ACCEPTED ARTICLE PREVIEW

Accepted Article Preview: Published ahead of advance online publication

Effects of aspartame-, monk fruit-, Stevia-, and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake

S L Tey, N B Salleh, J Henry, C G Forde

Cite this article as: S L Tey, N B Salleh, J Henry, C G Forde, Effects of aspartame-, monk fruit-, Stevia-, and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake, *International Journal of Obesity* accepted article preview 13 December 2016; doi: 10.1038/ijo.2016.225.

This is a PDF file of an unedited peer-reviewed manuscript that has been accepted for publication. NPG are providing this early version of the manuscript as a service to our customers. The manuscript will undergo copyediting, typesetting and a proof review before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers apply.

Accepted manuscript

Received 14 July 2016; revised 13 October 2016; accepted 13 November 2016; Accepted article preview online 13 December 2016

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

Effects of aspartame-, monk fruit-, Stevia-, and sucrose-sweetened beverages on postprandial glucose, insulin and energy intake

Siew Ling Tey[1], Nurhazwani B Salleh[1], Jeyakumar Henry[1,2] and Ciarán G. Forde[1,3*]

[1]Clinical Nutrition Research Centre, Singapore Institute for Clinical Sciences, Agency for Science, Technology and Research (A*STAR), Singapore; [2]Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, Singapore 117596; [3]Department of Physiology, Yong Loo Lin School of Medicine, National University of Singapore, 2 Medical Drive, Singapore 117597. *Correspondence and offprint requests: Dr Ciarán Forde, Clinical Nutrition Research Centre, Singapore Institute for Clinical Sciences, 14 Medical Drive, Singapore 117599. Tel: +6564070104. Fax: +6567766840. Email: Ciaran_Forde@sics.a-star.edu.sg

The authors declare no conflicts of interest.

This research was supported by the Singapore Institute for Clinical Sciences, A*STAR, Singapore.

Running title: Glycaemic response to non-nutritive sweeteners

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

ABSTRACT

BACKGROUND: Substituting sweeteners with non-nutritive sweeteners (NNS) may aid in glycaemic control and body weight management. Limited studies have investigated energy compensation, glycaemic and insulinaemic responses to artificial and natural NNS.

OBJECTIVES: This study compared the effects of consuming NNS (artificial vs. natural) and sucrose (65 g) on energy intake, blood glucose and insulin responses.

METHODS: Thirty healthy males took part in this randomised, crossover study with four treatments: aspartame-, monk fruit-, Stevia-, and sucrose-sweetened beverages. On each test day, participants were asked to consume a standardised breakfast in the morning and they were provided with test beverage as a preload in mid-morning and *ad libitum* lunch was provided an hour after test beverage consumption. Blood glucose and insulin concentrations were measured every 15 minutes within the first hour of preload consumption and every 30 minutes for the subsequent two hours. Participants left the study site three hours after preload consumption and completed a food diary for the rest of the day.

RESULTS: *Ad libitum* lunch intake was significantly higher for the NNS treatments compared to sucrose (P = 0.010). The energy "saved" from replacing sucrose with NNS was fully compensated for at subsequent meals, hence no difference in total daily energy intake was found between the treatments (P = 0.831). The sucrose-sweetened beverage led to large spikes in blood glucose and insulin responses within the first hour whereas these responses were higher for all three NNS beverages following the test lunch. Thus, there were no differences in total area under the curve (AUC) for glucose (P = 0.960) and insulin (P = 0.216) over three hours between the four test beverages.

CONCLUSIONS: The consumption of calorie free beverages sweetened with artificial and natural NNS have minimal influences on total daily energy intake, postprandial glucose and insulin compared to a sucrose-sweetened beverage.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

INTRODUCTION

The prevalence of diabetes and obesity has increased substantially worldwide in the last few decades[1, 2] and highlights the urgent need to devise interventions at population level to reduce the risks. Interventions such as limiting intake of added sugar[3-5] or substituting nutritive sweetener with non-nutritive sweeteners (NNS) have the potential to reduce energy intake[6-9] and to prevent and control diabetes.[10, 11]

There is a marked increase in the consumption of NNS in both foods and beverages in the recent years due to the increased demand for health and nutrition, enhanced palatability, "sugar free" label, and the relatively low cost of NNS compared to nutritive sweeteners.[12, 13] It has been estimated that the global market of NNS will be close to 10 billion dollars by the end of 2016.[14] The U.S. Food and Drug Administration (FDA) has approved six artificial NNS as food additives and two natural NNS extracted from plants, i.e. Stevia (GRAS Notice No. GRN 000252; *Stevia rebaudiana* Bertoni) and monk fruit (GRAS Notice No. GRN 000301; *Siraitia grosvenorii* Swingle), are Generally Recognised As Safe for consumption.[15] Due to the rapidly growing popularity of natural plant-derived compounds,[16] it will be of interest to determine whether natural NNS would be a healthier alternative to sugar and artificial NNS for consumers.

To date, only one study has investigated the effects of artificial and natural NNS on food intake, postprandial glucose and insulin.[17] This randomised crossover study utilized a double preload design to compare the effects of consuming two preloads of crackers and cream cheese sweetened with sucrose (986 kcal), aspartame (580 kcal) or Stevia (580 kcal); one prior to lunch and one before dinner, on energy intake, postprandial glucose and insulin concentrations. Daily energy intake was significantly lower when participants consumed preloads with aspartame and Stevia, compared to a sucrose preload. Stevia preload also showed additional benefit of lowering postprandial blood glucose and insulin concentrations, compared to aspartame and sucrose preloads. However, it is unknown whether the

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

beneficial effect of Stevia can be generalised to monk fruit and also whether the same result can be obtained if the preload contains no calories, e.g. diet beverage.

Monk fruit is native to China and it has been used as a traditional medicine and natural sweetening agent for several centuries among Asians.[18, 19] The consumption of monk fruit has been shown to exhibit antihyperglycaemic effect by stimulating insulin secretion in diabetic rats.[20, 21] However, no study has investigated the effects of monk fruit consumption on glucose and insulin responses and energy intake in humans.

The aim of the current study was to examine the effects of consuming four different types of sweeteners (aspartame, monk fruit, Stevia, and sucrose) in liquid form on postprandial glucose, insulin and total daily energy intake.

Accepted manuscript

SUBJECTS AND METHODS

Participants

Thirty-four males were recruited from the general public through advertisements placed around the National University of Singapore campus. The study inclusion criteria were healthy males aged between 21 and 50 years with normal BMI (18.5 to 25.0 kg/m^2). The exclusion criteria were people with major chronic disease, intolerances or allergies to study foods or test products, and taking any drug known to affect appetite, glucose or energy metabolism. Individuals whose body weight had changed more than 5 kilograms in the last 12 months and those who were currently dieting were also excluded.

The inclusion criteria for the present study was tightly controlled, i.e. males only. We focused our comparison on the differences in blood glucose response and energy compensation between the study beverages rather than gender differences in these outcomes. In addition, previous literature from our group has shown that males and females differ in their energy compensation for caloric beverages[22] and menstrual cycle of females may play a role in energy intake and basal metabolic rate[23], therefore only

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

males were included in this study. A relatively homogenous study population could reduce the variability of the data and increase the power to detect a meaningful difference in the outcome of interest.

The National Healthcare Group Domain Specific Review Board in Singapore approved all study procedures. All study participants provided written informed consent. This trial was registered at Australian New Zealand Clinical Trials Registry as ACTRN12615001321538.

Design

This was a randomised crossover study with four treatments. All participants were randomly assigned to the treatments, with the order balanced. They were required to have a minimum of five-day hiatus between the test days and they did not receive or consume any study beverage during this period. Participants were asked to continue their usual diet and physical activity during the study.

