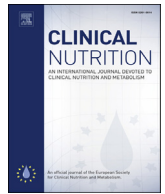




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## Randomized Control Trials

# Between-meal sucrose-sweetened beverage consumption impairs glycemia and lipid metabolism during prolonged sitting: A randomized controlled trial

Q5 Pia Varsamis<sup>a, b, \*</sup>, Melissa F. Formosa<sup>a</sup>, Robyn N. Larsen<sup>a</sup>, Medini Reddy-Luthmoodoo<sup>a</sup>,  
 Q4 Garry L. Jennings<sup>a, j</sup>, Neale D. Cohen<sup>a</sup>, Megan Grace<sup>a, b</sup>, John A. Hawley<sup>e</sup>,  
 Brooke L. Devlin<sup>e</sup>, Neville Owen<sup>a, c, g, h, i</sup>, David W. Dunstan<sup>a, c, d, e, f, g</sup>,  
 Paddy C. Dempsey<sup>a, h</sup>, Bronwyn A. Kingwell<sup>a, b, c</sup>

<sup>a</sup> Baker Heart & Diabetes Institute, Melbourne, Australia<sup>b</sup> Department of Physiology, Faculty of Medicine, Nursing & Health Sciences, Monash University, Melbourne, Australia<sup>c</sup> Central Clinical School, Faculty of Medicine, Nursing & Health Sciences, Monash University, Melbourne, Australia<sup>d</sup> Centre of Physical Activity and Nutrition Research, School of Exercise and Nutrition Sciences, Deakin University, Melbourne, Australia<sup>e</sup> Exercise & Nutrition Research Programme, Mary MacKillop Institute of Health Research, Australian Catholic University, Melbourne, Victoria, Australia<sup>f</sup> School of Public Health, The University of Queensland, Brisbane, Australia<sup>g</sup> School of Sport Science, Exercise and Health, The University of Western Australia, Perth, Australia<sup>h</sup> Swinburne University of Technology, Melbourne, Australia<sup>i</sup> School of Population and Global Health, The University of Melbourne, Melbourne, Australia<sup>j</sup> Sydney Medical School, University of Sydney, Sydney, Australia

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## SUMMARY

**Background & aims:** Chronic overconsumption of sugar-sweetened beverages (SSBs) is associated with unfavourable health effects, including promotion of obesity. However, the acute effects of consuming SSBs on glucose and lipid metabolism remain to be characterized in a real-world, post-prandial context of prolonged sitting. We quantified the acute effects of between-meal SSB consumption compared with water, on glucose and lipid metabolism in habitual soft drink consumers during prolonged sitting.

**Methods:** Twenty-eight overweight or obese young adults [15 males;  $23 \pm 3$  (mean  $\pm$  SD) years, body mass index (BMI)  $31.0 \pm 3.6$  kg/m<sup>2</sup>] participated. During uninterrupted sitting and following standardized breakfast and lunch meals, each participant completed two 7-h conditions on separate days in a randomized, crossover design study. For each condition, participants consumed either a sucrose SSB or water mid-morning and mid-afternoon. Peak responses and total area under the curve (tAUC) over 7 h for blood glucose, insulin, C-peptide, triglyceride and non-esterified fatty acid (NEFA) concentrations were quantified and compared.

**Results:** Compared to water, SSB consumption significantly increased the peak responses for blood glucose ( $20 \pm 4\%$  (mean  $\pm$  SEM)), insulin ( $43 \pm 15\%$ ) and C-peptide ( $21 \pm 6\%$ ) concentrations. The tAUC for all these parameters was also increased by SSB consumption. The tAUC for triglycerides was  $15 \pm 5\%$  lower after SSBs and this was driven by males ( $P < 0.05$ ), as females showed no difference between conditions. The tAUC for NEFAs was  $13 \pm 5\%$  lower after the SSB condition ( $P < 0.05$ ).

**Conclusions:** Between-meal SSB consumption significantly elevated plasma glucose responses, associated with a sustained elevation in plasma insulin throughout a day of prolonged sitting. The SSB-induced reduction in circulating triglycerides and NEFAs indicates significant modulation of lipid metabolism, particularly in males. These metabolic effects may contribute to the development of metabolic disease when SSB consumption is habitual and co-occurring with prolonged sitting.

**Abbreviations:** Body Mass Index, BMI; Sugar-Sweetened Beverages, SSBs; Total Area Under the Curve, tAUC; Moderate-to-Vigorous intensity Physical Activity, MVPA; Non-esterified Fatty Acid, NEFA; United States, US; World Health Organisation, WHO.

\* Corresponding author. Metabolic and Vascular Physiology, Baker Heart and Diabetes Institute, Level 4, 99 Commercial Rd., Melbourne, VIC 3004, Australia.

E-mail address: [pia.varsamis@baker.edu.au](mailto:pia.varsamis@baker.edu.au) (P. Varsamis).

