RESEARCH ARTICLE

Manipulation of glycemic response with isomaltulose in a milk-based drink does not affect cognitive performance in healthy adults

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Previous research suggests that glucoregulation and nutrient interventions, which alter circulating glucose, impact cognitive function. To examine the effect of modulating glycemic response using isomaltulose on cognitive function 24 healthy male adult participants consumed energy and macronutrient-matched milk-based drinks containing 50 g isomaltulose, 50 g sucrose or a water control in a counterbalanced within-subject design. Interstitial glucose was measured continuously in 12 subjects and all provided 9 capillary measures on each test day. A 30-min cognitive test battery was administered before and twice (+35 and + 115 min) after drink ingestion. Immediate, delayed, recognition, verbal and working memory, and psychomotor performance were assessed. Glycemic profiles induced by the drinks differed significantly during the first but not the second post-drink test battery. Neither administration of the sucrose nor isomaltulose drinks produced consistent effects on verbal or working memory, or psychomotor performance. This study used isomaltulose as an investigative tool to lower glycemic response. Importantly, it demonstrates a lack of effect of modulating glucose on cognitive performance based on reliable, continuously measured glycemia. It refutes the hypothesis that glycemia is associated with cognitive performance and questions the suggestion that isomaltulose has an effect on cognitive performance.

Keywords:
Cognitive function / Glycemic response / Isomaltulose / Memory / Psychomotor

1 Introduction

As glucose is the primary source of energy for the brain [1], the hypothesis that changes in blood glucose could influence cognitive performance has received a great deal of research interest. Release of glucose into the blood elicits an insulin response, which serves to return circulating glucose to normal levels and restore homeostasis. Impaired glucose tolerance occurs when this response begins to fail, leading to prolonged blood glucose excursions which have been associated with cognitive impairments [2].

Several studies have investigated the acute effect of manipulation of peripheral glycemic response on cognitive performance in healthy adults using oral glucose drink interventions accompanied by capillary blood glucose measures [3–8]. Improvements in memory or attention have been associated with increasing blood glucose levels in healthy young adults [3, 5] and falling blood glucose levels [6]. Tasks with a high cognitive load have been reported to induce a reduction in capillary blood glucose levels in healthy volunteers [7, 8]. Although a systematic review of the evidence from 31 studies in healthy young adults, which

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Abbreviations: AGD, ambulatory glucose-monitoring device; **VVLT,** visual verbal learning test

met the inclusion criteria of scientific quality, found beneficial effects on only half of the 134 outcome measures employed, these effects were most consistent for measures of delayed verbal memory [9].

Certain nutrient manipulations may influence glycemic response, *e.g.* GI and whey proteins [10, 11]. Any relationship between glucose and cognition may be a function of glycemic response and its metabolic consequences. Manipulations, which modulate glycemic and insulin response, may provide useful experimental models to examine cognitive effects. Recent studies have employed food interventions based on their purported rate of delivery of glucose from the small intestine to the blood [12–15]. Such interventions are more ecologically valid than pure glucose drink interventions and are typically based on the ratio of slowly to rapidly digested/available carbohydrate and/or glycemic index/load of a given food. While there is some evidence of beneficial effects on memory and attention of breakfasts with a low compared with a high glycemic index or load [13–15], this has not been consistently reported [12, 15]. Furthermore, the timing of cognitive effects relative to the temporal distribution of glycemic response within studies is inconsistent [16].

Isomaltulose, an isomer of sucrose, is a naturally occurring reducing disaccharide ($C_{12}H_{22}O_{11}$) composed of a glucose and fructose molecule linked by an α-1,6-glycosidic bond. It is digested at slower rate than sucrose [17] but is of similar taste and appearance [18]. It has also been shown to improve glycemic control in healthy men [19]. There is some evidence to suggest a beneficial cognitive effect of isomaltulose [20]. In this Japanese study, a significant increase in calculation ability measured at 90 min following consumption of 40 g of Palatinose® (isomaltulose) or 40 g sucrose was reported. In the sucrose condition, performance measured at 150 min decreased compared with performance at 90 min, whereas in the isomaltulose condition, performance at 150 min was only slightly lower than that reported at 90 min. However, the two treatments were not compared statistically.