Participants were blinded to the treatment allocation. Researchers who assessed the outcomes and those who analysed the data were also blinded to the treatment allocation.

Test food

Test beverages were: (i) 0.44 g aspartame, (ii) 0.63 g monk fruit extract (50% Mogroside V), (iii) 0.33 g Stevia (Steviol Glycoside, Rebaudioside A), and (iv) 65 g sucrose, in 500 mL water. A pilot test was carried out before the study to confirm that the test beverages were deemed perceptibly similar in their sweetness intensity, flavour, and liking (all $P \geq 0.070$). Sixty five grams of sucrose were chosen as this is the amount commonly found in commercial beverage. All test beverages were spiked with strawberry flavouring and pink colouring in order to mask any potential differences in taste and visual cues between the beverages.

The breakfast and lunch used in the current study are typical foods consumed among young Singaporeans and had been used in previous studies conducted on the same population at the Clinical

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

Nutrition Research Centre (CNRC).[24, 25] The standardised breakfast consisted of a small carton of Milo (Nestle: 250 mL), an apple (Fuji: 200 g), a packet of cheese sandwich biscuits (Julie's: 28 g) and one muesli bar (Uncle Toby's: 24 g) packing up a total of 529 kcal (88 g carbohydrate, 8 g protein, and 16 g fat). The *ad libitum* test lunch consisted of 800 g of fried rice (1256 kcal, 182.4 g carbohydrate, 52 g protein, and 35.2 g fat) and a glass of water (250 mL). Water intake was optional.

Procedure

All participants were asked to attend five sessions, consisting of one screening session and four test sessions. During the screening session, descriptive measures such as basic anthropometric measurements, body composition, blood pressure, and fasting blood glucose were collected after a minimum of 10-hour overnight fast. Anthropometric measurements were recorded which included height and weight (Seca 763 Digital Scale), waist and hip circumferences (luftkin W606PM measuring tape), and body composition (Cosmed BodPod 2007A). Blood pressure was taken in triplicate (Omron HEM-907) and fasting blood glucose was taken in duplicate (Hemocue® Glucose 201 Analyser) and the values were averaged. Participants were also asked to fill out a Dutch Eating Behaviour Questionnaire (DEBQ) and a screening questionnaire including questions such as food allergies or intolerance, chronic diseases, and basic health information.

Following this, participants were required to attend four test sessions where they were randomised to the test conditions. Prior to each test day, participants were asked to refrain from vigorous physical activity, consume an evening meal of similar composition and quantity and fast for ten hours. For instance, if the participants consumed a meat dish, a vegetable dish and a bowl of white rice on the evening before their first session, they were asked to consume something similar in terms of composition and quantity on the evenings for all subsequent sessions. An overview of the study protocol is shown in **Figure 1**. On each test day, participants were asked to consume a pre-package study

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

breakfast outside CNRC between 8 and 9 am. Participants arrived at CNRC between 11 am and 12 pm, where they were randomly allocated to receive a fixed portion of one of the study beverages as a preload. Baseline (i.e. 0 minute) blood samples and appetite measures were taken prior to beverage consumption. Participants were required to consume the entire beverage within 15 minutes. An hour after the commencement of study beverage consumption, participants were given an *ad libitum* portion of fried rice for lunch, where they were allowed to consume as little or as much as they wished until they felt comfortably full. Further blood samples were taken at 15, 30, 45, 60, 90, 120, 150, and 180 minutes after baseline. Appetite measures were taken immediately after the blood draws at each time point. Participants were allowed to leave CNRC after the last measurement at 180 minutes post baseline. During the three-hour test session, participants were asked to remain rested in the laboratory.

Participants were instructed to keep a diet record to note down as well as to take photos of any food and drinks they consumed after they left the study site. A nutritionist provided verbal instructions to each participant on the way to collect diet records. Written instructions were also provided in the diet record. A trained researcher reviewed all the diet records and photos of the food and drinks upon return for accuracy and completeness. A nutritionist entered all dietary data and the diet records were analysed using version 6.70 of the Diet Plan nutrient database (Forestfield Software Ltd, West Sussex, UK).

Accepted manuscript

Hedonic, sensory and appetite ratings

Prior to consuming the beverage, participants were asked to rate their appetite and mood states on a 100 mm visual analogue scale (VAS) anchored with "not at all *<rating>*" (0 mm) and "extremely *<rating>*" (100 mm). Appetite questions included desire to eat, hunger, prospective consumption, fullness, and thirst. In addition, several distractor questions such as happiness, clear-headed, and alertness were included in the appetite questionnaire. Following this, participants were asked to have a

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

sip of the beverage and rate various attributes such as pleasantness, thickness, desire to eat, bitterness, sweetness, filling, familiarity, overall liking of the flavour. Participants were asked to complete appetite questionnaires every 15 minutes for the first hour. Following this, participants received an *ad libitum* portion of fried rice and water (optional). Mood and appetite questions similar to those completed by participants during the beverage session were completed before and after lunch. Leftover fried rice and water was then weighed and recorded.

Blood analysis

Finger prick blood samples were obtained for glucose and insulin analyses. For glucose analysis, blood samples were collected directly into HemoCue® cuvettes and analysed using HemoCue® Glucose 201 analyser (Helsingborg, Sweden). For insulin analysis, 300 µL blood samples were obtained at each time point and collected into micro-tubes containing dipotassium EDTA. All blood specimens were separated by centrifugation at 8000 rpm for ten minutes at 4°C (ThermoFisher Scientific, Sorvall Legend Micro 17R) within two hours of being drawn and plasma aliquots were stored at -80°C until analysis. Blood insulin was analysed by using a commercial kit from Roche on Cobas e411 immunoassay analyser (Roche Diagnostics). The mean intra- and inter-assay CVs for insulin were 1.84% and 1.80% respectively. Total blood glucose and insulin responses were expressed as the incremental area under the curve (iAUC) above baseline and calculated using the trapezoidal rule.[26]

Data analysis

Based on our previous study looking at blood glucose response to liquids with different nutrient composition in a similar population group,[27] it has been calculated that in order to have 90% power at the two-sided 0.05 level to detect a difference of 30% in total AUC for glucose between the treatments,

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

25 participants would be required with full data. In addition, ten participants with complete data would be needed to detect a difference of 30% in total AUC for insulin between the treatments.[28]

Baseline characteristics of the study participants were reported as means and s.d. Linear mixed models were used to compare the effects of the study treatments on 'energy intake', 'appetite ratings', 'glycaemic response', and 'insulinaemic response'. Log transformations were used where this improved residual normality and/or homoscedasticity. All the outcome measurements, except for blood insulin, were assessed on all participants (n = 30). Blood insulin analyses were performed for ten participants who were randomly chosen. The linear mixed models also assessed the influence of 'age', 'body fat percentage', 'fat free mass', 'BMI', 'basal metabolic rate', 'restraint status', 'session number', and 'lunch intake' on all study outcomes. In addition, sensory ratings of the test beverages such as 'pleasantness', 'bitterness', 'sweetness', and 'overall liking of the flavour' were adjusted for in all the mixed models for food intake analyses. None of these variables significantly influenced the outcomes and hence they were excluded from the final models. Stata 11.2 (StataCorp LP, TX, US) was used for all analyses and two-sided $P < 0.05$ was considered significant.

Accepted manuscript

RESULTS

Participants' characteristics

Of the 34 participants recruited and screened for the study, three participants withdrew from the study prior to randomisation as they could no longer commit to all the test sessions and another participant withdrew due to personal reason. Thirty participants completed the study. Participants were young and relatively lean males with a mean (s.d.) age of 27.6 (5.5) years, BMI of 21.7 (1.9) kg/m^2, percent body fat of 17.6 (8.4) %, waist circumference of 75.1 (6.1) cm, hip circumference of 90.6 (5.4) cm, and DEBQ restraint score of 2.2 (0.8). Participants' fasting blood glucose and blood pressure were within the normal range (**Supplementary Appendix 1**).