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## 1. Introduction

Globally, sugar-sweetened beverages (SSBs) are the largest source of added sugars in Western diets [1]. SSB consumption is associated with the development of weight gain, fatty liver, type 2 diabetes and cardiovascular disease [2–6]. To date, most studies have focused on the relationships between sugary drink consumption and overweight/obesity. However, the large amount of added sugars that these drinks typically contain have additional implications beyond weight control, which may directly elevate risk for diabetes and cardiovascular disease [2,3]. These relate to chronic post-prandial glucose excursions which contribute to pancreatic  $\beta$ -cell failure and vascular complications as well as non-alcoholic fatty liver [7–9].

We have recently shown that there is significant variation across countries for identically-branded soft drinks, in their total concentration of glucose and fructose, as a result of global differences in primary industry sources of sugar [10]. Soft drinks in Australia and Europe are chiefly sweetened by sucrose (disaccharide composed of 50% glucose and 50% fructose), whereas formulations marketed under the same trade name in the United States (US) use high-fructose corn syrup [15]. It is unknown whether the difference in glucose-fructose ratio between sucrose (50:50) and high-fructose corn-syrup (typically 55:45) is sufficient to drive specific health effects, but given the global variation in soft drink composition, there is a need to quantify the magnitude by which sucrose-sweetened drinks elevate plasma glucose and insulin concentrations [11,12].

In addition to the adverse effects of SSBs on glycaemic responses, consumption in the context of prolonged uninterrupted sitting during the day would be expected to exaggerate glucose and insulin excursions. Through observational and experimental studies, we have shown that impaired glycaemic control is an important contributor to sitting-associated risk for chronic disease [13,14]. Such a perspective is important given current population trends for increasingly sedentary lifestyles, as characterized by time spent in prolonged sitting [15]. Indeed, SSB consumption has been demonstrated to co-occur with high sedentary time [13,16–18], making this behaviour a key driver of cardiometabolic risk in highly sedentary population groups [19].

Despite this, the acute metabolic effects of SSB consumption on both glucose and lipid metabolism in a real-world context that incorporates typical daily consumption levels, as well as meal

patterns and prolonged sitting, have not been investigated. For many young adults between-meal SSB consumption is a daily habit which challenges metabolic homeostasis and potentially seeds chronic cardiometabolic diseases. The purpose of this study was to quantify the acute effects of between-meal sucrose-sweetened beverage consumption compared with water during prolonged sitting on glucose and lipid metabolism in habitual soft drink consumers.

## 2. Materials and methods

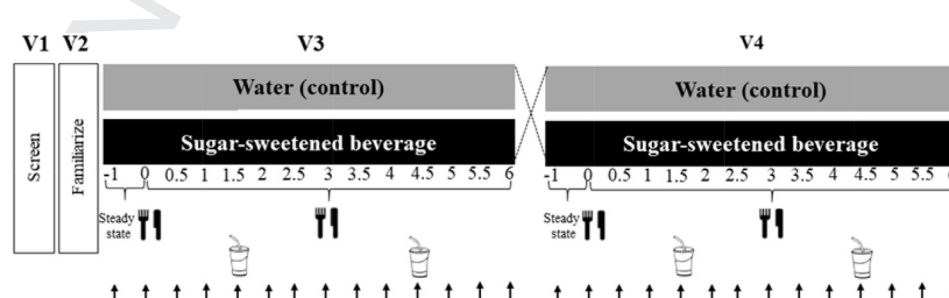
### 2.1. Participants

Twenty-eight inactive overweight/obese males ( $n = 15$ ) and females ( $n = 13$ ), who were habitual consumers of SSBs, participated in this study. Participants were recruited via posters, online advertisements, and social media. Eligibility included: age between 19 and 30 yr; body mass index (BMI)  $\geq 25$ , but  $\leq 40$  kg/m<sup>2</sup>, SSB consumption of  $>2$  L or more per week for at least the previous 3 months; self-reported sitting time  $> 5$  h/day, and no regular moderate-to-vigorous intensity physical activity (MVPA;  $\geq 150$  min/week for  $> 3$  months). Exclusion criteria included: being employed in a non-sedentary occupation (as characterized by low demand for sitting – e.g. tradesperson), currently using prescription medication that would confound interpretation of the data, pregnant or currently smoking. The study was approved by the Alfred Human Research Ethics Committee and all participants provided written informed consent. This trial was registered with the Australian New Zealand Clinical Trials Registry at <https://anzctr.org.au/Trial/Registration/TrialReview.aspx?ACTRN=12616000840482> as ACTRN12616000840482.

### 2.2. Study design

This randomized crossover trial was undertaken at the Baker Heart and Diabetes Institute between June 2016 and August 2017. Participants completed two acute single day (7 h) experimental conditions in random order with a minimum of 21 days wash-out between visits. Both conditions were performed on a background of uninterrupted sitting.

