 diabetes mellitus (T2DM) alone was estimated at over £21 billion in 2010/2011 and this number is expected to rise by at least 50% by 2035 (Hex et al., 2012).

The popularity of sugar-sweetened beverages (SSBs) in the Western diet has been previously linked to obesity and T2DM (Greenwood et al., 2014; van Baak and Astrup, 2009). Hence, both the World Health Organisation (WHO; 2015) and the UK Scientific Advisory Committee on Nutrition (2015) recommend that free sugars, defined as the monosaccharides and disaccharides added to food and drink as well as naturally occurring sugars in fruit juices (WHO, 2015), comprise no more than 5% of daily energy intake. Therefore, replacement of SSBs with low-energy, artificially-sweetened alternatives may aid individuals in reducing overall energy intake leading to potential weight loss (Miller and Perez, 2014), in addition to improving other aspects of wellbeing such as dental health (Fitch et al., 2012).

Non-nutritive sweeteners (NNS) are zero- to low-energy sugar substitutes often orders of magnitude sweeter than sucrose that may permit reduced sugar and energy intake while simultaneously preserving the palatability of foods and drinks (Burke and Small, 2015). According to the British Dietetic Association (2017), there are currently eleven types of artificial sweeteners licensed for use in the UK, including sucralose, aspartame, saccharin and acesulfame-K. Consumables containing NNS are not limited to beverages only; NNS may be found in common food products such as cereals and sugar-free condiments in addition to household items such as toothpaste and dental floss (Sylvetsky and Rother, 2016). Although the ubiquity of NNS makes it difficult to ascertain consumption at a population level, an estimated 2.3 million UK adults (aged ≥ 15 years) consumed artificial sweeteners at least four times per day in 2017 (Statista, 2018). It is therefore of paramount importance to determine the potential benefits or deleterious effects associated with NNS consumption.

Positive effects of NNS include the potential for decreased daily energy intake and improved glycaemic control as reported by Raben and Richelsen (2012) in a recent review of the scientific literature. However, the research in this area is equivocal at present regarding the physiological and metabolic consequences of NNS, with many studies unable to present clear evidence of either positive or negative effects (Mattes and Popkin, 2009; Wiebe et al., 2011). However, some recent evidence points towards deleterious effects of NNS consumption such as the disruption of glucose metabolism and glycaemic homeostasis (Swithers, 2013).

Consumption of NNS has been consistently linked epidemiologically with an increased likelihood of overweight and obesity, metabolic syndrome, and type 2 diabetes mellitus (Azad et al., 2017; Imamura et al., 2015). Evaluating the robustness of these observational associations is difficult. The additional risk of
obesity and other adverse metabolic outcomes with increased NNS consumption may be owing to reverse causality (Mattes and Popkin, 2009). That is, individuals with higher pre-existing BMI wishing to reduce adiposity may choose to increase intakes of NNS in order to lessen sugar and energy intakes. Prospective studies show that individuals consuming large quantities of NNS frequently have a higher body mass index at baseline than those who do not consume NNS (de Koning et al., 2011). However, the positive relationship between NNS and risk of obesity and metabolic dysfunction persists even in studies adjusting for baseline BMI (Bhupathiraju et al., 2013; Fowler et al., 2008).

The physiological mechanisms that underlie the association between consumption of NNS and metabolic dysfunction are poorly understood (Pereira, 2014). The digestion, metabolism and assimilation of ingested nutrients are optimised through gastrointestinal changes resulting from cephalic phase (e.g. gustatory/olfactory) signals (Smeets et al., 2010). Insulin levels, for example, may be raised after gustatory exposure to foods (Zhu et al., 2014). This cephalic phase insulin response is strongest after oral exposure to carbohydrates (Dušková et al., 2013), and may be necessary to predict energy intake and optimise digestion and metabolism for the maintenance of glucose homeostasis (Ahrén and Holst, 2001). Through a Pavlovian lens, chronic consumption of sweet-tasting yet zero-energy foods (i.e. NNS) may disrupt these cephalic phase responses, lessening the association of sweet taste with energy intake and resulting in the potential dysregulation of metabolism and glycaemic homeostasis (Swithers, 2013; Yang, 2010).