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

Hedonic, sensory and appetite ratings of the test beverages and lunch

Participants were asked to make hedonic, sensory, and appetite ratings of test beverages after consuming a sip of the beverage (**Table 1**). All test beverages had similar thickness and were expected to deliver the same fullness. Small but statistically significantly differences were found in sweetness and bitterness. Beverages sweetened with monk fruit and Stevia were rated as slightly less sweet and more bitter than the aspartame- and sucrose-sweetened beverages. Participants repeated many of these ratings after consuming a spoonful of the lunch meal and the results are summarized in **Table 1**.

Figure 2 (a-d) shows the appetite ratings over a three-hour period, where 0 minute indicates the time prior to preload consumption and 60 minutes represents the time before lunch. Desire to eat, hunger, and prospective consumption ratings from 30 to 60 minutes were significantly higher whereas fullness rating was lower for the three NNS treatments compared to the sucrose treatment. There was very little difference in appetite ratings between the four beverages after lunch. No significant differences were found between the treatments for thirst or distractors such as happiness, clear-headed, and alertness (data not shown).

Accepted manuscript

Energy intake

Figure 3 shows the breakdown of energy consumed at each meal for all the treatments. Although *ad libitum* lunch intake was significantly higher (ranging from 57 to 82 kcal) in the NNS treatments compared to sucrose, the subsequent meal intake after participants left the study site did not differ between the four treatments (**Table 1**). Mean (s.e.) total daily energy intake was 2330 (108) kcal, 2306 (114) kcal, 2241 (78) kcal, and 2312 (76) kcal for aspartame, monk fruit, Stevia, and sucrose treatment, respectively. Energy compensation score was 107% for aspartame, 98% for monk fruit, and 73% for Stevia.

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

Glycaemic and insulinaemic responses

The sucrose-sweetened beverage consumption resulted in large spikes in glucose and insulin concentrations whereas these measures were relatively stable with NNS sweetened beverages within the first 60 minutes. On the other hand, there were sharper rise in glucose and insulin responses after lunch with the three NNS beverages compared with sucrose-sweetened beverage (**Figure 4** and **Figure 5**). The differences in glucose and insulin responses between NNS- and sucrose-sweetened beverages persist, even after adjusting for lunch intake and other potential confounders. Blood glucose for all three NNS preloads peaked at 120 minutes and range from 2.4 to 2.7 mmol/L above baseline, which was two times higher than the sucrose preload (1.3 mmol/L above baseline). There were no differences in total AUC for glucose (P = 0.960) and insulin (P = 0.216) over the three-hour period between the four treatments (**Table 1**). Temporal curves and total AUC for blood glucose and insulin were not statistically significantly different between the NNS treatments.

DISCUSSION

This study is the first to compare the effects of consuming preloads sweetened with sucrose, artificial NNS (aspartame) and natural NNS (monk fruit and Stevia) on energy intake, glycaemic and insulinaemic responses. The findings showed a surprising result, where there was no difference in total daily energy intake across all four treatments, with full compensation of the energy obtained from sucrose. In addition, glucose and insulin responses were significantly higher for all three NNS preloads following the test lunch compared to sucrose preload. Energy intake, blood glucose and insulin responses did not differ between the three NNS treatments, suggesting the source of NNS e.g. artificial or natural, does not influence these outcomes.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

One of the concerns with NNS consumption is the increased appetite, which may lead to overcompensation for the energy saved. The present study found that although desire to eat, hunger and prospective consumption ratings were higher after consuming NNS preloads compared to sucrose preload, there was no evidence of overconsumption on NNS preload days. This is supported by a recent review which reported that NNS consumption does not increase liking and wanting for sweet tasting foods and lead to higher energy intake.[29] In the present study, partial energy compensation (22 to 32%) was observed at lunch time and the energy saved from switching to NNS was fully compensated for by the end of the test day. This is also contrary to previous findings which suggest that NNS consumption may reduce overall energy intake and aid in body weight management.[6, 7] Nevertheless, it is important to highlight that the current study investigated the simple exchange of one serving of sucrose-sweetened beverage (65 g of sucrose) with either natural or artificial NNS over a day. A recent meta-analysis, which included twelve randomised controlled trials conducted in adults, showed that consuming NNS for four to 26 weeks led to a significant weight loss of 0.72 kg and most importantly none of the randomised controlled trials included in this meta-analysis showed weight gain with NNS consumption.[30] When the results are taken together, the evidence seems to suggest that the use of NNS does not lead to overconsumption. Furthermore, if NNS is used to replace nutritive sweeteners and in the absence of any compensatory eating behaviour, this will in theory lead to a net deficit in overall energy intake, which may promote weight loss over a long period of time[6, 29], although this cannot be confirmed in the current study due to the acute nature of the trial.

Postprandial glycaemia is a significant risk factor for diabetes and cardiovascular disease.[31, 32] The present study showed large spikes in glucose and insulin responses within the first hour following sucrose-sweetened beverage consumption whereas no such changes were seen with NNS consumption. However the rise in glucose concentration after lunch was higher with NNS consumption compared to the sucrose-sweetened beverage. Hence the total AUC for glucose were similar across all four

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

treatments. A previous study also reported that NNS consumption did not stimulate the release of insulin, GIP or GLP-1 in healthy humans.[33] A recent study provided participants with a preload as (i) water, or water sweetened with (ii) sucrose, (iii) sucralose, (iv) sucrose and sucralose, an hour before breakfast. Results showed an increase in blood glucose 30 minutes after consuming sucrose-containing preloads but not sucralose or water preload while the opposite was observed after breakfast, i.e. blood glucose was significantly higher in sucralose or water preload but not sucrose-containing preloads.[34] This could be in part explained by the higher insulin response following the consumption of sucrose-containing preload and the flat insulin response after consuming preloads with NNS within the first hour.[34]

It is noteworthy that consuming a sucrose-sweetened beverage prior to the lunch did not lead to a cumulative effect on blood glucose and result in a much higher glucose response after lunch. Interestingly, the blood glucose peak at 120 minutes was significantly higher with the NNS preloads compared to the sucrose preload. There are some suggestions from animal studies that NNS may activate sweet taste receptors and in turn enhance glucose uptake via upregulation of transporters.[35-37] A previous study conducted in 17 obese insulin sensitive predominantly non-Hispanic black women who were not regular users of NNSs reported that consuming NNS ten minutes before a 75 g oral glucose tolerance test (OGTT) led to higher peak glucose compared to water consumption before OGTT, although no significant differences were found in the iAUC for glucose.[38] However, another study did not show any differential effect on glucose when participants consumed a preload sweetened with NNS before a meal compared with water.[39] The increase in glucose absorption was also not observed in human studies when participants consumed NNS with sugar or other caloric foods. For instance, when participants consumed a preload of crackers with cream cheese sweetened with NNS twenty minutes before lunch, postprandial glucose concentration following lunch was significantly lower with NNS compared to a preload sweetened with sucrose.[17] Overall the evidence seems to suggest that

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

consuming NNS alone without any energy (empty calorie) before a meal may lead to larger spikes in glucose and insulin responses after the meal. The preload used in the current study resembles a pre-meal beverage such as a mid-morning sugar-sweetened beverage prior to lunch or a midafternoon soft drink prior to dinner.