**SSB and uninterrupted sitting:** Participants sat upright in a comfortable lounge chair and consumed a commercially available



**Fig. 1.** Experimental randomized, cross-over study design and study day protocol for each condition with measurement time-points (in hours). Participants visited the laboratory on four separate occasions. The two trial conditions (visits 3 & 4) were completed in a randomized order separated by a minimum 21-day washout. All participants consumed standardized breakfast and lunchtime meals (F) at 0 h and 3 h. At 1.5 h and 4.5 h, a water or a SSB (C) was consumed. Blood (↑) was collected half hourly for glucose, insulin and C-peptide and hourly for triglycerides and non-esterified fatty acids (NEFAs).

sucrose-sweetened beverage 90 min after a standardized breakfast and lunch meal (Fig. 1).

**Water and uninterrupted sitting:** Participants sat upright in a comfortable lounge chair and consumed a volume of water equal to that consumed during the SSB condition, 90 min after the breakfast and lunch meal.

Participants attended the laboratory on four separate occasions. During their first visit, a general screening was conducted for baseline physical (height, weight, waist: hip ratio, blood pressure), and biochemical (glucose, insulin, HbA1c, lipids) characteristics against inclusion/exclusion criteria. During the second visit, conducted seven days prior to the first experimental trial condition (visit 3), participants were familiarized with each experimental condition and were instructed to complete physical activity and dietary records. Participants also received (written and verbal) instruction regarding the pre-experimental evening meal (including overnight fasting) and physical activity restriction prior to each trial condition. Female participants were asked to provide details regarding their menstrual cycle, to permit scheduling of each experimental visit within the follicular phase (between days 3–10). Standardized email, text message prompts and phone calls were used to maximize participant compliance. To eliminate potential bias, trial condition order was randomly assigned by a third party using computer-generated random numbers, stratified by sex. Study personnel and participants were blinded to the condition order until the morning of the first trial condition. Study investigators PV and BAK, the pathology technicians, and team statisticians were blinded throughout data collection and analysis.

### 2.3. Beverages

The SSB was a commercially-available carbonated soft drink containing sucrose (8.8 g/100 mL), free glucose (1.1 g/100 mL) and free fructose (1.1 g/100 mL) [10]. This corresponded to a total glucose (calculated final monosaccharide concentration) of 5.5 g/100 mL and a total fructose (calculated final monosaccharide concentration) of 5.6 g/100 mL [10]. The two SSBs serves each provided 6% of estimated energy requirements (Schofield equation, 1.5 physical activity factor) and approximated a discretionary food serve (Australian dietary guidelines) [20]. The total volume of soft drink consumed on experimental days was reflective of levels reported among SSB consumers' aged 19–30 year-olds in the recent Australian Health survey [21]. The average volume per serve was  $376 \pm 12$  mL and the average total volume consumed per experimental day was  $752 \pm 23$  mL.

### 2.4. Standardization of diet and physical activity

Participants were provided with a 'food pack' containing an evening meal for consumption the night before each experimental day, while breakfast and lunch meals were individually prepared and provided in the laboratory. Meal plans were individualized to meet estimated energy requirements (Schofield equation, 1.5 physical activity factor) and were based on Australian dietary intakes [21]. For breakfast, lunch and dinner, the macronutrient profile (as a percentage of total energy) was 14–18% for protein, 48–52% for carbohydrate, and 29–32% for fat. Meals were identical across conditions for each individual and provided ~33% of estimated daily energy requirements. For the evening meals, participants received verbal and written instructions and reminders to consume only those items within the 'food packs'. They were also instructed and reminded to record their dietary intake in the provided diary and to refrain from consuming alcohol and caffeine in the 24 h preceding each experimental condition. Weighed/measured food records were individually completed and dietary

intakes assessed using Australian-specific dietary analysis software (FoodWorks: Xyris Software, Version 8, AUS).

To minimize any potential effects of physical activity, participants were instructed to avoid moderate and/or vigorous exercise for at least 48 h prior to each experimental condition. To confirm this, participants kept an activity diary and wore a triaxial accelerometer (GTx3+; Actigraph, Pensacola, FL) to objectively assess their activity levels during waking hours for seven consecutive days before the condition (defined as the habitual period) and during the experimental condition day. They were instructed to wear the accelerometer on the right hip during all waking hours, unless doing water-based activities. The 1-min epoch activity data (for waking hours) were then processed using a cut off  $< 100$  counts/min define sedentary time [22,23]. Freedson's cut offs were used to differentiate moderate-to-vigorous-intensity activity (counts/min  $\geq 1952$ ) from light-intensity activity (100–1951 counts/min) [24]. Total time was calculated as the sum of time spent in all activities (sedentary, light and MVPA). Data are reported as averages for valid days (days with  $> 10$  h wear and no minutes with counts  $\geq 20,000$ ).

### 2.5. Study protocol

After a minimum 10 h overnight fast, participants reported to the laboratory at 0715 h. After voiding, and once anthropometric measurements were obtained, an indwelling catheter was inserted into an antecubital vein and fasting blood samples were collected before (–1 h) and after (0 h) a 1 h seated steady-state period.

