Addition of an alginate hydrogel to a carbohydrate beverage enhances gastric emptying

Shaun Sutehall[1], Stuart D.R. Galloway[2], Andrew Bosch[1] and Yannis Pitsiladis[3]

[1]Division of Exercise Science and Sports Medicine, University of Cape Town, Cape Town, South Africa; [2]Faculty of Health Sciences and Sport, University of Stirling, Stirling, United Kingdom; [3]Collaborating Centre of Sports Medicine, University of Brighton, Eastbourne, United Kingdom

Corresponding author:

Professor Yannis Pitsiladis

Collaborating Centre of Sports Medicine,

University of Brighton,

Denton Road,

Eastbourne,

BN20 7SH,

United Kingdom

Email: Y.Pitsiladis@brighton.ac.uk

Abstract

Purpose. To examine the effect of altering osmolality or adding sodium alginate and pectin to a concentrated carbohydrate (CHO) beverage on gastric emptying (GE) rate. **Methods.** 500 mL boluses of three drinks were instilled double-blind in eight healthy males while seated and GE measured using the double sampling method for 90 min and blood samples collected regularly. Drinks consisted of glucose and fructose (MON, 1392 mOsmol/kg), maltodextrin and fructose (POLY, 727 mOsmol/kg) and maltodextrin, fructose, sodium alginate and pectin (ENCAP, 732 mOsmol/kg) with each providing 180 g/L CHO (CHO ratio of 1:0.7 maltodextrin/glucose:fructose). **Results.** Time to empty half of the ingested bolus was faster for ENCAP (21±9 min) than POLY (37±8 min), both were faster than MON (51±15 min). There were main effects for time and drink in addition to an interaction effect for the volume of test drink remaining in the stomach. There were no differences between MON or POLY at any timepoint. ENCAP had a smaller volume of the test drink in the stomach than MON at 30 min (193±62 vs 323±54 mL), which remained less up to 60 min (93±37 vs 210±88 mL). There was a smaller volume of the drink remaining in the stomach in ENCAP compared with POLY 20 min (242±73 vs 318±47 mL) and 30 min (193±62 vs 304±40 mL) after ingestion. Although there was a main effect of time, there was no effect of drink or an interaction effect on serum glucose, insulin or non-esterified fatty acid (NEFA) concentrations. **Conclusion.** The addition of sodium alginate and pectin to a CHO beverage enhances early GE rate but did not effect serum glucose, insulin or NEFA concentration at rest.

Key words: Gastric emptying, sodium alginate, pectin, half emptying time, carbohydrate

Introduction

Consuming both carbohydrate (CHO) and fluid during prolonged endurance exercise is necessary for optimal performance (1). Firstly, it is well documented that the ingestion of CHO during exercise improves endurance performance primarily through the maintenance of blood glucose concentration (2). This has been shown to attenuate the decline in endogenous carbohydrate stores, namely liver glycogen (3), permitting a higher exercise intensity to be sustained for a longer duration. Secondly, the ingestion of water during prolonged endurance exercise prevents significant losses of plasma volume due to dehydration and thus cardiovascular function is maintained, allowing the constant dissipation of metabolic heat to the environment (4). The current guidelines for athletes recommend ingesting CHO at a rate of up to 90 $g \cdot h^{-1}$ and fluid at a rate of 600 - 800 $mL \cdot h^{-1}$ when performing prolonged endurance exercise exceeding 2.5 hrs (5,6). While these recommendations are supported by more recent evidence (3,7), it is rarely adhered to by athletes drinking *ad libitum* in "real-life" competition (8,9). This may, in part, be caused by the occurrence of gastrointestinal (GI) distress when ingesting a CHO beverage in excess of ~10% CHO (10,11), among other factors such as the difficulties of trying to drink while running at ~20 $km \cdot hr^{-1}$.