Although a direct relationship between glucose levels and cognitive function has been demonstrated in many studies of healthy volunteers and interpreted in relation to glucoregulation, these studies are often based on infrequent capillary sampling of glucose with changes inferred between temporally distant measurement points, often only before and after cognitive testing. We have recently validated the use of interstitial glucose measurement using microdialysis against arterialised venous blood which is the best available proxy for blood glucose delivery to the brain, and capillary samples, measured by finger prick [21], which are commonly used in studies of glucose–cognition relationships.

In the present study, we investigated the relationship between glycemic response (measured continuously in interstitial tissue and periodically in capillaries) and cognitive function, using isomaltulose or sucrose in a milk-based vehicle to manipulate blood glucose levels and a water control.

2 Materials and methods

2.1 Participants

A total of 24 healthy men (mean age 23 ± 4 years; range 18–32 years) with a BMI in the normal range (range 19–25 kg/m^2) were recruited from the University of Leeds campus. All participants were regular breakfast consumers, dietary unrestrained and low habitual caffeine consumers (defined as ≤ 100 mg caffeine in the morning and/or ≤ 250 mg caffeine per day). The study was approved by the University of Leeds, Institute of Psychological Sciences Research Ethics Committee (IPS Ref. 07049-01). Participants gave prior written informed consent to participate in the study.

2.2 Design

A within-subject, placebo-controlled, repeated measures design was employed. Each subject underwent three study conditions in a counterbalanced order, separated by a 1-wk washout period.

2.3 Test drinks

Participants consumed milk-based drinks containing either isomaltulose (50 g), sucrose (50 g) or a water control (429 mL; 0 Kcal). The water control was included as a negative control as this should not produce a change in blood glucose levels at either of the two post-ingestion cognitive test sessions in contrast to the active treatments. Therefore, any decline in blood glucose during the cognitive test sessions should be attributable to the demand for glucose induced by the cognitive tests [5, 8]. The isomaltulose and sucrose drinks were matched on volume (429 mL), energy (325 Kcal) and macronutrient composition (2.1% fat, 1.3% protein, 11.2% carbohydrate). The isomaltulose drink contained artificial sweeteners to match the sweetness of the sucrose drink. Both caloric drinks were served cold and had a similar colour and taste.

2.4 Cognitive tests

2.4.1 Visual verbal learning test

The Visual verbal learning test (VVLT), a visual analogue of the Rey Auditory-Verbal Learning Test [22] that measures verbal learning and memory and is a hippocampal task, was used. Three trials of 16 words (list A) were presented in the

same order in the centre of a computer screen at the rate of one word every 2 s. At the end of each trial, participants verbally recalled as many words as possible in a free recall task. Trial 3 was followed by a presentation of a 16-word interference list (list B) and a subsequent free recall of those words. A free recall of list A immediately succeeded this. After 20 min, participants recalled list A (delayed recall). They also performed a word recognition test. This consisted of 16 words from list A of the VVLT, 16 words from list B of the VVLT and 16 new distracter words (list C). Half of the words from each list were presented visually and half aurally. Participants responded by pressing a key to indicate whether the word was from the previously seen list A, list B or a new word (regardless of modality). Ten different versions of each word list (A, B, C) matched for word frequency, concreteness and imagery were used. Order was randomised across test days and sessions such that each word list was seen once only by each participant.

2.4.2 Psychomotor test

A small square target appeared on the screen at random locations, and the participant was required to click the target as quickly and accurately as possible. A new target appeared immediately after each mouse click, regardless of accuracy. After each response, the mouse cursor returned to the screen centre point. The task continued for 3 min. Response time for accurate trials was the dependent variable for this task which involves cerebellar activation.

2.4.3 Serial sevens

A random three-digit number appeared on the screen and the participant was required to subtract 7 from this number, entering the answer using the keypad. The subtractions continued from the previous number entered, for a period of 2 min. The number of correct subtractions was the dependent variable. This is a working memory task invoking the hippocampus.