At 0 h participants consumed the standardized breakfast meal with the time taken to consume ( $< 20$  min) replicated in subsequent conditions (Fig. 1). At 3 h participants consumed lunch ( $< 20$  min). Ninety-minutes after each meal, participants consumed individualized volumes of the SSB or water within 10 min. Postprandial blood samples were collected at 30-min intervals over each 7 h experimental condition. A total of 273 mL of blood was taken from each participant during an experimental trial. Participants had access to internet services, standardized television and DVD viewing and reading materials (newspapers and magazines) during the two experimental conditions. To minimize unscheduled physical activity, standardized lavatory visits were incorporated into the protocol immediately following SSB or water consumption (1.5 h and 4.5 h) for each trial.

### 2.6. Biochemical analyses

All blood for screening and the experimental conditions was collected in to appropriate tubes (BD Vacutainer™, Franklin Lakes, NJ, USA) for determination of concentrations of glucose, insulin, C-peptide, HbA1c, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, NEFAs and human chorionic gonadotrophin (for females). All analyses except for NEFAs were conducted at the Alfred Hospital, Department of Pathology according to clinical diagnostic standards (National Association of Testing Authorities accredited). Plasma glucose was measured using the hexokinase method. Serum insulin and C-peptide were measured using a chemiluminescent microparticle immunoassay (Architect ci16200; Abbott Diagnostics, Santa Clara, CA). At visits 3 and 4, baseline and hourly samples were drawn into EDTA tubes, centrifuged ( $2000 \times g$  for 15 min at  $4^\circ\text{C}$ ) and the plasma stored at  $-80^\circ\text{C}$  for later analysis of NEFAs using a commercially available kit (Waco Diagnostics, Richmond, VA, USA). Insulin resistance was estimated from fasting glucose by using a computer-based homeostasis model assessment system (HOMA2-IR) provided by the Oxford Centre for Diabetes, Endocrinology, and Metabolism (<http://www.dtu.ox.ac.uk/homa>). Similar previous studies have used and validated this approach [25].

## 2.7. Statistical analyses

Study data were collated and managed using REDCap electronic data capture tools hosted at [Baker Heart and Diabetes Institute] [26]. Physical characteristics were compared between males and females using an unpaired two-tailed Student's *t* test. Anthropometric, dietary, and accelerometer-derived physical activity data before each of the respective trial conditions are presented in [Supplementary Table 1](#). The small but statistically significant difference in sedentary time (48 h prior to experimental visits) had no effect on endpoint analyses and was therefore not included as a covariate.

Plasma glucose in both the morning and the afternoon was the primary outcome measure, with sample size determined by power calculations based on our previous studies [13,14]. To allow examination of differential effects of the interventions throughout the day, the study was powered at a  $\beta$  value of 80% to detect a 15% minimum difference in glycaemia (based on a standard deviation of the difference of 25%) after both the morning and the afternoon drink at an alpha level of 0.025 (to accommodate dual endpoints). Peak plasma glucose in response to each drink in the morning [(1.5–3 h (Drink 1))] and afternoon [(4.5–6 h (Drink 2))] was calculated. Total area under the curve (AUC) (trapezoidal method using a baseline of zero) over the 7 h intervention was also calculated for glucose, insulin, C-peptide, triglycerides and NEFAs. Generalized linear mixed models (with random intercepts) were used to evaluate the differential effects of the experimental conditions on the selected outcomes using Stata 14 (StataCorp LP, College Station, Texas, USA). All models were adjusted for potential covariates explaining residual outcome variance (age, sex, and BMI), baseline values, and period effects (treatment order). Residuals were examined for serial correlation, heteroscedasticity and normality. Substantial departures from model assumptions were not observed. Sex-by-condition, interactions were performed for each tAUC outcome measure. Statistical significance was set at  $P < 0.05$ . Data are expressed as mean  $\pm$  SEM unless otherwise stated.

## 3. Results

### 3.1. Participant characteristics

Thirty-three participants were randomized and familiarized, but five withdrew prior to the first experimental condition ([Supplementary Fig. 1](#)). As such, twenty-eight participants [15 males, 13 females;  $23 \pm 3$  years, BMI  $31.0 \pm 3.6$  kg/m<sup>2</sup> (mean  $\pm$  SD)] commenced and completed all trial conditions ([Table 1](#)). There were no significant differences in baseline variables between sexes except for HDL-cholesterol which was higher in women.

### 3.2. Glycaemic responses

The average volume of the SSB and water, and average amount of sugars (sucrose, glucose, fructose, total glucose and total fructose, calculated final monosaccharide concentration) consumed during the trial conditions are presented in [Supplementary Table 2](#). For the SSB intervention, the average amount of total glucose (calculated final monosaccharide) was  $20.7 \pm 0.6$  g per serve and  $41.4 \pm 1.3$  g per trial day and total fructose (calculated final monosaccharide) was  $21.1 \pm 0.7$  g per serve and  $42.1 \pm 1.3$  g per trial day.