The present study showed that although sucrose led to significant higher glycaemic and insulinaemic responses, this did not translate into a higher energy intake at the subsequent meal. In line with this finding, previous research reported that consuming sucrose-sweetened beverage as a preload an hour before the meal led to greater glycaemic response, suppressed appetite and resulted in lower meal energy intake in young men, compared to preloads with lower glycaemic response such as amylose or fructose-glucose mixture.[40, 41] Similar result was also reported in another acute study conducted in overweight restrained and unrestrained women.[42] It is important to point out that Henry et al. reported that consuming a low GI beverage at three main meals significantly reduced mean 24-hour glucose and iAUC for glucose, compared to a control sucrose beverage.[43] In addition, a previous study showed that consuming a diet high in sucrose (2 g per kg of body weight) for ten weeks led to significantly higher postprandial glucose response compared to a diet rich in NNS.[44] It is possible that a higher number of exposures or a longer intervention period may be required in order for the beneficial effects of NNS on glycaemia and insulinaemia to occur.

The strengths of the current study include its robust study design, the comparison of one artificial NNS and two natural NNS with sucrose, and being the first study to investigate the effects of monk fruit consumption on energy intake, glycaemic and insulinaemic responses in humans. However, this study was not without limitations. One limitation is that the study relied on self-reported dietary data for subsequent meals after participants left the study site. It should be noted that the observed and weighed lunch intake at the study site was the primary meal of interest. However, results from diet records were also reported in order to determine whether participants compensated later in the day.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

Extensive efforts had been placed to ensure the accuracy and completeness of participants' diet records. For instance, participants were asked to take photos and record down all the foods and drinks they consumed in details. Trained researchers checked all the food photos and diet records upon return and a single nutritionist entered all the dietary data for consistency. In addition, although the sensory results were similar across all treatments in the pilot test, small but significant differences in the pleasantness, bitterness, sweetness, and overall liking of the flavour ratings were found between the treatments in the actual trial, which may have influenced the food intake results. It is important to highlight that these sensory qualities were controlled for in all the mixed models for the food intake analyses and results remained unchanged after controlling for these variables. Another limitation is that the study duration was only one day. Longer-term studies are warranted to determine the prolonged effects of nutritive and non-nutritive sweeteners on glycaemic control and body weight. Lastly, participants in the present study were males with normal BMI, limiting the extrapolation of the study results. Future research should extend the study populations to females and participants with a range of BMI, including both overweight and obese individuals.

Accepted manuscript

CONCLUSION

In conclusion, the consumption of calorie free beverages sweetened with non-nutritive sweeteners has minimal influences on total daily energy intake, glucose and insulin responses compared to a sucrose-sweetened beverage in healthy lean males. It appears that the source of non-nutritive sweeteners (artificial or natural) does not differ in their effects on energy intake, postprandial glucose and insulin.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

ACKNOWLEDGEMENTS

The authors would like to thank all the participants for taking part in this study and Mr Edwin Chia for his assistance in entering all the diet records.

AUTHOR CONTRIBUTIONS

SLT, JH, and CF designed the research; SLT and NS conducted the research; SLT performed statistical analyses and drafted the manuscript; all authors approved the final manuscript.

Supplementary Information is available at International Journal of Obesity's website (http://www.nature.com/ijo)

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

REFERENCES

1. N. C. D. Risk Factor Collaboration. Worldwide trends in diabetes since 1980: a pooled analysis of 751 population-based studies with 4·4 million participants. *The Lancet* 2016; **387**: 1513-1530.
2. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C *et al.* Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. *The Lancet* 2014; **384**: 766-781.
3. Johnson RK. Reducing intake of sugar-sweetened beverages is vital to improving our nation's health. *Circulation* 2016; **133**: 347-349.
4. Mitka M. New dietary guidelines place added sugars in the crosshairs. *JAMA* 2016; **315**: 1440-1441.
5. World Health Organization. Guideline: Sugars intake for adults and children. Geneva: World Health Organization; 2015.
6. Gardner C. Non-nutritive sweeteners: evidence for benefit vs. risk. *Curr Opin Lipidol* 2014; **25**: 80-84.
7. Mattes RD, Popkin BM. Nonnutritive sweetener consumption in humans: effects on appetite and food intake and their putative mechanisms. *Am J Clin Nutr* 2009; **89**: 1-14.
8. Rogers PJ, Hogenkamp PS, de Graaf C, Higgs S, Lluch A, Ness AR *et al.* Does low-energy sweetener consumption affect energy intake and body weight? A systematic review, including meta-analyses, of the evidence from human and animal studies. *Int J Obes* 2016; **40**: 381-394.
9. Zheng M, Allman-Farinelli M, Heitmann BL, Rangan A. Substitution of sugar-sweetened beverages with other beverage alternatives: a review of long-term health outcomes. *J Acad Nutr Diet* 2015; **115**: 767-779.
10. Franz MJ, Powers MA, Leontos C, Holzmeister LA, Kulkarni K, Monk A *et al.* The evidence for medical nutrition therapy for type 1 and type 2 diabetes in adults. *J Am Diet Assoc* 2010; **110**: 1852-1889.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

11. Johnston CA, Stevens B, Foreyt J. The role of low-calorie sweeteners in diabetes. *US Endocrinol* 2013; **9**: 13-15.
12. Gardner C, Wylie-Rosett J, Gidding SS, Steffen LM, Johnson RK, Reader D *et al.* Nonnutritive sweeteners: Current use and health perspectives: A scientific statement from the American Heart Association and the American Diabetes Association. *Circulation* 2012; **126**: 509-519.
13. Sylvetsky AC, Rother KI. Trends in the consumption of low-calorie sweeteners. *Physiol Behav* 2016; **164**: 446-450.
14. Ibrahim OO. How sweet it is: Chemicals structure, properties and applications of high intensity sweeteners. *EC Nutrition* 2015; **1**: 57-66.
15. U.S. Food and Drug Administration. High-intensity sweeteners. 2014. Available from: http://www.fda.gov/Food/IngredientsPackagingLabeling/FoodAdditivesIngredients/ucm397716.htm.
16. Pawar RS, Krynitsky AJ, Rader JI. Sweeteners from plants—with emphasis on Stevia rebaudiana (Bertoni) and Siraitia grosvenorii (Swingle). *Anal Bioanal Chem* 2013; **405**: 4397-4407.
17. Anton SD, Martin CK, Han H, Coulon S, Cefalu WT, Geiselman P *et al.* Effects of stevia, aspartame, and sucrose on food intake, satiety, and postprandial glucose and insulin levels. *Appetite* 2010; **55**: 37-43.
18. Jin J-S, Lee J-H. Phytochemical and pharmacological aspects of Siraitia grosvenorii, luo han kuo. *Orient Pharm Exp Med* 2012; **12**: 233-239.
19. Li C, Lin L-M, Sui F, Wang Z-M, Huo H-R, Dai L *et al.* Chemistry and pharmacology of Siraitia grosvenorii: A review. *Chin J Nat Med* 2014; **12**: 89-102.
20. Qi X-Y, Chen W-J, Zhang L-Q, Xie B-J. Mogrosides extract from Siraitia grosvenori scavenges free radicals in vitro and lowers oxidative stress, serum glucose, and lipid levels in alloxan-induced diabetic mice. *Nutr Res* 2008; **28**: 278-284.
21. Suzuki YA, Tomoda M, Murata Y, Inui H, Sugiura M, Nakano Y. Antidiabetic effect of long-term supplementation with Siraitia grosvenori on the spontaneously diabetic Goto–Kakizaki rat. *Br J Nutr* 2007; **97**: 770-775.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