2.5 Glycemic response

Glycemic response was measured using finger prick capillary samples (all participants) and interstitial continuous glucose monitoring ($n = 12$). Capillary glucose was measured using the same pre-calibrated GlucoMen® Meter (A Menarini Diagnostics) for each subject. Finger prick samples were taken at 40 and 5 min prior to consumption of the test drinks and at 30, 65, 80, 95, 110, 145 and 165 min post-consumption. Interstitial glucose was measured *via* continuous ambulatory monitoring using the Glucoday® microdialysis technique previously validated against arterialised and capillary samples in non-diabetic males [21]. The Glucoday® device consists of a microdialysis fibre, which is inserted into the subcutaneous tissue, and a biosensor, which consists of a glucose oxidase membrane. The device provides 3-min interstitial glucose estimates for up to 48 h. The ambulatory glucose-monitoring device (AGD) was calibrated according to manufacturer's instructions using the capillary sample taken just prior to consumption of the test drinks.

2.6 Procedure

On the day before each test day, participants were instructed to consume a standardised evening meal before 21:00, after which they were asked to fast and only drink water until the test procedure. The energy content of the meal was kept constant across test conditions within subjects. Participants were also instructed to refrain from exercise and alcohol and to maintain a standard pattern of activity on the preceding day.

On the morning of each test day, AGD participants attended the Research Unit at 7:00, while those who underwent capillary measures arrived 2 h later. The same measurement interval schedule was followed for both the groups. Fasted capillary glucose was measured by finger prick on arrival and AGD participants were then fitted with an AGD. Two additional finger prick measures were taken at 40 and 5 min prior to consumption of the test drinks; one of which was used to calibrate the AGD. All participants consumed the test drink at 9:50 with nothing further to eat or drink until the end of the test procedure.

A cognitive test battery comprising tests of verbal memory, psychomotor speed and mental arithmetic (a measure of working memory) was administered 35 min before consumption (baseline) and at 35 and 115 min post-consumption. The timing of the test battery was based on glycemic response profiles obtained during a pilot study. Each test battery did not exceed 30 min. Ratings of subjective performance and mental effort in relation to the cognitive tests along with subjective mood and appetite were measured throughout the test day using visual analogue scales administered using the electronic appetite rating system [23, 24] (data not reported). Additionally, participants were asked to identify which test within the battery was most/least difficult and required the most/least amount of effort. All participants were offered a drink and snack before leaving the test centre.

2.7 Statistical analysis

All data were entered in Excel and examined for outliers using SPSS (Version 14.0, SPSS, Chicago, IL). Data analysis was performed using SAS (Version 9.1.3, SAS Institute,

Cary, NC). ANCOVA models using PROC MIXED were used to examine the effects of the study drinks on indices of cognitive performance. All tests were two-tailed with an alpha level of 0.05. Drink was a within-subject factor in all analyses with baseline performance included as a covariate. Session reflected the post-ingestion test session (within-subject factor), and order of drink presentation and visit were included to ensure there were no carryover or practice effects. The difference between trial 1 and trial 3 within each cognitive test battery was examined to indicate change in the rate of learning in the immediate verbal learning task.

3 Results and discussion

3.1 Glycemic response

Capillary glucose was measured nine times across the test day. Values rose sharply on ingestion of sucrose, were less elevated but more sustained following isomaltulose and unchanged after consumption of water. The changes in capillary glucose induced by water vary within 1 mmol, the limit of detection generally accepted as the margin of error for capillary devices in the euglycemic range. Changes in capillary glucose induced by isomaltulose spanned 1.5 mmol/L while sucrose induced twofold changes.

Differences in interstitial glucose levels induced by the three beverages were analysed during three epochs, corresponding to the baseline, and the two post-ingestion cognitive test batteries which included baseline (time point –11 which corresponds to the start of the baseline cognitive test battery) as a covariate.

There was no significant difference between the glycemic profiles during the baseline cognitive test battery (session 1), which was performed in a fasted state ($F(2,170) = 2.00$, $p = 0.14$). There was no decline in interstitial glucose during this demanding cognitive test battery. This does not support the view that demanding mental performance tasks lead to increased utilisation of glucose reflected by a peripherally discernible drop in circulating glucose [8]. The same mentally demanding working memory task (serial sevens) was used in both the studies but previous studies used capillary measures pre- and post-task [3–8]. Interstitial measures were obtained at 3-min intervals in this study and any drop in circulating glucose during the test battery would be more likely to be detected by our technique because of the frequent within task sampling of glucose.