[Figure 2 \(A–C\)](#) shows the plasma glucose, insulin and C-peptide concentrations during each of the experimental conditions. Compared to water, between-meal SSB consumption significantly increased peak plasma glucose, insulin and C-peptide

concentrations both in the morning by  $20 \pm 4\%$  (mean  $\pm$  SEM),  $43 \pm 15\%$  and  $21 \pm 6\%$ , and in the afternoon by  $8 \pm 3\%$ ,  $35 \pm 14\%$  and  $15 \pm 6\%$ ; all  $P < 0.05$  ([Fig. 2; D–F](#)). The tAUCs were significantly higher for the SSB intervention compared to water for glucose, insulin and C-peptide by  $5 \pm 1\%$ ,  $26 \pm 9\%$  and  $11 \pm 3\%$ , respectively; all  $P < 0.05$ .

### 3.3. Lipid responses

[Figure 3](#) shows the plasma triglyceride concentrations during each of the experimental conditions for males and females. The tAUC for plasma triglycerides was significantly lower after SSB consumption compared to water by  $15 \pm 5\%$  ( $P < 0.05$ ). The reduction for the morning period was  $13 \pm 4\%$  and the afternoon was  $18 \pm 6\%$  ( $P < 0.05$  for both). There was a significant sex-by-condition interaction effect for the triglyceride tAUC which corresponded to a  $24 \pm 5\%$  reduction in males after the SSB compared to the water condition ( $P < 0.05$ ). Females had significantly lower triglyceride levels than males at baseline but showed no difference between conditions ([Fig. 3](#)).

[Figure 4](#) shows the NEFA concentrations during each of the experimental conditions. There was a trend for higher baseline NEFA concentration in the SSB trial, but after adjustment (see Statistical Analysis), the tAUC for NEFA concentrations was significantly lower after SSB consumption compared to water (by  $13 \pm 5\%$ ;  $P < 0.05$ ). This was evident in both the morning ( $10 \pm 4\%$ ) and in the afternoon ( $21 \pm 9\%$ ;  $P < 0.05$ ). There was no significant sex-by-condition interaction for NEFA concentration, nor any other outcome.

## 4. Discussion

Consumption of a sucrose-sweetened beverage in a pattern typical of habitual soft drink consumers, and in the context of normal meals and prolonged sitting, elevated peak plasma glucose concentration by 20% compared to water. This was associated with a sustained 26% elevation in plasma insulin throughout the day. These effects were observed in parallel with modulation of parameters associated with lipid metabolism. Plasma triglyceride concentration was 15% lower after sucrose-sweetened beverage consumption compared to water, an effect limited to men, where values were reduced by 24%. In addition, NEFAs were reduced by 13% after sucrose-sweetened beverage consumption compared with water. These effects are relevant to typical daily consumption levels [27], are in the context of real world behaviour patterns (regular meals and prolonged sitting) and are quantitated in comparison to water which is considered the optimal alternative to SSB consumption in the general community [28].

The effects of sucrose-sweetened beverage consumption on glucose and fat metabolism are of interest because these formulations are higher in glucose than high-fructose corn syrup formulations [10]. The differential effects of glucose and fructose consumption are most likely due to glucose being absorbed from the small intestine into the blood where it elevates blood glucose concentration and stimulates the pancreas to produce insulin [29]. In contrast, fructose is primarily metabolized in the liver [30], stimulating glycogenesis, gluconeogenesis and lipogenesis. Distinct from glucose, fructose does not acutely increase blood glucose or insulin concentration [31].

Specific effects of sucrose-sweetened drinks, which we have shown to be 22% higher in glucose than high-fructose corn syrup sweetened drinks, may relate to induction of high and variable plasma glucose and insulin levels [10]. Chronic post-prandial glucose excursions and variability contribute to pancreatic  $\beta$ -cell failure and progression to late-stage diabetes [32].

**Table 1**  
Participant characteristics.

Characteristic	Total population	Males <sup>a</sup>	Females	P Value <sup>b</sup>
Sex, n (%)		15 (54)	13 (46)	0.411
Age, y	23 ± 3	23 ± 3	24 ± 3	0.226
BMI, kg/m <sup>2</sup>	31.0 ± 3.6	30.7 ± 3.1	31.3 ± 4.2	0.678
Waist circumference, cm	98.2 ± 13.4	102.3 ± 11.5	93.5 ± 14.3	0.084
HbA <sub>1c</sub> , %	5.3 ± 0.3	5.3 ± 0.3	5.3 ± 0.2	0.881
HbA <sub>1c</sub> , mmol/mol	34.8 ± 3.0	34.9 ± 3.8	34.8 ± 1.9	0.934
Fasting glucose, mmol/L	4.9 ± 0.4	5.0 ± 0.4	4.8 ± 0.4	0.750
Fasting insulin, μU/mL	13.7 ± 6.0	13.7 ± 5.8	13.6 ± 6.4	0.993
Fasting cholesterol, mmol/L				
Total	4.6 ± 1.1	4.7 ± 1.1	4.4 ± 1.1	0.537
LDL	2.8 ± 0.9	3.0 ± 0.9	2.7 ± 0.9	0.347
HDL	1.2 ± 0.3	1.0 ± 0.2	1.3 ± 0.4	0.004
Fasting triglycerides, mmol/L	1.2 ± 0.7	1.5 ± 0.9	1.0 ± 0.4	0.067
HOMA2%B	86 ± 42	92 ± 51	80 ± 28	0.431
HOMA2%S	135 ± 45	125 ± 45	147 ± 42	0.205
HOMA2-IR value, AU	0.9 ± 0.7	1.0 ± 0.8	0.8 ± 0.5	0.356
Systolic blood pressure, mmHg	112 ± 12	115 ± 12	109 ± 10	0.122
Diastolic blood pressure, mmHg	70 ± 8	68 ± 7	73 ± 8	0.102
Heart rate, bpm	73 ± 10	70 ± 9	77 ± 11	0.089