GI distress can occur for a variety of reasons and is especially apparent when ingesting a hypertonic, highly concentrated CHO beverage (12). In an attempt to prevent the rapid appearance of large quantities of CHO in the intestine from an ingested CHO beverage, gastric emptying (GE) rate declines when the solution contains ≥6% CHO (13) and thus, decreases the rate of CHO delivery to the intestine. While the GE rate of a CHO beverage is inversely proportional to the energy density, the delivery of CHO to the intestine is increased in beverages containing high concentrations of CHO despite a slower emptying rate and

smaller volume emptied (14). Due to this, either fluid or CHO delivery must be prioritized when deciding on which concentration of CHO to provide to an athlete during prolonged strenuous exercise. In hot conditions, where an athlete is expected to lose a significant volume of body fluid via sweating, fluid delivery is prioritized by using a beverage containing a relatively low concentration of CHO (e.g. <6%) (15). Conversely, in a cold environment, a higher concentration of CHO is favored (16) although this is not universally agreed upon (17). Osmolality is an additional factor that must been considered when formulating a CHO beverage since it has been reported that hyperosmolality can slow the GE rate of a CHO beverage (18,19). Current consensus suggests that osmolality plays a minimal role in the GE rate at low (i.e. 40 $g \cdot L^{-1}$) concentrations of CHO but may have a more marked effect at higher (i.e. 188 $g \cdot L^{-1}$) concentrations (19,20). Recent research suggests that there exists an osmotic threshold of ~350 $mOsm \cdot kg^{-1}$, after which GE will be inhibited (21)

Alginate is a natural biopolymer extracted from seaweed and is used as an excipient in a variety of different applications such as oral drug delivery (22) and within the food industry (23). The release of these compounds from the alginate gel is dependent on the local environmental pH and temperature (24) and can be tailored to release in a desired environmental condition (22). Recently, it was theorized that the addition of a sodium alginate-pectin hydrogel to a CHO beverage may allow a higher concentration of CHO to be emptied from the stomach during exercise by masking the CHO from the various receptors located in the duodenum that slow GE (25). It is believed that the addition of sodium alginate and pectin to a CHO beverage will encapsulate the CHO within the pH sensitive hydrogel when in contact with the acidic gastric juice in the stomach. This encapsulated CHO will then pass into the duodenum, where the relatively rapid elevation in pH (26) will initiate the release of CHO to the intestinal lumen for absorption, but depending on the speed of this

release, may result in a lag phase in the activation of the inhibitory control of GE. Currently there is no empirical evidence to support this notion, only anecdotal evidence for a lack of GI distress in strenuously exercising athletes when ingesting beverages containing ≥14% CHO (25,27).

Therefore, the aim of this study is twofold; firstly, to investigate the effect of adding sodium alginate and pectin to a concentrated CHO beverage on the rate of GE and secondly, to investigate the effect of manipulating the osmolality of a highly concentrated CHO beverage on the rate of GE.

Methods

Participants

Eight healthy, active but untrained males volunteered to participate in this study (age; 27 ± 5 yrs, height; 178 ± 6 cm, mass; 83.3 ± 5 kg). None of the participants required any medication or had any condition affecting gastric function. Once the study information had been given to participants in verbal and written form, all participants signed an informed consent form. All procedures were approved by the University of Stirling (Scotland) ethics committee.

Experimental design

Participants arrived at the laboratory following an overnight fast and abstinence from alcohol, caffeine and unaccustomed exercise for 24 hrs. While seated, participants self-positioned a nasogastric tube (French Levine, 14 gauge, Vygon, Wiltshire, UK) orally into the stomach. The stomach was emptied, washed and a recovery test carried out similar to that described elsewhere (28). Briefly, 100 mL of distilled water was instilled into the participant's stomach and the contents immediately aspirated. If >90 mL was aspirated, the tube was deemed in the correct position. Following this, 500 mL of a test drink, containing an additional 5 mL of a 15 $g \cdot L^{-1}$ nonabsorbable marker (phenol red; Sigma-Aldrich, Dorset, UK), was instilled rapidly into the stomach. All test drinks were provided at room temperature and the participants remained seated throughout the sampling period. Each visit to the laboratory was separated by a minimum of 48 hrs. Participants attended the laboratory on three occasions, with the drink allocation randomized using a Latin square design. All of the drinks were provided in a double-blind fashion, with participants and investigator performing the measurement of gastric volume blinded to the drink randomization.