During the first post-ingestion cognitive test battery (session 2), the interstitial glucose profiles induced by the drinks differed significantly ($F(2,285) = 5.29$, $p = 0.0056$). Sucrose ingestion resulted in significantly higher glycemic responses during the test battery than isomaltulose, which in turn produced a significantly higher overall glycemic response than water. Sucrose facilitated a rapid elevation of glucose compared with the stable profile seen for water and the attenuated rise induced by isomaltulose. The glucose response following all drinks had returned to near baseline levels by the time the second post-ingestion cognitive test battery began (+115 min), and there was no significant difference between the three drinks during session 3 ($F(2,285) = 2.26$, $p = 0.1064$).

Elevated glycemic responses following consumption of rapidly digested carbohydrates, such as sucrose, are commonly followed by a rapid decline in blood glucose,

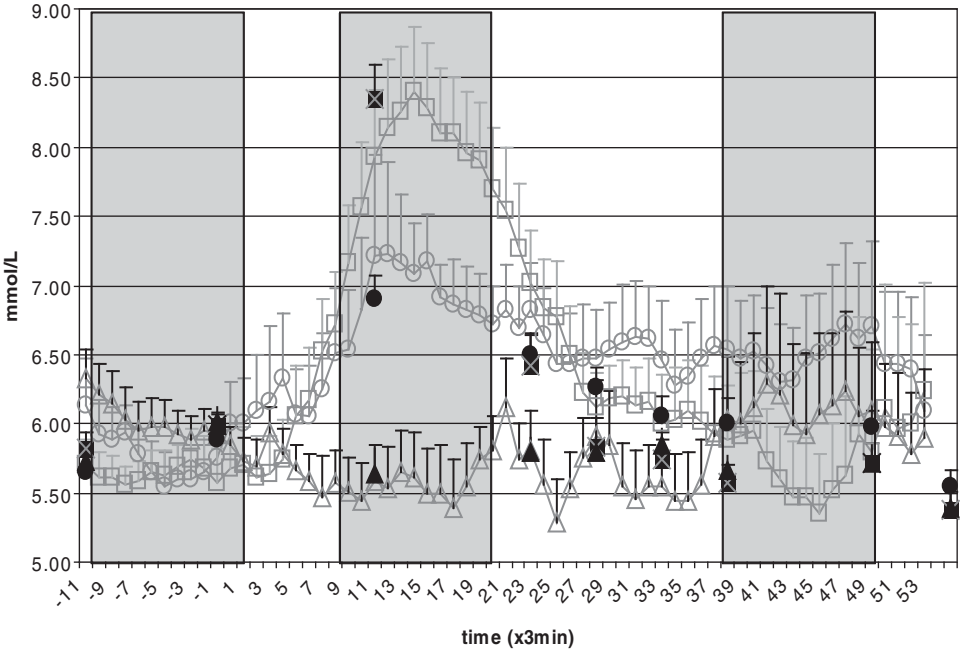

Figure 1. Mean (+SE) interstitial glucose profiles (measured using AGD) and capillary glucose, pre- and post-consumption of water, isomaltulose and sucrose drinks. Capillary glucose measures are indicated by filled symbols and interstitial glucose by open symbols. Triangles represent water, squares sucrose and circles isomaltulose drinks. Cognitive test periods are indicated by the shaded areas. The glucose profile following the sucrose drink is significantly higher than following isomaltulose which is also significantly higher than the water control during the second cognitive test period.

often to below baseline levels. This glycemic undershoot could trigger a counter-regulatory response of lipolysis and free fatty acids [25, 26]. As apparent from Fig. 1, we observed a very mild glycemic undershoot in the sucrose condition around the time of the second cognitive test battery, whereas no such undershoot was observed in the isomaltulose condition, although a high dose (50 g) was used. The glycemic response to sucrose is slightly less than that to glucose, and the insulin response is about 50% [27]. Isomaltulose is hydrolysed to glucose and fructose more slowly than sucrose, in the small intestine, leading to smaller elevations of blood glucose and lower insulin [28] and free fatty acid release [19]. It is likely that the caloric drink conditions elicited differential metabolic effects (*e.g.* insulin, free fatty acids) particularly at session 3; however, these were not measured in the current study. It is plausible that the insulinotrophic properties of the milk protein in both the isomaltulose and the sucrose drinks influenced glycemic response at this stage [26, 29]. Any difference in cognitive performance at session 3 could be due to the different metabolic consequences of the sucrose and isomaltulose drinks, although blood glucose responses were similar.