Some experimental trials have noted no additional glycaemic impact of NNS when consumed simultaneously with glucose (Little et al., 2009; Ma et al., 2010). A recent systematic review and meta-analysis concluded that consumption of NNS was not associated with significant glycaemic effects (Nichol et al., 2018). Acesulfame-K, an NNS used with aspartame in UK diet cola, was not included in the review’s analysis. A study by Bryant et al. (2014) has shown that, when co-ingested with a glucose load, acesulfame-K elevated the glycaemic excursion following a glucose load further than glucose consumption alone. However, investigating the impact of acesulfame-K and aspartame together in diet cola when co-ingested with glucose, Solomi et al. (2019) observed no significant effects on glycaemia.

Experimental evidence has highlighted that NNS may exert an acute effect on the glycaemic response to a glucose load when consumed pre-prandially, albeit inconsistently (Tucker and Tan, 2017). For example, Temizkan et al. (2015) observed that the glycaemic area under the curve (AUC) following glucose consumption was significantly lower in those consuming aspartame compared with a water control as a preload. Aspartame added to test meals with matched energy/macronutrient content has also been shown to elevate the insulin response (Prat-Larquemin et al., 2000), although literature is conflicting in this area, with other studies noting no effects (Teff et al., 1995). With regards to acesulfame-K, Sylvetsky et al. (2016) observed a 25% increase in insulin AUC following acesulfame-K consumption prior to an oral glucose tolerance test, although this did not reach statistical significance. In contrast, Brown et al. (2012) noted no glycaemic effects of a preload containing sucralose and acesulfame-K. However, the AUC for glucagon-like protein-1 (GLP-1) was 34% higher in the NNS versus the control condition. Similar increases in GLP-1 are reported in Sylvetsky et al. (2016), indicating that
NNS are not necessarily invariably metabolically inert as previously thought and may indeed exert an effect on metabolism.

Worldwide, the market for low-energy sweeteners is expanding. Global annual growth for NNS from 2008-2015 has been estimated at upwards of 5% per year and is expected to reach a total of $2.2 billion (£1.7 billion) by 2020 (Sylvetsky and Rother, 2016). With the recent introduction of the 2018 Soft Drinks Industry Levy (Her Majesty’s Revenue & Customs, 2016) and the resulting greater cost of SSBs to UK consumers, nationwide purchasing and consumption of artificially-sweetened beverages may be likely to increase. While many studies have administered doses of NNS at levels that exceed reasonable or typical consumption (e.g. Hall et al., 2003; Tey et al., 2017), this study aims to determine the acute glycaemic impact of NNS at commercially available levels when consumed prior to a glucose load. Having reviewed the literature, no study to date has focused on the glycaemic effects of aspartame and acesulfame-K in UK diet cola when ingested pre-prandially. Hence, this specific combination and its effect on glycaemia when consumed prior to a glucose load is a unique and novel area of research.

**Methodology**

**Sample**
A total of ten students volunteered to participate (50% female, mean age 26.9 ± 3.3 years, mean BMI 24.7 ± 1.1 kg·m⁻²). Sample size was based on methodology in past primary research and in a seminal review in this area (Brouns et al., 2005). A priori analysis revealed that ten participants was sufficient to detect a large (0.8) effect size with 95% power at a two-tailed significance level of 5%. Criteria for participation in the study included an age ≥ 18 years and a body mass index of 18 to 30 kg·m⁻². Criteria for exclusion were known pregnancy, diabetes diagnosis, and use of glucocorticoids, thyroid medication, gastrointestinal motility enhancers or any other form of medication known to interfere with intestinal absorption.

All study procedures were approved by the Research Ethics & Integrity Committee of the University of Plymouth Faculty of Science and Engineering. Participants completed a short health screening questionnaire and provided written informed consent prior to the start of the test protocol.