22. Ranawana DV, Henry CJK. Are caloric beverages compensated for in the short-term by young adults? An investigation with particular focus on gender differences. *Appetite* 2010; **55**: 137-146.
23. Elliott SA, Ng J, Leow MK-S, Henry CJK. The influence of the menstrual cycle on energy balance and taste preference in Asian Chinese women. *Eur J Nutr* 2015; **54**: 1323-1332.
24. McCrickerd K, Salleh NB, Forde CG. Removing energy from a beverage influences later food intake more than the same energy addition. *Appetite* 2016; **105**: 549-556.
25. Tey SL, Chia EME, Forde CG. Impact of dose-response calorie reduction or supplementation of a covertly manipulated lunchtime meal on energy compensation. *Physiol Behav* 2016; **165**: 15-21.
26. Brouns F, Bjorck I, Frayn K, Gibbs A, Lang V, Slama G *et al.* Glycaemic index methodology. *Nutr Res Rev* 2005; **18**: 145-171.
27. Tey SL, Van Helvoort A, Henry CJ. Glycaemic responses to liquid food supplements among three Asian ethnic groups. *Eur J Nutr* 2016: In Press.
28. Sun L, Ranawana DV, Leow MK-S, Henry CJ. Effect of chicken, fat and vegetable on glycaemia and insulinaemia to a white rice-based meal in healthy adults. *Eur J Nutr* 2014; **53**: 1719-1726.
29. Bellisle F. Intense sweeteners, appetite for the sweet taste, and relationship to weight management. *Curr Obes Rep* 2015; **4**: 106-110.
30. Miller PE, Perez V. Low-calorie sweeteners and body weight and composition: a meta-analysis of randomized controlled trials and prospective cohort studies. *Am J Clin Nutr* 2014; **100**: 765-777.
31. Monnier L, Colette C, Owens D. Postprandial and basal glucose in type 2 diabetes: assessment and respective impacts. *Diabetes Technol Ther* 2011; **13**: S25-S32.
32. Dickinson S, Brand-Miller J. Glycemic index, postprandial glycemia and cardiovascular disease. *Curr Opin Lipidol* 2005; **16**: 69-75.
33. Ma J, Bellon M, Wishart JM, Young R, Blackshaw LA, Jones KL *et al.* Effect of the artificial sweetener, sucralose, on gastric emptying and incretin hormone release in healthy subjects. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G735-G739.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

34. Brown AW, Bohan Brown MM, Onken KL, Beitz DC. Short-term consumption of sucralose, a nonnutritive sweetener, is similar to water with regard to select markers of hunger signaling and short-term glucose homeostasis in women. *Nutr Res* 2011; **31**: 882-888.
35. Mace OJ, Affleck J, Patel N, Kellett GL. Sweet taste receptors in rat small intestine stimulate glucose absorption through apical GLUT2. *J Physiol* 2007; **582**: 379-392.
36. Margolskee RF, Dyer J, Kokrashvili Z, Salmon KSH, Ilegems E, Daly K *et al.* T1R3 and gustducin in gut sense sugars to regulate expression of Na+-glucose cotransporter 1. *Proceedings of the National Academy of Sciences* 2007; **104**: 15075-15080.
37. Renwick AG, Molinary SV. Sweet-taste receptors, low-energy sweeteners, glucose absorption and insulin release. *Br J Nutr* 2010; **104**: 1415-1420.
38. Pepino MY, Tiemann CD, Patterson BW, Wice BM, Klein S. Sucralose affects glycemic and hormonal responses to an oral glucose load. *Diabetes Care* 2013; **36**: 2530-2535.
39. Wu T, Bound MJ, Standfield SD, Bellon M, Young RL, Jones KL *et al.* Artificial sweeteners have no effect on gastric emptying, glucagon-like peptide-1, or glycemia after oral glucose in healthy humans. *Diabetes Care* 2013; **36**: e202-e203.
40. Anderson GH, Catherine NL, Woodend DM, Wolever TM. Inverse association between the effect of carbohydrates on blood glucose and subsequent short-term food intake in young men. *Am J Clin Nutr* 2002; **76**: 1023-1030.
41. Anderson GH, Woodend D. Effect of glycemic carbohydrates on short-term satiety and food intake. *Nutr Rev* 2003; **61**: S17-S26.
42. Burton-Freeman BM, Keim NL. Glycemic index, cholecystokinin, satiety and disinhibition: is there an unappreciated paradox for overweight women? *Int J Obes* 2008; **32**: 1647-1654.
43. Henry CJK, Newens KJ, Lightowler HJ. Low-glycaemic index sweetener-based beverages reduce 24-h glucose profiles in healthy adults. *J Hum Nutr Diet* 2009; **22**: 77-80.
44. Raben A, Møller BK, Flint A, Vasilaras TH, Christina Møller A, Juul Holst J *et al.* Increased postprandial glycaemia, insulinemia, and lipidemia after 10 weeks' sucrose-rich diet compared to an artificially sweetened diet: a randomised controlled trial. *Food Nutr Res* 2011; **55**: 5961.

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

FIGURE LEGENDS

Figure 1 Flow chart for the methods on each test day.

Figure 2 (**a**) Desire to eat, (**b**) hunger, (**c**) prospective consumption, and (**d**) fullness ratings overtime (mean ± s.e.) (*n* = 30). *Linear mixed models showed statistically significant differences in these appetite measures between the treatments at those time points, $P < 0.05$. Aspartame (filled square, solid line), Monk fruit (open diamond, short dashed line), Stevia (open triangle, dotted line), Sucrose (filled circle, long dashed line).

Figure 3 Energy intake consumed at each meal on aspartame, monk fruit, Stevia, and sucrose test days (mean ± s.e.) (*n* = 30). A linear mixed model showed no significant difference in total daily energy intake between the treatments ($P = 0.831$). Fixed portion study breakfast (light gray bars), *Ad libitum* study lunch (dotted bars), Free-living subsequent meals (dark gray bars), Test beverage (large grid bars).

Figure 4 Temporal curves of the blood glucose response for the test beverages (mean ± s.e.) (*n* = 30). *Linear mixed models showed statistically significantly differences in blood glucose between the treatments at those time points, $P < 0.05$. Aspartame (filled square, solid line), Monk fruit (open diamond, short dashed line), Stevia (open triangle, dotted line), Sucrose (filled circle, long dashed line).

Figure 5 Temporal curves of the blood insulin response for the test beverages (mean ± s.e.) (*n* = 10). *Linear mixed models showed statistically significantly differences in blood insulin between the treatments at those time points, $P < 0.05$. Aspartame (filled square, solid line), Monk fruit (open diamond, short dashed line), Stevia (open triangle, dotted line), Sucrose (filled circle, long dashed line).

Accepted manuscript

© 2016 Macmillan Publishers Limited. All rights reserved.