Abbreviations: AU, arbitrary units; BMI, body mass index; HDL, high-density lipoprotein; HOMA2%B homeostasis model assessment of estimated beta cell function; HOMA2%S, homeostasis model assessment of insulin sensitivity; HOMA2-IR homeostasis model assessment of insulin resistance index; LDL, low-density lipoprotein.

<sup>a</sup> Data are mean ± SD or number (%).

<sup>b</sup> Males and females compared with unpaired two-tailed Student's t test (continuous variables) and Chi-square test (categorical variables).

#### 4.1. Glucose metabolism

The greatest difference in post drink plasma glucose in the current study was observed after the first drink. Moderation of the increase in plasma glucose after the second drink was achieved through elevated insulin levels established after the first drink and sustained throughout the day (second meal effect) [6,9,33,34].

Previous research has focused predominantly on the relationship between SSBs and weight gain [35–37]. Some studies examining physiological responses to sugar consumption have examined single doses of individual sugars (e.g. sucrose, glucose or fructose) on a fasting background and over relatively short follow up periods of two hours or less [12,38,39]. These studies demonstrate large excursions in blood glucose (up to 60%) and insulin in response to consumption of glucose drinks, but are less relevant to the real-world scenario of mixed sugar consumption associated with SSBs in the context of meals [11,29,39,40]. Other studies have considered SSB consumption in the context of meals, but have examined very high SSB 'doses' supplying 25% of daily energy requirements [39].

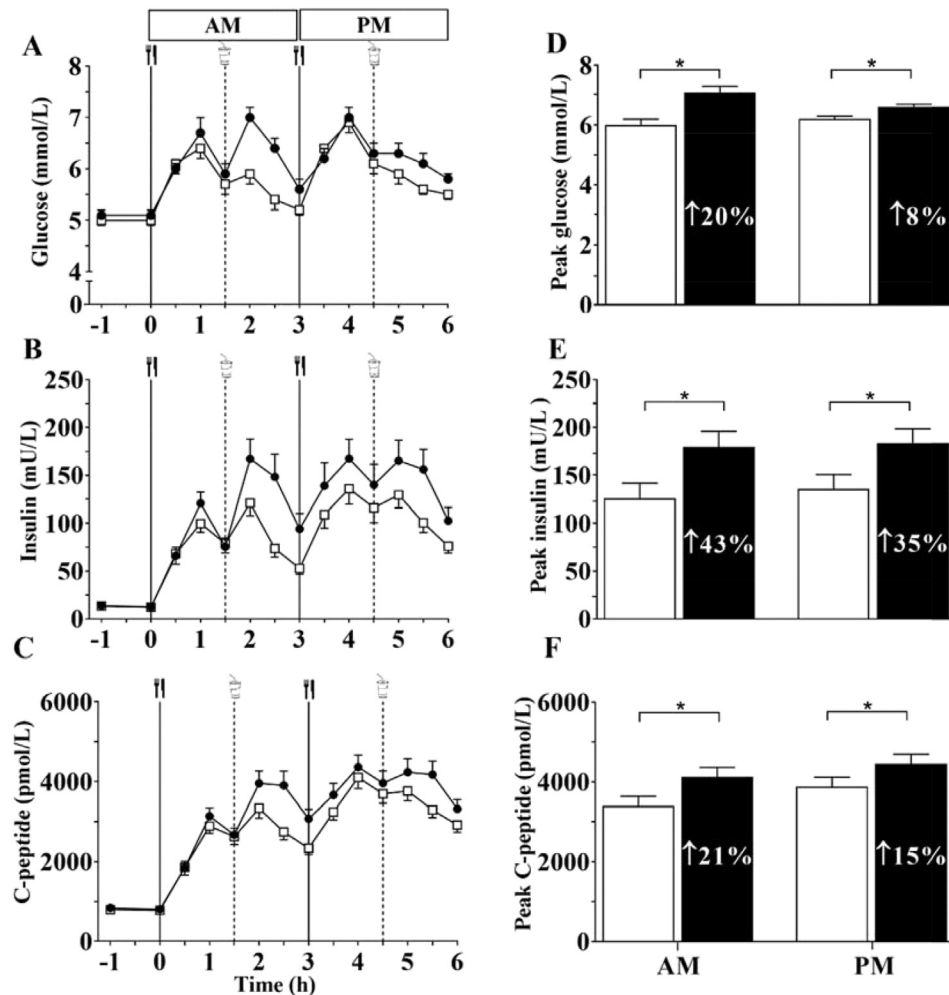
The SSB "dose" delivered in our study (12% of daily energy requirements, Supplementary Table 2) is highly relevant to current global consumption trends. In the age group corresponding to the current study (19–30 years), where SSB consumption is greatest, the top 10% highest consumers in the Australian Health Survey drank more than 1 L of SSBs, peaking at 1.5 L (28 teaspoons or 110 g) for males on the day prior to interview [21]. Alarmingly, these consumption levels far exceed the current World Health Organisation (WHO) recommendations to limit intake of total sugars to less than 50 g (approximately 12 teaspoons) per day [41]. Compounding the negative health impact of sugar over-consumption are concurrent population trends for low levels of physical activity and prolonged periods of sedentary time that are characterized by the absence of skeletal muscle contractile activity [16]. Despite strong evidence indicating that exercise can mitigate some of the detrimental effects of high sugar intake, independently of energy balance [42,43], recent estimates suggest that sitting occupies the majority of the waking hours in adults (between 7 and 10 h per day) [18]. Consistent with these findings, our study participants spent approximately 10 h per day sedentary (Supplementary Table 2). We have established through recent observational and experimental studies that