Consideration of the capillary profiles superimposed on Fig. 1 suggests that intermittent capillary sampling misses a good deal of information about the glycemic response during testing. One cannot infer a linear relationship between the two capillary sampling points and indeed our data suggests that infrequent capillary sampling may miss potentially important fluctuations and lead to incorrect inferences about the temporal profile of glycemic response to nutritional manipulations.

3.2 Cognitive performance

Table 1 lists the least square mean values (+SE) after consuming sucrose, water or isomaltulose on the various measures of cognitive performance employed in this study.

3.2.1 Verbal memory

3.2.1.1 Immediate recall

Rate of learning within each session was reflected by the difference in the number of words recalled from trial 1 to trial 3. Baseline did not predict post-ingestion performance ($F(1,110) = 0.07$, $p = 0.7920$) and there was no effect of drink (Table 1). When order and visit were included in the analysis, these were also not significant.

3.2.1.2 Delayed recall

Delayed memory was the primary outcome variable on which the power calculations were based. It was identified as the performance measure most sensitive to glucose manipulations based on a systematic review with strict quality criteria [9]. Baseline delayed recall performance was a significant predictor of subsequent performance ($F(1,108) = 7.68$, $p = 0.0066$), but there was no effect of drink on delayed recall of the verbal learning task ($F(2,108) = 0.25$, $p = 0.78$) nor drink by session interactions. Including order and visit as factors had no impact.

3.2.2 Recognition

The number of correct responses for each list as well as the reaction time to identify the words correctly *via* each sensory modality was analysed. Trials for which the reaction time fell 1.96SD below the mean for each participant were excluded, as they were unlikely to be perceived or were potential guessing trials. This amounted to less than 3% of all recognition trials.

There were no effects of drink on the number of correctly identified words presented visually or aurally, although response time was faster for visually presented stimuli ($F(2,763) = 20.21$, $p < 0.0001$) which is compatible with encoding. Baseline was a significant predictor for both number of correct responses ($F(1,763) = 41.56$, $p < 0.0001$) and response time ($F(1,763) = 45.14$, $p < 0.0001$). Recognition was best for the novel word list (list C) than for

Table 1. Least squares mean values after ingestion of sucrose, isomaltulose and water for each cognitive performance measure

Performance measure	Sucrose	Isomaltulose	Water
VVLT immediate recall: no. of correct responses	4.44	4.33	4.25
VVLT delayed recall: no. of correct responses	7.9	8.1	8.5
VVLT recognition: no. of correct responses	5.63	5.47	5.73
VVLT recognition: response time (msec)	1525	1530	1515
Psychomotor: no. of correct responses	141.1	142.8	139.2
Psychomotor: reaction time (msec)	829.3	925.2	893.6
Psychomotor: no. of correct responses (excluding low baseline)	151.9	151.9	153.2
Psychomotor: reaction time (excluding low baseline) (msec)	865.3	957.1	923.5
Serial sevens: no. of correct responses	26.9*	24.9	24.9
Serial sevens: reaction time (msec)	4574	5027	4776

Least square mean values are adjusted for the effect of the baseline.
*Significant difference between sucrose and water/isomaltulose is $p < 0.05$.

list A (which had been seen three times in the learning phase) and was worse for list B which was seen only once ($F(2,763) = 7.26$, $p = 0.0008$). For recognition and reaction time, there was no main effect of order or visit.