ACCEPTED ARTICLE PREVIEW

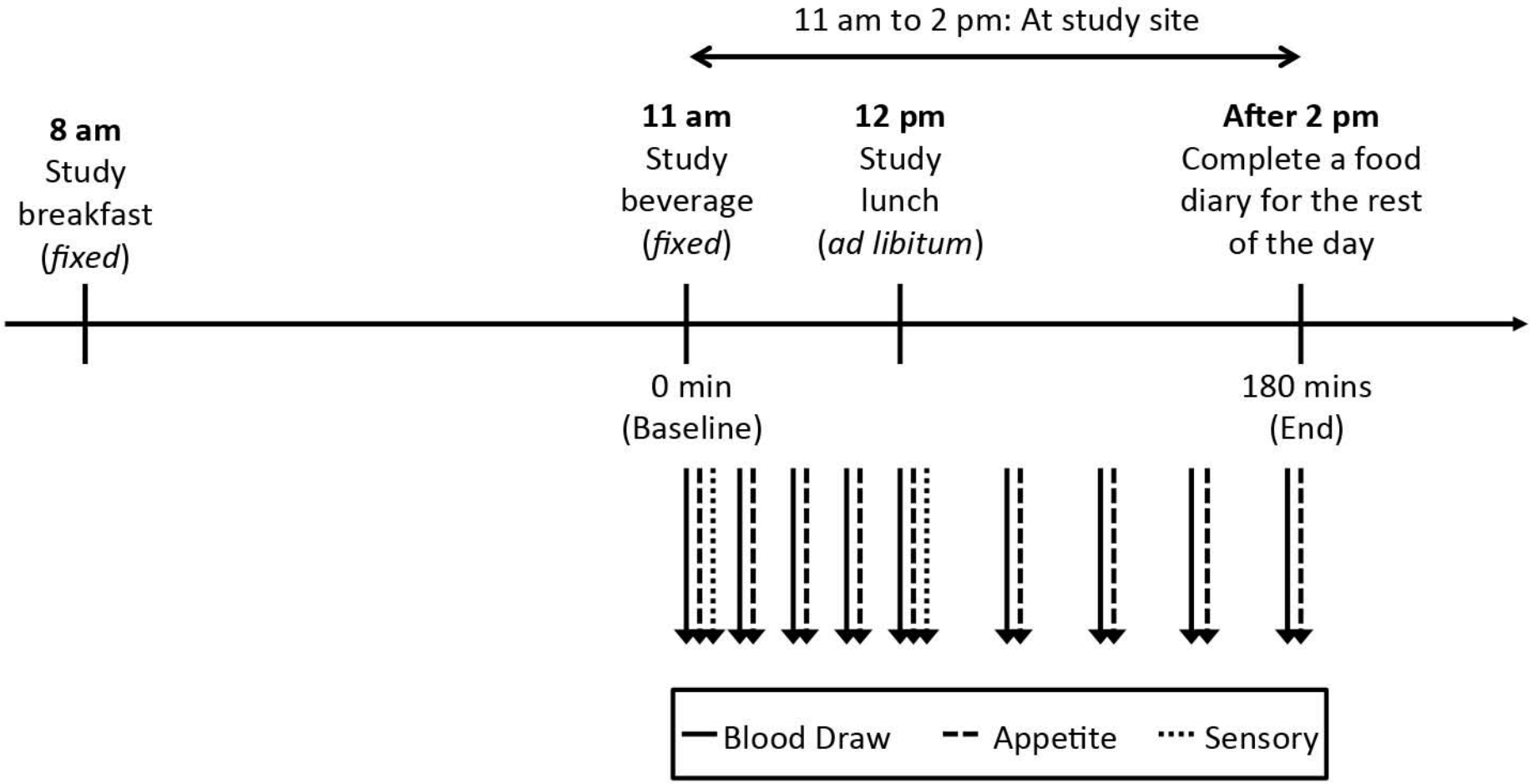
Table 1. Hedonic, sensory, appetite ratings and intake for each treatment (mean ± s.e.) (n = 30)

	Aspartame	Monk Fruit	Stevia	Sucrose	P value
Drink					
Pleasantness (mm)	54.9 ± 4.0 [a]	41.4 ± 4.1 [b]	43.9 ± 4.0 [b]	56.9 ± 3.6 [a]	<0.001
Thickness (mm)	34.7 ± 3.8	41.0 ± 3.8	36.6 ± 4.5	35.9 ± 4.6	0.464
Desire to eat (mm)	60.2 ± 3.5	52.4 ± 3.8	57.9 ± 4.6	55.1 ± 4.0	0.298
Bitterness (mm)	22.0 ± 4.3 [a]	42.2 ± 5.2 [b]	39.2 ± 5.1 [b]	11.7 ± 2.6 [a]	<0.001
Sweetness (mm)	76.3 ± 2.5 [a]	64.6 ± 3.6 [b]	60.2 ± 4.2 [b]	76.6 ± 3.1 [a]	<0.001
Fillingness (mm)	57.7 ± 4.5	54.5 ± 4.0	53.2 ± 3.7	56.4 ± 4.1	0.684
Familiarity (mm)	67.8 ± 3.6	60.3 ± 4.7	54.9 ± 5.1	67.2 ± 3.7	0.064
Overall liking of the flavour (mm)	51.5 ± 3.8 [a]	36.4 ± 4.4 [b]	37.6 ± 3.9 [b]	53.4 ± 3.8 [a]	<0.001
Lunch					
Pleasantness (mm)	69.0 ± 2.8 [ab]	72.8 ± 2.8 [b]	72.0 ± 2.8 [b]	66.5 ± 2.6 [a]	0.005
Sweetness (mm)	37.8 ± 4.7	37.0 ± 4.5	37.0 ± 4.7	31.4 ± 4.3	0.146
Saltiness (mm)	47.1 ± 3.8	49.0 ± 3.6	48.7 ± 3.2	40.8 ± 3.7	0.053
Familiarity (mm)	77.2 ± 3.4	81.2 ± 3.3	74.9 ± 4.3	77.6 ± 2.8	0.333
Hunger (mm)	73.6 ± 3.5 [ab]	78.0 ± 2.2 [a]	72.1 ± 3.2 [bc]	67.4 ± 3.2 [c]	0.005
Desire to eat (mm)	74.5 ± 3.4 [a]	78.0 ± 2.6 [a]	77.8 ± 2.3 [a]	68.2 ± 3.0 [b]	<0.001
Prospective consumption (mm)	76.4 ± 2.9 [a]	77.6 ± 2.2 [a]	78.0 ± 2.2 [a]	70.7 ± 2.4 [b]	0.004
Thirst (mm)	44.7 ± 4.4	43.9 ± 4.6	47.6 ± 4.2	48.4 ± 3.9	0.539
Fullness (mm)	23.6 ± 3.4 [a]	25.9 ± 3.4 [a]	26.8 ± 3.5 [ab]	32.2 ± 3.5 [b]	0.025
Energy Intake					
Ad libitum lunch (kcal)	799 ± 44.4 [a]	824 ± 42.6 [a]	821 ± 41.5 [a]	742 ± 44.8 [b]	0.010
Subsequent meals (kcal)	1002 ± 96.1	953 ± 102.2	892 ± 73.9	781 ± 71.4	0.114
Total daily intake (kcal)	2330 ± 108	2306 ± 114	2241 ± 77.8	2312 ± 76.0	0.831
Glucose Total Area Under the Curve					
Glucose AUC 0 to 60 mins	6.79 ± 1.39 [a]	4.64 ± 1.22 [a]	5.40 ± 1.21 [a]	78.3 ± 6.95 [b]	<0.001
Glucose AUC 60 to 180 mins	199 ± 14.4 [a]	214 ± 15.6 [a]	223 ± 15.7 [a]	83.7 ± 12.6 [b]	<0.001
Glucose AUC 0 to 180 mins	196 ± 16.6	204 ± 19.2	194 ± 20.3	203 ± 19.4	0.960
Insulin Total Area Under the Curve					
Insulin AUC 0 to 60 mins	42.1 ± 37.8 [a]	10.1 ± 9.93 [a]	6.22 ± 3.72 [a]	2024 ± 368 [b]	<0.001
Insulin AUC 60 to 180 mins	5035 ± 675 [a]	5814 ± 1173 [a]	6359 ± 1240 [a]	2958 ± 667 [b]	<0.001
Insulin AUC 0 to 180 mins	4193 ± 624	4662 ± 871	5097 ± 1301	5741 ± 1060	0.216