impaired glycaemic control is an important contributor to sitting-associated risk [13,14]. The current findings are thus highly relevant in terms of characterizing the metabolic impact of SSB consumption against a background of high levels of daily sitting time.

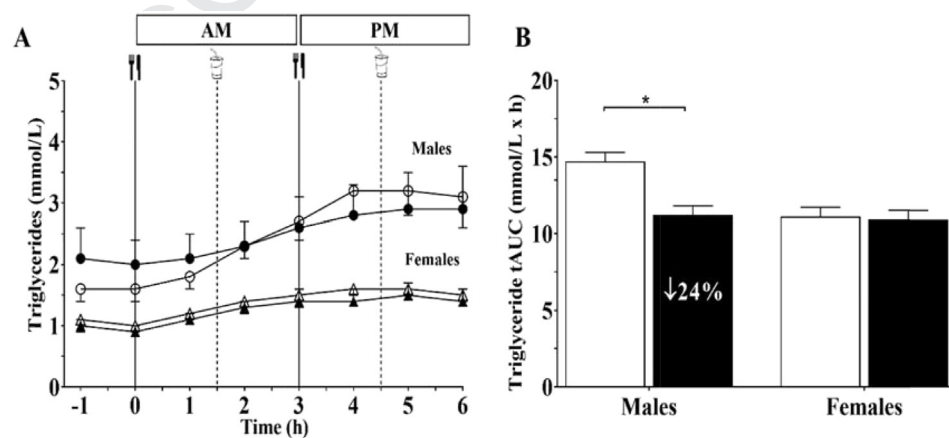
Our results contrast with a previous study employing a very similar protocol and SSB consumption pattern (two 355 mL sucrose-sweetened beverages; 75 g of sucrose), but examining interstitial glucose rather than plasma glucose [44]. The finding that sucrose-sweetened beverage consumption in this previous study did not affect interstitial glucose compared with water brings into question the reliability of interstitial glucose measures to monitor acute glucose changes [45,46]. The current study clearly demonstrates substantial effects of sucrose-sweetened beverage consumption on both plasma glucose and insulin which are likely to be detrimental in regular consumers of sucrose-sweetened soft drinks and particularly in those with elevated cardiometabolic risk factors.

#### 4.2. Lipid metabolism

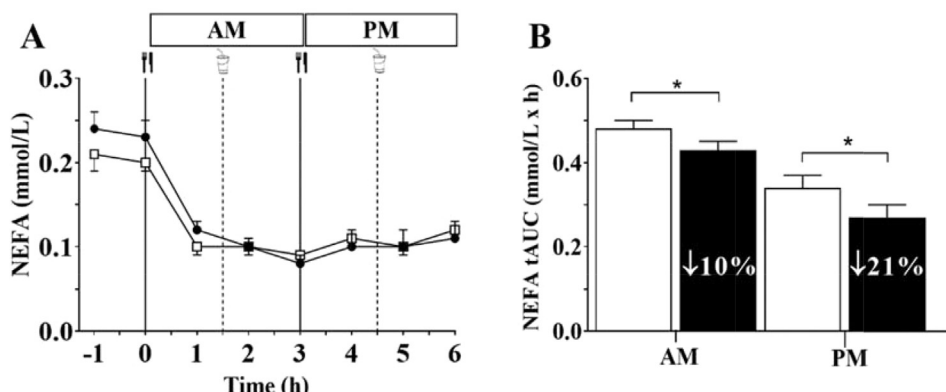
Glucose-induced insulin elevation also has consequences for fat metabolism, as a result of insulin-mediated suppression of liver triglyceride production and lipolysis in favour of glucose catabolism [38]. Through this mechanism, excess glucose consumption may contribute to liver fat accumulation. We observed a large SSB-induced elevation in insulin, which may further exacerbate the detrimental effects of fructose on lipid metabolism and liver fat accumulation [47]. The elevation in insulin was associated with lower plasma triglycerides, an effect driven entirely by the response seen in males and consistent with suppressed liver triglyceride production in very low-density lipoprotein (VLDL). These data align with known sex differences in liver insulin sensitivity, in that obese men are more sensitive to glucose and insulin induced suppression of liver VLDL-triglyceride production than obese women [48]. Alternatively, other potential explanations include that reduced adipose tissue lipolysis and NEFA flux could also contribute to reduced hepatic VLDL production [11]. These possibilities suggest that sucrose-sweetened beverage consumption may predispose men to an elevated risk for fatty liver disease. This is particularly concerning given that young males lead SSB consumption in terms