Experimental drinks

The three drinks assessed consisted of 180 $g \cdot L^{-1}$ glucose and fructose (MON, 1392 ± 45 $mOsm \cdot kg^{-1}$), 180 $g \cdot L^{-1}$ maltodextrin and fructose (POLY, 727 ± 22 $mOsm \cdot kg^{-1}$), and 180 $g \cdot L^{-1}$ maltodextrin, fructose, sodium alginate and pectin (ENCAP, 732 ± 33 $mOsm \cdot kg^{-1}$). The composition of each drink is presented in Table 1. The total amount of sodium alginate and pectin represented 0.2% of the formulated drink with a ratio of sodium alginate to pectin of 3:2. All drinks also contained an additional 1.5 $g \cdot L^{-1}$ of sodium chloride and had a CHO ratio of 1:0.7 (maltodextrin or glucose:fructose). The sodium derived from the sodium alginate present in ENCAP (0.06 g) was not accounted for in MON and POLY as sodium alginate is classified as a resistant CHO and subsequently only digested in the lower parts of the intestine. The bioavailability of this sodium is, therefore, very low and unlikely to influence any outcome measure in the present study. None of the drinks used in the present study are commercially available.

Assessment of gastric emptying rate

Gastric emptying of the test drink was assessed using the double sampling gastric aspiration method of George (29) as modified by Beckers et al. (30). Specifically, a 2.5 mL sample of the test drink was taken from the 500 mL bolus with the remaining volume instilled into the participant (<2 min) with the aid of gravity using two 60 mL syringes. Although the test drink was instilled, it will be refereed to hereon in as ingested. Following ingestion, the stomach contents were mixed using a 60 mL syringe to aspirate 30-40 mL and subsequently re-inject into the stomach at least 10 times. A 2.5 mL sample was again taken from the gastric aspirate so that the volume of gastric secretions and swallowed saliva could be measured and accounted for in subsequent time points. Nine min after ingestion of the test drink, the stomach contents were mixed, a 2.5 mL sample of the gastric aspirate taken, 5 mL of phenol

111 red dye added, mixed and a further 2.5 mL sample of the gastric aspirate taken at 11 min.
112 Although these samples were taken at 9 and 11 min after ingestion, the calculated volumes
113 are referred to as those collected at 10 min. This procedure was repeated every 10 min until
114 90 min after ingestion of the test drink. To improve the accuracy of the measurement, the
115 concentration of the 5 mL of phenol red dye added at each 10 min timepoint increased
116 progressively (0.25 $g \cdot L^{-1}$ at 10 and 20 min, 0.5 $g \cdot L^{-1}$ at 30 and 40 min, 1.0 $g \cdot L^{-1}$ at 50 and 60
117 min, 1.75 $g \cdot L^{-1}$ at 70 and 80 min and 2.0 $g \cdot L^{-1}$ at 90 min). The concentration of the phenol red
118 dye present in the gastric samples was measured spectrophotometrically following dilution
119 (1:20) with NaOH:$NaHCO_3$ (250:500 $mmol \cdot L^{-1}$) buffer. The time taken to empty half the
120 volume ($t_{1/2}$) of the ingested beverage was calculated using linear interpolation between each
121 10 min value identifying the point at which half the volume of the ingested beverage had
122 been emptied.
123
124 *Blood and urine collection and analysis*
125 Arterialized venous blood samples were collected from the dorsum of the hand while
126 submerged in a ~40°C water bath for the duration of the 90 min and aliquoted into a 6 mL
127 clot activating tube. A sample was taken 10 and 5 min prior to the ingestion of the test drink
128 and subsequently every 10 min after the ingestion of the test drink until 60 min, thereafter
129 blood was collected every 15 min until the 90 min was completed. The 6 mL blood samples
130 were allowed to clot and then centrifuged at 2000 g, for 10 min while at 4°C with the
131 resulting serum stored at -20°C until further analysis. Serum osmolality was measured prior
132 to sample freezing via freezing point depression (Type 15 Osmometer, Löser Messtechnik,
133 Berlin, Germany).
134

Serum glucose and non-esterified fatty acid (NEFA) concentration were measured using enzymatic assay methods on an automated analyzer (iLab Aries, Instrumentation Laboratory, Warrington, UK). Serum insulin was measured using a commercially available ELISA kit (Dimedic International, Hamburg, Germany) according to the manufacturer's instructions. The between assay coefficient of variation (CV) for these analyses are 2.5% and 6%, respectively.

Urine was collected immediately prior and immediately after the experimental procedure and its osmolality measured via freezing point depression (Type 15 Osmometer, Löser Messtechnik, Berlin, Germany) to determine hydration status.