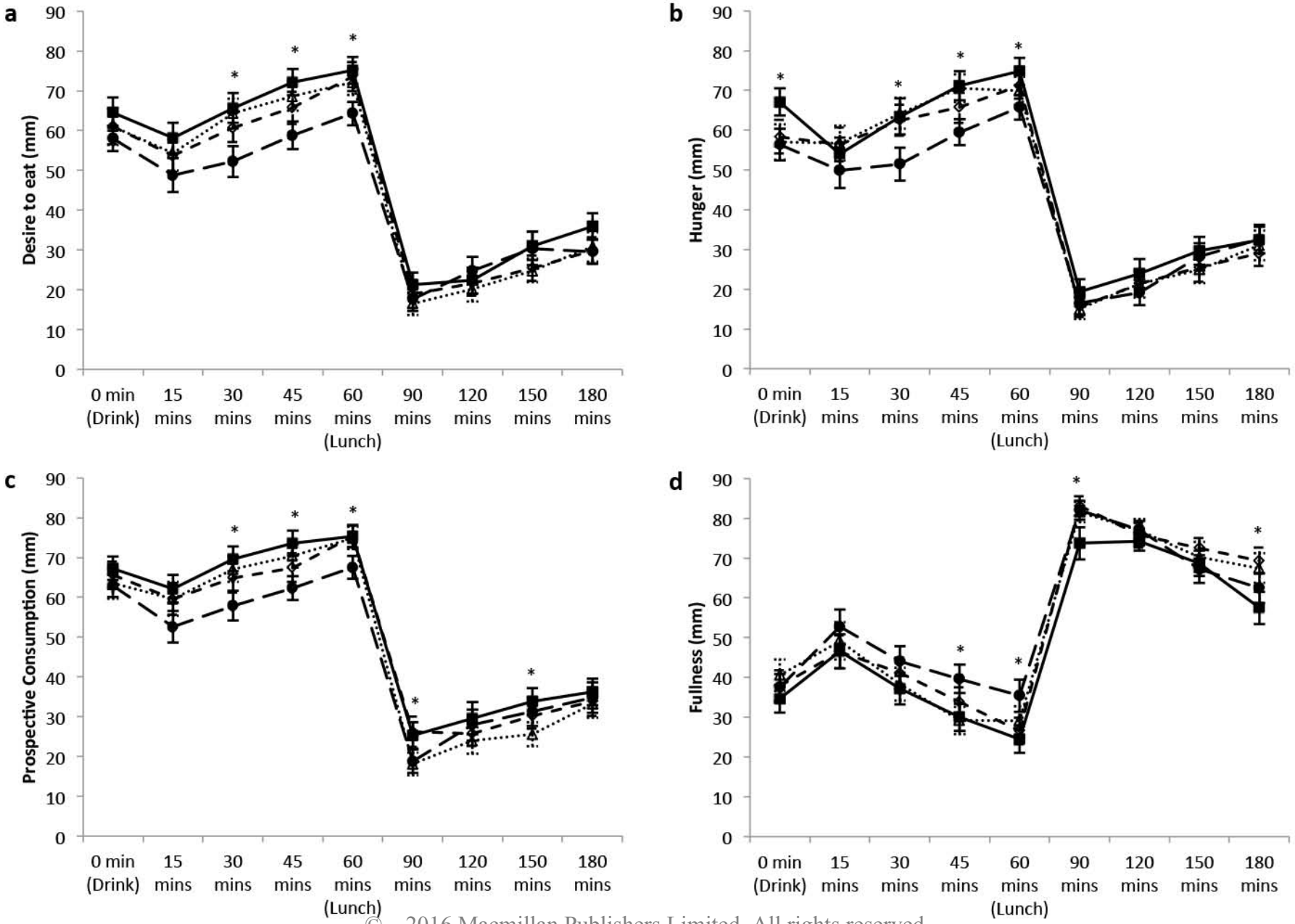
Rows with different lower case letters are significantly different from each other (P < 0.05).

Accepted manuscript

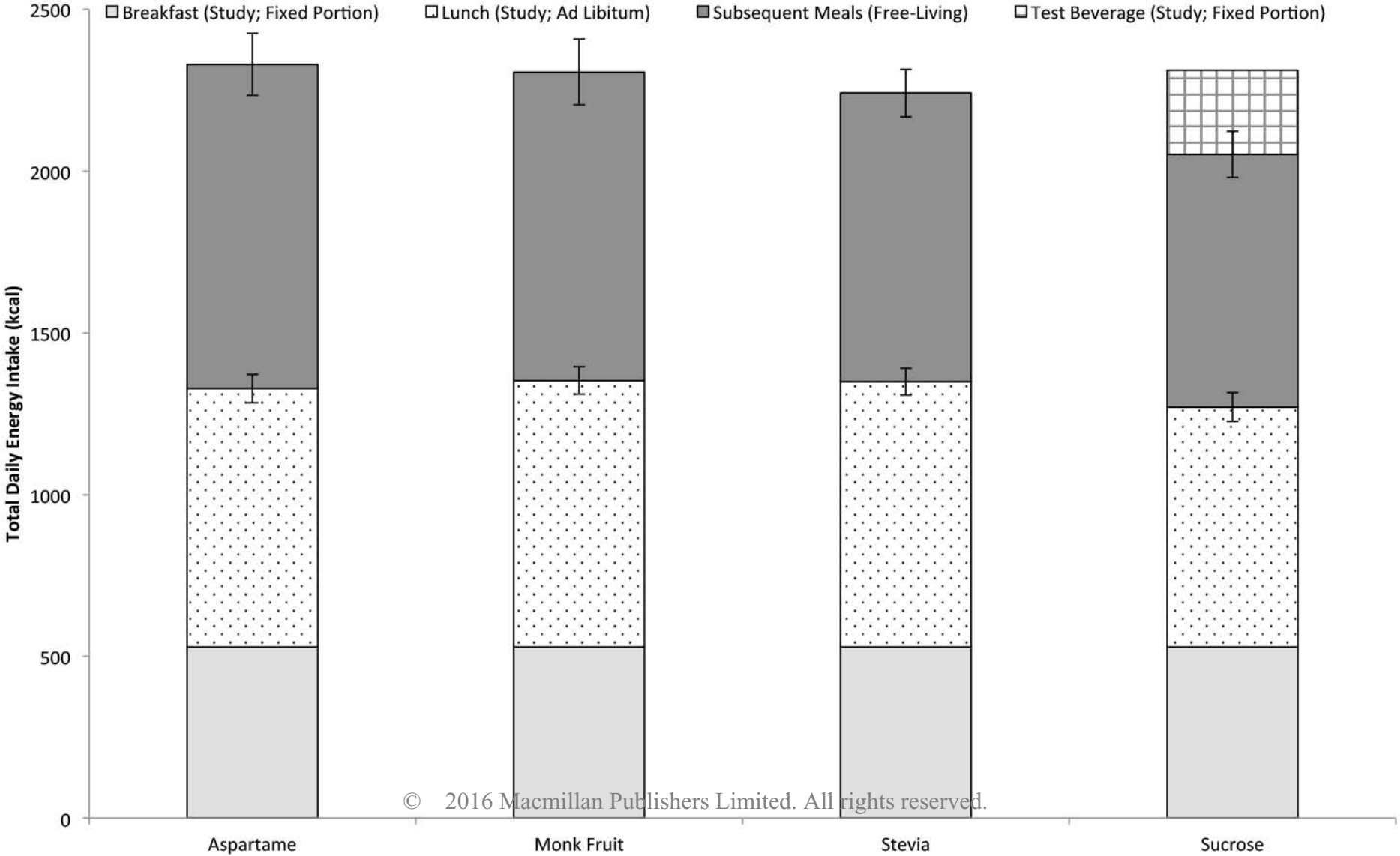
© 2016 Macmillan Publishers Limited. All rights reserved.