**Study design**
A cross-over design was selected on the basis of proposed objectives. Each participant underwent both treatments, thereby acting as his/her own control and controlling for inter-participant variations in metabolism. Individuals attended the laboratory on two separate mornings at 09:00 having been instructed to fast from 22:00 the previous evening and abstain from strenuous physical activity in the 24 hours preceding the test. Individuals consumed a 25 g glucose beverage (Bulkpowders, Colchester, UK) dissolved in 125 mL water on both test days. Ten minutes prior to glucose beverage consumption, participants consumed one of two preloads: either 250 mL diet cola sweetened with aspartame and acesulfame-K (Caffeine-Free Diet Coke, Atlanta, Georgia, USA) or a 250 mL unsweetened carbonated water control (Sainsbury’s Supermarkets Limited, London, UK). Precise quantities of the NNS aspartame and acesulfame-K in UK diet cola are currently unknown as the information is of a proprietary nature and not currently made
available by the manufacturer. Portable glucometers (Accu-Chek Aviva, Roche, Welwyn Garden City, UK) were used to measure blood glucose from fingertip capillaries immediately before preload consumption and again prior to consumption of the 25 g glucose beverage. Blood glucose was then measured thereafter in 15-minute intervals over a 120-minute period.

Data analysis
The incremental area under the blood glucose curve (AUC) over the test period following a glucose load and both the carbonated water and diet cola preloads was ascertained using the trapezoid method as described in Brouns et al. (2005). As it was hypothesised that the NNS in diet cola would elevate insulin levels (subsequently depressing the glycaemic curve), the decremental AUC for blood glucose following consumption of the glucose beverage after each preload was calculated using similar methods as abovementioned. Descriptive statistical tests were performed to test for significant differences in blood glucose increments between the two preloads at each time point; paired t tests or Wilcoxon matched pairs signed rank tests were used as appropriate. Statistical analyses were performed using Minitab Express (Version 1.5.1) or SPSS (Version 24) for non-parametric data sets. Data are reported as mean ± SE or median ± semi-interquartile range ([75th percentile minus 25th percentile]/2) for non-parametric data sets.

Results
Baseline blood glucose values from both carbonated water (CW) and diet cola (DC) preload conditions are displayed in Table 1. A paired t test revealed that baseline blood glucose measurements were significantly different across the two conditions at the -10 minute point (i.e. prior to preload consumption; 5.2 ± 0.1 versus 6.0 ± 0.2 mmol·L⁻¹ for CW and DC respectively, \( P < 0.05 \)). A Wilcoxon matched pairs signed rank test detected a difference approaching statistical significance in blood glucose values at the 0-minute time point (i.e. following preload consumption but prior to glucose beverage ingestion; 5.1 ± 0.1 compared with 6.1 ± 0.2 mmol·L⁻¹ for CW and DC respectively, \( P = 0.053 \)).

Glycaemic excursions following a 25 g glucose beverage after each preload are depicted in Figure 1. No significant difference in the incremental area under the curve for blood glucose was detected in response to the glucose load after carbonated water and diet cola consumption (AUC 117.8 ± 16.1 versus 115.1 ± 13.4 mmol·L⁻¹·120 min⁻¹ for CW and DC respectively, \( P > 0.05 \)). Glucose consumption

<table>
<thead>
<tr>
<th>Time point (min)</th>
<th>Preload (blood glucose, mmol·L⁻¹)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbonated water (control)</td>
<td>Diet cola (sweetened with NNS)</td>
</tr>
<tr>
<td>-10</td>
<td>5.2 ± 0.1</td>
<td>6.0 ± 0.2</td>
</tr>
<tr>
<td>0</td>
<td>5.1 ± 0.1</td>
<td>6.1 ± 0.6</td>
</tr>
</tbody>
</table>

*denotes statistically significant difference.
following both preloads elicited near-superimposable excursions above pre-prandial levels for the first 60 minutes, each peaking at the 30-minute point. Glycaemia fell to below baseline after 75 minutes in both conditions, persisting for the remainder of the test period.

Figure 1. Comparison of incremental blood glucose values after ingestion of a glucose beverage preceded by (1) carbonated water or (2) caffeine-free diet cola, sweetened with aspartame and acesulfame-K. Values shown are mean increments and error bars denote SEM.

The diet cola preload elicited a non-significant greater decline below baseline when determined via the decremental AUC (-22.1 ± 5.3 compared with -40.7 ± 9.9 mmol·L⁻¹·120 min⁻¹ for CW and DC respectively, P>0.05). Incremental blood glucose values were significantly lower in the diet cola compared with the carbonated water preload condition at 120 minutes only (-0.8 ± 0.2 compared with -0.2 ± 0.1 mmol·L⁻¹, P<0.05; Table 2). Blood glucose increments were not significantly different between conditions at any other time point (all P>0.05; Table 2).