Statistical analysis

All data are presented as mean ± SD and considered statistically significant at $P \leq 0.05$. All data were assessed for normal distribution using the Shapiro-Wilks test for normality as well as visually accessing the data's distribution. A two-way repeated measures ANOVA (time*trial) was performed to assess the differences in gastric emptying rates, serum metabolite concentrations, serum osmolality and gastric aspirate osmolality at each time point, between the different drinks. Significant interactions were followed by a paired t-test using Bonferroni correction for multiple comparisons. The $t_{1/2}$, urine osmolality, peak, time to peak and the area under the curve (AUC) for glucose and insulin concentration were assessed using a one-way ANOVA. The Statistical Package for the Social Science 25.0 software (SPSS, IBM Corp, NY, USA) was used for all ANOVA and *post hoc* analysis. The trapezoid method was used for calculation of the area under the curve (AUC) for serum glucose and insulin concentration using GraphPad (Version 8.0, GraphPad Software Inc, CA, USA). A Pearson correlation coefficient was also performed comparing $t_{1/2}$ between experimental

drinks using GraphPad. It is common in GE studies to find very large differences in $t_{1/2}$ between experimental drinks. For example, comparing the GE rate of a glucose polymer drink (237 mOsmol·kg^{-1}) with a glucose monomer drink (1300 mOsmol·kg^{-1}) resulted in a difference in $t_{1/2}$ of ~65 min (19). Considering the differences in drink composition used in the present study were more modest, we assumed a smaller effect in our study. Therefore, to determine the sample size required, we estimated a minimum difference in the $t_{1/2}$ of 15 min between conditions with a common SD of 10 min (Cohen's *d*=1.5). Using a one-way ANOVA, with a 95% confidence interval and at 80% power, it was estimated that seven participants would be required. We compared three groups and assumed equal variance between groups. Power calculations were performed using G*Power 3.1 (31). Following the completion of the study, the observed power of the two-way ANOVA to detect differences in the volume of test drink in the stomach was estimated. It was found that this study achieved 68% power, with two additional participants required to reach 80% power, indicating that this study is underpowered and may be unable to detect small differences in GE rate over time.

Results

Gastric emptying of test drink

The volume of the test drink remaining in the stomach in all experimental trials is presented in Fig 1. There was a significant main effect for time (P<0.01), trial (P<0.01) in addition to a time*trial interaction (P<0.05) for the volume of test drink remaining in the stomach. Specifically, there was a significantly smaller volume of the test drink in the stomach with the ingestion of ENCAP compared with MON at 30, 40 and 50 min but not after 60 min (P=0.063, Fig 1). Thereafter, there were no significant differences in the volume of the test drink in the stomach between MON and ENCAP. There were no significant differences in the

volume of the test drink in the stomach between ENCAP and POLY 10 min after ingestion. However, 20 and 30 min after ingestion, there was a significantly smaller volume of the test drink remaining in the stomach on the ENCAP trial (Fig 1). From 40 min onward, there were no differences between ENCAP and POLY. MON and POLY were not significantly different at any time point throughout the experimental period, however, the $t_{1/2}$ for POLY was significantly faster than MON (37 ± 8 vs 51 ± 15 min, $P<0.05$, respectively). The $t_{1/2}$ for ENCAP (21 ± 9 min) was significantly faster than both POLY ($P<0.05$) and MON ($P<0.01$). The volume of gastric secretion and swallowed saliva increased over time ($P<0.01$) but there was no main effect of trial nor a time*trial interaction. There was a strong positive correlation between the $t_{1/2}$ of MON and POLY (r=0.8, $P=0.02$) and weak, non-significant correlations between MON and ENCAP (r=0.2, $P=0.6$) and POLY and ENCAP (r=-0.1, $P=0.9$).

Blood metabolites and urine osmolality

There was a significant main effect of time for serum glucose ($P<0.01$, Fig 2A), NEFAs ($P<0.01$, Fig 2B) and insulin ($P<0.01$, Fig 2C) concentrations. However, there was no main effect of trial or a time*trial interaction for any of these variables. Similarly, there were no significant differences in serum glucose or insulin peak concentration, time to peak concentration or the AUC between any drink.