3.2.3 Psychomotor performance

There were 160 targets in each version of this task. Performance was generally good with an overall mean of 143 correct targets achieved over all test days and sessions. For the number of correct responses, there was a clear effect of baseline ($F(1,101) = 1039$, $p = 0.0001$) and a main effect of drink (isomaltulose > sucrose and water, $F(2,101) = 3.66$, $p = 0.0293$) with respect to the intercepts for the relationship between baseline and post-intervention score. The least square mean values, *i.e.* the mean values corrected for differences in baseline, showed that significant effects of drink could not be detected. The number of correct responses fell as the morning progressed ($F(3,101) = 2.97$, $p = 0.0353$), which indicates increased fatigue rather than practice on this task. There was also evidence that performance improved significantly with subsequent visits ($F(2,101) = 16.68$, $p = 0.0001$), which would suggest a practice effect. However, when 24 observations (4 subjects) associated with low levels of baseline performance were excluded, these effects were negated indicating that they were not robust.

For reaction times on the psychomotor test, no main effect of drink was observed ($F(2,101) = 1.51$, $p = 0.2276$). Baseline was a highly significant predictor ($F(1.101) = 19.13$, $p = 0.0001$). The reaction time for correct responses decreased, *i.e.* performance improved significantly, with each visit ($F(2,101) = 3.26$, $p = 0.0425$). When 24 observations (4 subjects) associated with low levels of baseline performance for the number of correct responses were excluded, again these effects were negated. This clearly indicates that the effects observed for both number of correct answers and response time are attributable to the challenging nature of the task.

3.2.4 Serial sevens

Performance on this working memory task was measured by the number of correct subtractions, the response time for these and the total number of subtractions attempted in each test battery. Baseline performance was a strong and significant predictor of subsequent performance ($F(1,97) = 9.17$, $p = 0.0032$). For the number of correct responses, there was no baseline–drink interaction but there was a main effect of drink ($F(2,99) = 6.03$, $p = 0.0034$) such that performance following sucrose was superior to both water and isomaltulose (Table 1). A trend for increased correct responses relative to baseline observed over the test day (baseline by session within visit interaction: $F(3,97) = 2.42$, $p = 0.0706$) which was further related to consecutive visits 2 and 3 (baseline by visit interaction: $F(2,97) = 3.84$, $p = 0.025$) could indicate a practice effect.

Figures 2 and 3 show response times at baseline plotted against response times in the first (Fig. 2) and second (Fig. 3) post-ingestion sessions. Comparing these figures suggests that the relationship between baseline and response time is influenced by the drink consumed, but that this influence differs according to session. The effect of the drinks on response time depends on baseline performance ($F(1, 97) = 5.88$, $p = 0.0172$). There is no effect of drink at

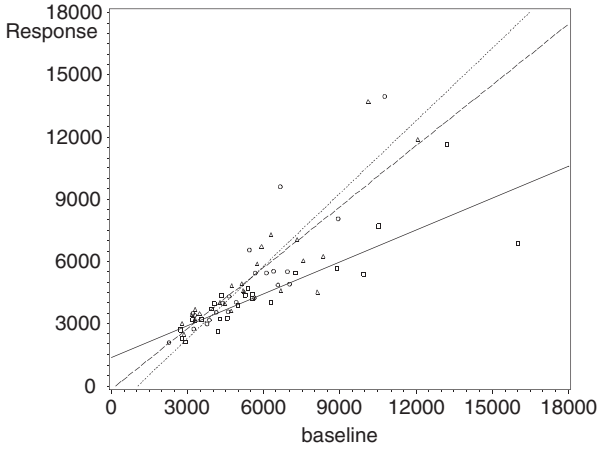

Figure 2. Serial sevens regression of baseline reaction time on reaction time for correct responses in the first post-ingestion test battery Triangles represent water, squares sucrose and circles isomaltulose drinks. The relationship between baseline and response is given by the solid line for sucrose, dashed line for isomaltulose and dotted line for water.