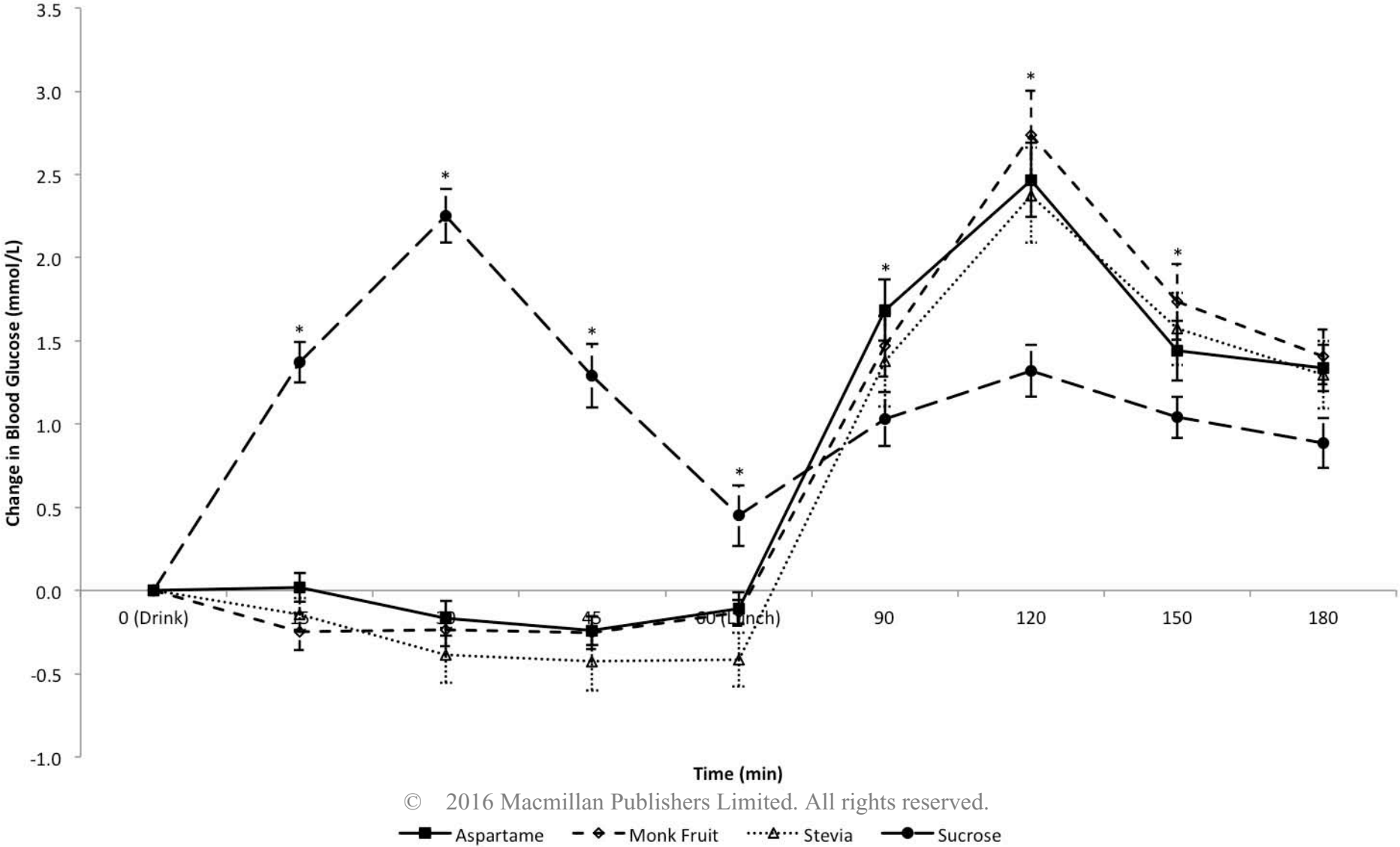
© 2016 Macmillan Publishers Limited. All rights reserved.



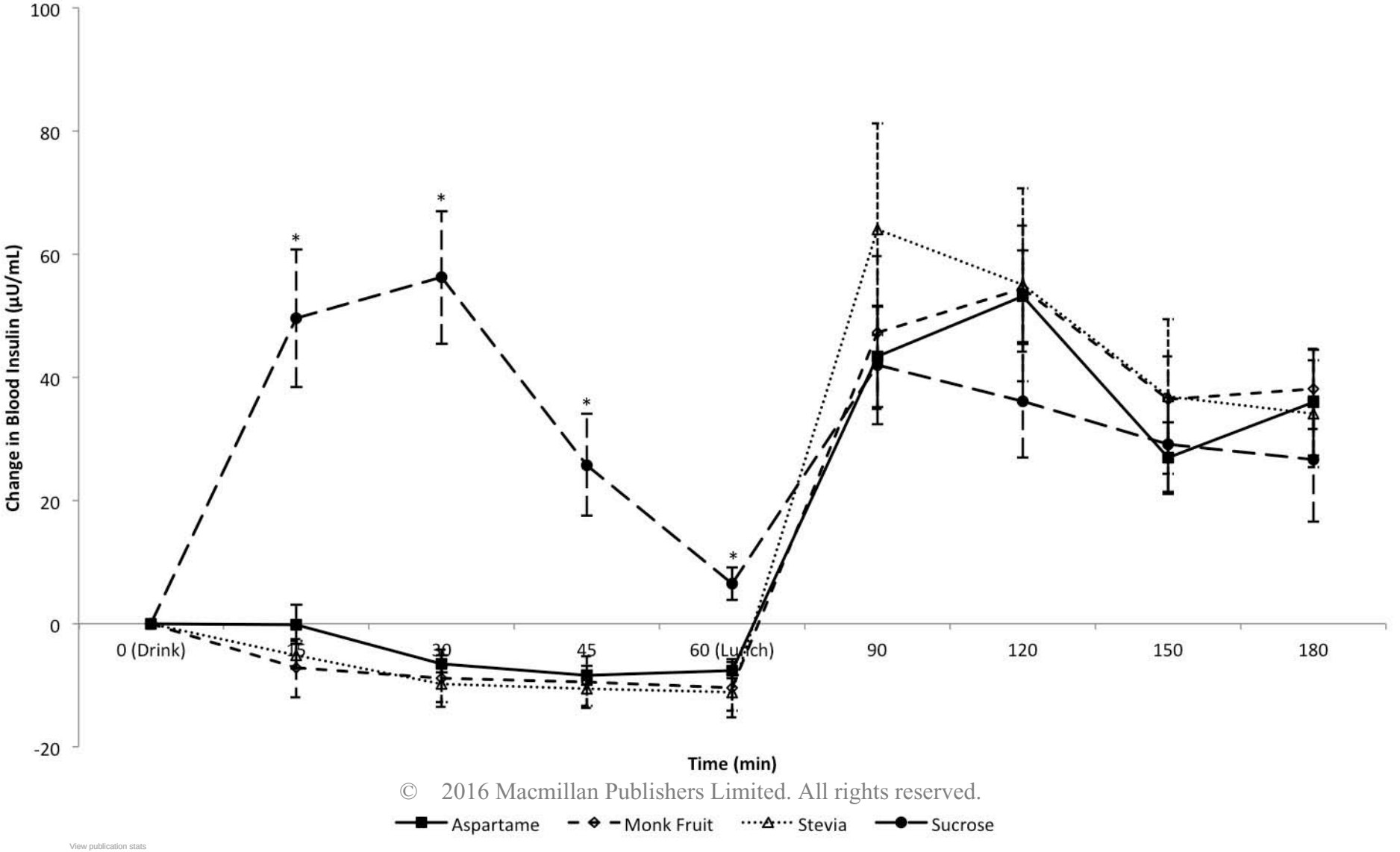
© 2016 Macmillan Publishers Limited. All rights reserved.



© 2016 Macmillan Publishers Limited. All rights reserved.



© 2016 Macmillan Publishers Limited. All rights reserved.



© 2016 Macmillan Publishers Limited. All rights reserved.

View publication stats