Discussion
Results showed that there were no significant differences in the incremental area under the blood glucose curve between preloads. Similar results have been observed in the literature previously. Temizkan et al. (2015) noted comparable glycaemic AUCs following consumption of an aspartame compared with carbonated water preload prior to a 75 g oral glucose tolerance test (OGTT) in healthy males and females. Pre-treatment with acesulfame-K has also been shown to elicit no additional glycaemic or insulin response (Wu et al., 2013). Similarly, Brown et al. (2012) noted no effects of a preload containing sucralose and acesulfame-K (compared with a carbonated water control) on the glycaemic response to a 75 g OGTT.

Findings are conflicting in this area, however. Pepino et al. (2013) administered an NNS pre-treatment to metabolically healthy adults with obesity. The NNS preload condition was associated with a statistically significant 20% increase in insulin AUC following a glucose load compared to a water control, indicating that the NNS were
Data are presented as mean ± SE (time points 15 to 90 minutes) or median ± semi-interquartile range (time points 105 and 120 minutes). Paired t tests were employed to test for significant differences between groups where normally distributed (time points 15 to 90 minutes). Wilcoxon matched pairs signed rank tests were used for time points with skewed datasets (time points 105 and 120 minutes). * denotes statistically significant difference.

impacting on insulin secretion. Sucralose was the NNS used in Pepino et al. (2013) and participants were individuals with obesity. Results are thus not directly comparable. In a more recent trial by Sylvetsky et al. (2016), healthy participants were administered various beverages with varying types and quantities of NNS prior to a 75 g OGTT. Pre-treatments sweetened with both aspartame and acesulfame-K (as in the present study) were associated with a 22-25% rise in insulin AUC compared with controls, although this did not reach statistical significance.

The cephalic phase insulin response (Ahrén and Holst, 2001) is a potential mechanism that may underpin the (non-significant) lower blood glucose trajectory after 75 minutes in the diet cola condition in the present study. As alluded to earlier, NNS-containing foods and drinks may signal a preliminary insulin release. This may be as a result of NNS action on pancreatic β-cell sweet taste receptors, consequently inducing insulin secretion directly (Sylvetsky et al., 2016), a hypothesis supported by in vitro research (Nakagawa et al., 2009). Alternatively, NNS may bind to enteroendocrine L-cells in the gastrointestinal tract (Li et al., 2002), initiating a signal cascade that culminates in the release of the incretin hormone GLP-1 (Brown et al., 2012). Higher plasma insulin levels facilitate the assimilation of blood glucose into peripheral tissues via insulin-dependent glucose transporter channels (GLUT4; Huang and Czech, 2007), potentially depressing the incremental blood glucose curve following the diet cola pre-treatment as seen in Figure 1. Owing to the role of

<table>
<thead>
<tr>
<th>Time point (min)</th>
<th>Preload (Δ blood glucose, mmol·L⁻¹)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carbonated water</td>
<td>Diet cola</td>
</tr>
<tr>
<td>15</td>
<td>1.8 ± 0.3</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>30</td>
<td>2.8 ± 0.4</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>45</td>
<td>1.9 ± 0.4</td>
<td>1.7 ± 0.4</td>
</tr>
<tr>
<td>60</td>
<td>0.7 ± 0.4</td>
<td>0.5 ± 0.5</td>
</tr>
<tr>
<td>75</td>
<td>-0.1 ± 0.2</td>
<td>-0.2 ± 0.3</td>
</tr>
<tr>
<td>90</td>
<td>-0.3 ± 0.2</td>
<td>-0.6 ± 0.2</td>
</tr>
<tr>
<td>105</td>
<td>-0.4 ± 0.3</td>
<td>-0.4 ± 0.2</td>
</tr>
<tr>
<td>120</td>
<td>-0.2 ± 0.1</td>
<td>-0.6 ± 0.2</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SE (time points 15 to 90 minutes) or median ± semi-interquartile range (time points 105 and 120 minutes). Paired t tests were employed to test for significant differences between groups where normally distributed (time points 15 to 90 minutes). Wilcoxon matched pairs signed rank tests were used for time points with skewed datasets (time points 105 and 120 minutes). * denotes statistically significant difference.
insulin as an anabolic hormone, marginally higher insulin levels sustained for long periods of time (i.e. for regular consumers of NNS) may potentially result in eventual weight gain. However, the quantification of insulin levels was beyond the scope of this study and remains a key avenue for future research.