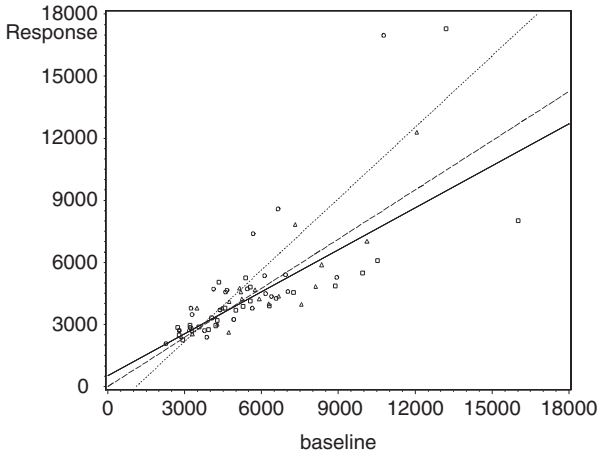

Figure 3. Serial sevens regression of baseline reaction time on reaction time for correct responses in the second post-ingestion test battery. Triangles represent water, squares sucrose and circles isomaltulose drinks. The relationship between baseline and response is given by the solid line for sucrose, dashed line for isomaltulose and dotted line for water.

either session in subjects with faster baseline response times. However, an effect is detectable for those with slower baseline response times, which is reflected by the significant baseline by drink interaction ($F(2, 97) = 3.90$, $p = 0.0236$).

In Fig. 2, the lines showing the relationship between baseline and response time for water and isomaltulose are close together suggesting little difference between isomaltulose and water immediately post-ingestion and a strong correlation with baseline performance. However, sucrose has a different slope indicating a beneficial effect of sucrose on response time compared with the other two drinks in those participants with slower baseline performance. At the second post-ingestion session, sucrose and isomaltulose regression lines are close together, while there is a different slope for water, indicating that both the isomaltulose and the sucrose drinks result in faster performance at session 3 than water in those participants whose baseline performance was slow. Hence, while sucrose improved performance at both test sessions, the effect of isomaltulose was confined to the later test session and both drinks only exerted a significant influence on the performance of those participants whose baseline performance was slowest.

Taken together these data demonstrate that despite large and significant fluctuations in frequently measured circulatory glucose, induced by sucrose and attenuated in amplitude and prolonged in duration by isomaltulose, few effects on cognitive performance were observed. These findings are commensurate with those of [12], who found that overall performance did not differ with consumption of different test foods, all of which elicited significant differences in glucose response curves. In line with these data, glucose ingestion may not influence complex memory [30].

In two studies that reported a beneficial effect on memory, blood glucose levels had returned to baseline before a treatment effect was observed [13, 31]. In other studies without blood glucose measures [14, 32], cognitive effects were mostly reported between 2 and 4 h after the intervention, which is probably also after the return of blood glucose levels to baseline. These temporal relationships implicate other metabolic events induced by the nutrient ingestion rather than glucose response *per se*. A strength of our study is the fact that we employed a robust balanced design which was preceded by a pilot study which established the distribution of glycemic response to the drinks and ensured that we positioned the cognitive test batteries at periods when differences in glycemic response were maximal and confirmed that the drinks were well matched for palatability, which can affect glycemic response [33]. Glycemic response was confirmed by a continuous glucose monitor validated against the arterialised venous blood [34], and reflective of glucose excursion in the cortex [35]. To our knowledge, this is the first study in this field to adopt this approach. Findings such as these indicate that peripheral measures of blood glucose *per se* might not be a reliable biomarker of performance measures, and question the traditional and intuitively appealing hypothesis that ingested glucose improves memory by directly increasing uptake of glucose to the brain.