**Fig. 2.** Fasting (–1 and 0 h) and postprandial plasma glucose (A), serum insulin (B) and serum C-peptide (C) concentrations measured during water (open squares) and SSB conditions (closed circles). Solid vertical lines indicate timing the breakfast (0 h) and lunch (3.0 h) meals. Vertical dashed lines indicate the timing of drink 1 (1.5 h) and drink 2 (4.5 h). Peak drink responses in the morning (AM; 1.5–3 h) and afternoon (PM; 4.5–6 h) for plasma glucose (D), serum insulin (E) and serum C-peptide (F) concentrations measured during water (white bars) and SSB conditions (black bars). Values within the bars indicate the percentage change compared to the water condition. All data are presented as mean  $\pm$  SEM. \* Difference between water and SSB condition ( $P < 0.05$ ).



**Fig. 3.** Fasting and postprandial plasma triglyceride concentrations measured during water (open circle and open triangle) and SSB (closed circle and closed triangle) conditions for males ( $n = 15$ ) (circles) and females ( $n = 13$ ) (triangles) (A). Triglyceride total area under the curves (tAUC) per trial condition for males and females (B) [Water (white bars) and SSB (black bars)]. All data are presented as mean  $\pm$  SEM. \* Sex-by-condition interaction effect ( $P < 0.05$ ).



**Fig. 4.** Fasting and postprandial NEFA concentrations measured during water (open square) and SSB (closed circle) conditions (A). Solid vertical lines indicate timing the breakfast (0 h) and lunch (3.0 h) meals. NEFA total area under the curves (tAUC) responses per trial condition in the morning (AM; 0–3 h) and afternoon (PM; 3–6 h) [Water (white bars) and SSB (black bars) (B)]. Values within the bars indicate the percentage change compared to the water condition. All data are presented as mean  $\pm$  SEM. \* Difference between water and SSB condition ( $P < 0.05$ ).

of both population prevalence and volumes consumed [21]. In addition to the gender-specific effects on plasma triglycerides, NEFAs were also reduced by sucrose-sweetened beverage consumption suggesting suppression of lipolysis in both men and women [11].

#### 4.3. Strengths and limitations

This was an appropriately powered, controlled randomized cross-over study incorporating young adult male and female participants, who were typical consumers of SSBs. Participants were their own controls, enhancing both the internal validity and reliability of our data, and demonstrated good compliance with consumption of all standardized meals and beverages during lead-in periods. Additionally, there was stringent control of potential confounding variables such as diet, sedentary behaviour and physical activity, through use of weighed food records and objectively measured sedentary and physical activity behaviours. Nevertheless, in interpreting these findings it is important to consider some limitations that future studies could address. First, due to the acute nature of this study we cannot speculate on the possible longer-term effects of sustained sucrose-sweetened beverage consumption. Second, blinding of research participants to experimental conditions (water, SSB) was not possible due to the nature of the intervention. Third, our sex-specific analysis was exploratory, however, the results suggest that future research on these differences is warranted. Finally, the acute effects observed in habitual SSB consumers cannot be generalized amongst other populations including the non-obese, children/adolescents (<19 years), and middle aged/older adults (>31 years).

## 5. Conclusion

Compared with water, consumption of sucrose-sweetened beverages significantly elevates post-drink plasma glucose in association with a sustained elevation in plasma insulin throughout a day of prolonged sitting. The SSB-induced reduction in circulating triglycerides and NEFAs indicates significant suppression of lipid metabolism, particularly in males. These metabolic effects may contribute to the development of metabolic disease when SSB consumption in the context of prolonged sitting is habitual.

#### Statement of authorship

The authors' responsibilities were as follows—BAK, NO, DWD, RNL, JH, BLD, MG, GLJ, PCD, and MFF: designed the research; PV,

MFF, and MRL: conducted the research; PV, PCD and BAK, analysed data or performed statistical analysis; GLW and NDC provided clinical support during data collection; PV, PCD, NO, DWD, RNL, JH, GLJ and BAK: wrote and participated in critical revision of the manuscript for intellectual content.; and PV: had primary responsibility for final content of the manuscript. None of the authors had any conflicts of interest regarding this manuscript.

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#### Conflicts of interest

The authors declare that they have no conflict of interest.

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#### Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.clnu.2018.08.021>.

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