The decremental area under the blood glucose curve (that is, the degree to which blood glucose excursions fell below baseline) was not significantly different between conditions, although incremental blood glucose values were significantly different at the 120-minute point only. Blood glucose trajectories began to diverge towards the end of the test, and it is possible that further divergence may have occurred at later stages beyond the end of the test. Therefore, an extension of the test period to 180 minutes might allow for elucidation of differences in glycaemic response. Additional possibilities for future research include quantifying satiety levels throughout and particularly at the end of the test period (e.g. via an ad libitum food task) to ascertain whether NNS consumption as a preload shortens or weakens feelings of satiety.

The finding that blood glucose was significantly lower (-0.6 ± 0.2 compared with -0.2 ± 0.1 mmol·L⁻¹) in the diet cola preload condition at 120 minutes warrants further investigation, as it indicates that NNS consumption impacted on the glycaemic response to a glucose load. Pepino et al. (2013) noted similar findings in that glycaemia following NNS pre-treatment was significantly lower at 180 minutes, although this was following a 75 g OGTT. However, raw fasted blood glucose values at baseline (-10 minutes) were significantly (15%) higher in the diet cola group compared with the carbonated water preload condition in the present study. Similarly, values at the 0-minute point were 20% higher in the diet cola pre-treatment condition and showed a trend towards statistical significance. Higher baseline glycaemia may provide greater room for depression of blood glucose levels throughout the test. Since statistical tests attempting to ascertain differences in blood glucose at each of the time points were conducted on blood glucose increments, it is possible that the higher blood glucose values in the diet cola condition at baseline may have negatively impacted on the ability of the study to detect differences in response to NNS consumption. Indeed, when comparing raw blood glucose values at 120 minutes with a Wilcoxon matched pairs signed rank test, this difference was no longer significant (P=0.401; data not shown). It is therefore recommended that findings are treated with caution, and further investigation is required.

Results add to the current body of literature demonstrating the absence of acute effects of NNS on glycaemia when consumed prior to a glucose beverage (e.g. Brown et al., 2012; Temizkan et al., 2015; Wu et al., 2013). Findings have potential implications for individuals with obesity electing to lessen sugar or energy intake. However, there is evidence to suggest that the replacement of SSBs with artificially-sweetened beverages may elicit a compensatory effect (Mattes and Popkin, 2009), resulting in a proportion of the energy intake avoided through substitution with NNS being consumed at a later point. Regarding aspartame, this compensatory effect is reduced when beverages (compared with solid foods) are substituted and seldom reaches 100% (de la Hunty et al., 2006). Therefore, the substitution of SSBs and replacement with those sweetened with NNS may be an effective means of reducing energy intake and achieving weight loss for those with obesity.
However, research by Brown et al. (2012) and Temizkan et al. (2015) indicates that the hormonal response to NNS and a glucose load may vary depending on metabolic health and may differ for those with type 2 diabetes. As the present study was conducted in healthy participants without a T2DM diagnosis, findings cannot necessarily be generalised to this population. In addition, differences in type and quantity of NNS used in studies can make comparisons difficult. Acesulfame-K has been shown to activate bitter-taste receptors (Kuhn et al., 2004) whereas aspartame and sucralose do not (Dotson et al., 2008). Aspartame is also metabolised differently to sucralose. Whereas sucralose is non-metabolisable (Grotz and Munro, 2009), aspartame undergoes proteolysis in the gastrointestinal tract into its constituent parts: phenylalanine, aspartic acid and methanol (Rycerz and Jaworska-Adamu, 2013). The effects of these metabolites on glycaemic response in the present study cannot be ruled out.

Although the absence of acute glycaemic effects of NNS administered as a preload in the present study is promising, longer-term adverse consequences cannot be overlooked. A recent scoping review of 372 studies focusing on health outcomes resulting from NNS consumption noted that the evidence base is inconsistent and inconclusive in this area (Lohner et al., 2017). However, a review of randomised controlled trials and prospective cohort studies by Azad et al. (2017) concluded that although chronic intakes of NNS were not associated with changes in glycaemia, long-term NNS consumption predicted higher BMI and adverse cardiometabolic outcomes. In a comprehensive review of the scientific literature, Sylvetsky and Rother (2018) report a positive association between NNS consumption and poor metabolic health. Yet, sugar consumption is itself predictive of adverse long-term health outcomes including metabolic syndrome, T2DM (Malik et al., 2010), cardiovascular disease (Yang et al., 2014) and all-cause mortality (Tasevska et al., 2014). The replacement of SSBs with those containing NNS may therefore still be associated with health benefit, particularly in ameliorating the risk of adverse outcomes. However, the long-term effects of NNS are currently poorly understood, and further research including prospective cohort studies and robust randomised controlled trials in this area is required.