Under conditions of adequate nutrition, glucose supply to the brain is prioritised. Brain glucose metabolism is increased at fasting insulin concentrations and is supplied preferentially to ''critical'' regions which differ in insulin sensitivity [36, 37]. Basal insulin stimulates glucose metabolism in brain regions with a high density of insulin receptors, *e.g.* hypothalamus and to a lesser degree the cerebellum [38]. Insulin resistance, impaired glucose tolerance and obesity are associated with cognitive impairments of tests which engage these regions [2, 39, 40]. When circulating insulin is raised above the fasting range, such as might occur after a glucose load to remove the excess glucose from the circulation, the neuronal response is to stimulate neurotransmitter release [41] which could indirectly increase glucose uptake in these areas of brain [41]. Tasks which require involvement of these specific brain areas, where there are a high proportion of GLUT transporters, insulin and glucose receptors, should be most sensitive to dietary interventions which affect glycemic response. However, absolute glucose levels cannot be measured in the human brain *in vivo* at present. Imaging techniques such as MRI can be used as proxy measure of glucose metabolism and are likely to have greater sensitivity than the peripheral glucose measures used in the current study. However, there are no MRI studies combining brain glucose metabolism during cognitive performance with blood glucose level measurements in healthy participants comparable to those in the current studies; the majority of MRI studies in this area are based on Alzheimer's patients. Positron emission tomography can also be used as a proxy for glucose metabolism by the brain. An increase in glucose uptake by the brain in young males undertaking a complex visuo-spatial motor task was observed in a positron emission tomography study [42], and in rats, a decrease in hippocampal interstitial glucose levels proportional to the difficulty of the maze was observed [43]. However, in both the studies peripheral glucose concentrations remained unchanged. This suggests that cognitive demand will be accompanied by increased local glucose metabolism in those brain areas engaged in specific tasks. Candidate tasks to detect such effects include those that engage the hippocampus (differential memory tasks), the cerebellum (psychomotor performance), both of which were included in this study, and tasks which are stressful, as these increase cortisol, which is linked to peripheral glucose regulation [44]. These cognitive domains were examined in this study using isomaltulose and sucrose to modulate glycemic response with little effect on performance in these young healthy males. One possible explanation of the lack of effects (other than that the interventions did not alter cognitive function) is that the tests used were not sensitive. Appropriate test selection is difficult because not all tasks which engage the hippocampus are similarly affected by the ingestion of glucose and performance may also be influ-

enced by age and glucoregulation [30]. Choosing cognitive measures is, therefore, never routine and should not be driven by convenience but rather with reference to the target population and proposed mechanism of action of the intervention [45, 46].

An alternative explanation for the lack of cognitive effects rather than test insensitivity is that such effects are unlikely to be detected in healthy subjects with good glucoregulation. Two recent reviews have both suggested that there are inconsistencies in the relationship between blood glucose patterns and performance measures [9, 16]. Benton [3–6] has shown that the best predictor of cognitive performance is the rate of falling blood glucose using capillary measures. This implies good glucose regulation and underlies our choice of young lean males for this study. In such healthy populations, the amount of glucose ingested does not influence glycemic response [27].

These data also raise some important issues in relation to study design. Inter-individual differences in glucose tolerance [12, 47] are important potential mediators of effects but within-subject designs with strong experimental control for second meal effects on glycemic response such as implemented here are rare, *cf*. Hoyland's inclusion criteria [9]. These effects are probable even in healthy young adults with good glucoregulation. Morgan *et al*. [10] and Lamport *et al*. [48] have demonstrated effects of evening meals varying in glycemic index on response to breakfast on the following day with suggestive effects on cognitive performance. Failure to control for these influences or the use of between-subject designs may increase the likelihood of detecting cognitive effects which are actually spurious. Our findings also highlight the importance of controlling for potentially influential covariates such as baseline performance. Practice effects can be short term occurring over sessions or long term carrying over visits. Such effects are seldom evaluated [45] or the tests on which they occur were identified [7, 8], in studies of nutrient effects on cognitive function. Nevertheless, these effects are not irrelevant [49] and illustrate the need for careful within-subject designs to reduce inter-subject variability on such tasks in studies of this nature.

4 Concluding remarks

This study is an important demonstration of cognitive performance under different levels of glycemia using isomaltulose as an investigative tool to lower glycemic response. It clearly refutes the hypothesis that glycemia is associated with cognitive performance. These data question the suggestion that isomaltulose has any effect on objective cognitive performance. However, evidence of potentially beneficial effects of isomaltulose on a number of other health indicators is mounting. Isomaltulose has been demonstrated to improve glucoregulation, free fatty acid levels and body composition [19, 50], and its lower exogenous oxidation has been shown to increase fat oxidation during exercise [51]. In our study, 50 g isomaltulose attenuated glycemic response even in a milk vehicle and further studies to explore the dose-dependent metabolic and cognitive effects of isomaltulose in a neutral vehicle with continuous measures of glucose and insulin in healthy and metabolically vulnerable individuals is suggested.

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