A number of limitations exist in the present study. All participants consumed the diet cola pre-treatment first. It is possible that the higher fasting blood glucose in the diet cola condition was as a result of the novelty of the test and/or anxiety regarding an initial fingertip pinprick. Anxiety is associated with elevated catecholamines (Paine et al., 2015) some of which facilitate hepatic glycogenolysis (Sherwin and Saccà, 1984) and subsequent release of glucose into the blood. It is acknowledged that this may have contributed to the discrepancy in baseline blood glucose values. Therefore, recommendations are that future research employ a more robust study design, requiring half of participants to consume a different pre-treatment in the first condition (e.g. as in Brown et al., 2012). It is possible that this may minimise differences in fasting blood glucose between conditions.

Furthermore, participants were not given a standardised meal on the evening prior to the test. Evidence suggests that evening meal macronutrient composition (Robertson et al., 2002) and glycaemic index (Wolever et al., 1988) may affect glycaemic and hormonal responses the following morning. Although every effort was made to ensure adherence to test instructions (such as remaining fasted and
abstaining from strenuous activity in the 24 hours preceding the test), minor deviations from protocol (which may include chewing gum in the morning, for example) cannot be ruled out. In addition, many household items including toothpaste and dental floss may contain NNS and it is not possible to rule out the acute effects of these on study findings. Additionally, regular NNS consumption has been shown to affect sweet taste perception (Appleton and Blundell, 2007). The habitual NNS use by participants in the present study may have therefore affected the acute glycaemic response to an NNS pre-treatment. While future research could employ a screening questionnaire to include only those with or without a history of habitual NNS consumption, the ubiquity of NNS (Sylvetsky and Rother, 2016) would render this impracticable.

The use of only one specific brand of diet beverage may negatively impact on the external validity and practical application of results. Future work may therefore include a number of common/popular diet beverages sweetened with NNS (e.g. lemonade, citrus soda, or diet energy drinks). Additionally, the possible effect of other ingredients (e.g. flavourings) in the diet cola or minerals in carbonated water cannot be disregarded. The specific quantities of the NNS aspartame and acesulfame-K used to sweeten UK diet cola may also impact on the external validity of findings, particularly in other parts of the world where the specific types and quantities of NNS used to sweeten diet beverages may be different. However, this study has a number of strengths. Firstly, the unique combination of NNS in the quantities present in diet cola and their effect on glycaemia represents an unexplored area of research. Having reviewed the literature, this study is the first to examine the acute glycaemic effects of the NNS in this specific brand of diet cola when consumed prior to a glucose load. Secondly, the study may to an extent replicate real-world conditions. For example, the 250 mL beverage amount represents an average glass size and NNS are unlikely to be consumed in isolation (i.e. it is probable that NNS are consumed in combination with other ingredients/nutrients).

Conclusion
This unique study adds to the body of research demonstrating the glycaemic inactivity of NNS when consumed prior to a glucose load and may have wide-reaching implications when viewed in the context of current literature. For example, findings support the suitability of this particular brand of artificially sweetened diet cola for individuals with obesity or those wishing to reduce energy intake and achieve weight loss. However, the observation that glycaemia following diet cola declined towards the end of the test period reiterates the need for additional robust studies in this area. The long-term health effects of NNS consumption remain unclear at present. Before any definitive conclusions may be drawn, further research is required regarding the efficacy and safety associated with long-term use of non-nutritive sweeteners as sugar substitutes.

Acknowledgements
The author wishes to thank Dr Gail Rees, for her support and guidance throughout, and Victoria Cammack and Natalie Sweet from the Nutrition, Exercise and Health laboratories at the University of Plymouth for their technical support.
References

Ahrén, B. and Holst, J.J. (2001). The cephalic insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycaemia. *Diabetes*, 50(5), pp.1030-1038.