There was a main effect of time for serum osmolality ($P<0.01$) over the 90 min but no main effect of trial or a time*trial interaction. There were no disparities in urine osmolality before the ingestion of the test drinks (MON; 607 ± 285, POLY; 703 ± 292 and ENCAP; 652 ± 183 $mOsmol \cdot kg^{-1}$). There was a significant decrease in urine osmolality following ingestion of both POLY and ENCAP ($P<0.05$) but not after MON (MON; 435 ± 233, POLY; 338 ± 110 and ENCAP; 326 ± 100 $mOsmol \cdot kg^{-1}$). There were significant main effects for time and trial

(P<0.01), in addition to a time*trial interaction (P<0.01) for the gastric aspirate osmolality. Specifically, from 10 min after ingestion until 90 min, the osmolality of the gastric aspirate following ingestion of MON was significantly higher than both POLY and ENCAP (Fig 3) with the exception of 90 min, where ENCAP was not significantly different from MON. There were no differences between POLY and ENCAP at any timepoint (Fig 3).

Discussion

There are two main findings of the present study. Firstly, encapsulating CHO within a hydrogel significantly enhances the early GE rate of an ingested, hypertonic, beverage at rest. Secondly, greatly increasing the osmolality of a CHO solution (i.e. glucose monomer vs glucose polymer) had a small (i.e. $t_{1/2}$ only) effect on the GE rate when both ingested beverages were extremely hypertonic (e.g. >700 mOsm·kg^{-1}). Despite a significantly faster GE rate with ENCAP, there were only minor alterations in subsequent serum glucose, NEFAs and insulin, suggesting that subsequent CHO absorption was not different between trials. The precise mechanism of this enhanced GE but delayed absorption with ENACP may be a result of a lower detection of energy density/osmolality and/or a delayed dissipation of the gel and subsequent hydrolysis of the maltodextrin.

In the present study, despite a large difference in osmolality between MON and POLY (~1400 vs ~730 mOsmol·kg^{-1}, respectively), there was minimal alteration in the GE rate, only detectable with $t_{1/2}$. This finding is similar to a previous study, which demonstrated that the GE rate is not affected by osmolality (32). However, this study (32) only investigated a range of drinks which differed in osmolality by ~130 mOsm·kg^{-1} (243 to 374 mOsmol·kg^{-1}) and therefore their results may only apply to drinks which are hypotonic or marginally

hypertonic. When the osmolality differed significantly by comparing a 15% glucose monomer and a glucose polymer beverage (739 vs 117 mOsmol·kg^{-1}, respectively), the glucose monomer solution emptied significantly slower (18). Notably, at lower concentrations, osmolality did not affect the rate of GE. Such findings have led to the perception that intestinal osmoreceptors are situated distally in comparison to the location of glucose polymer hydrolysis and therefore the osmolality of a glucose polymer solution will be similar to that of a glucose monomer of the same concentration detected by these receptors (19). With this in mind, should the rate of CHO delivery to the intestine or the concentration of the glucose polymer be exceptionally high, it is possible that the capacity of the small intestine to hydrolyze all the delivered CHO is exceeded, and therefore a higher osmolality will be detected, influencing GE through negative feedback (19). It has been suggested that this saturation point occurs when the ingested beverage is hypertonic (i.e. >350 mOsmol·kg^{-1}, (21)) and the results from the present study suggest that further increases in osmolality when the beverage is already markedly hypertonic will only have a minimal impact on GE rate. Another important consideration when comparing the effects of osmolality on GE, which may partially explain the lack of any differences, other than $t_{1/2}$ observed in the current study, is the type of CHO being investigated. Within this study, each drink contained glucose or maltodextrin in addition to fructose (in a ratio of 1:0.7) whereas the aforementioned studies investigating the effect of osmolality on GE used a single source of CHO only (e.g. (18,19)). The decision to include fructose was made in line with recent recommendations for the formulation of a CHO beverage to be used during exercise (7) and will have reduced the difference in osmolality between MON and POLY yet, the difference in osmolality still remained large (~1400 vs ~730 mOsm·kg^{-1}, respectively).

The volume of the test drink present in the stomach was significantly lower 20 and 30 min after ingestion of ENCAP compared with POLY (Fig1). This suggests that encapsulating CHO within a hydrogel somewhat decreases the inhibition of GE expected with a highly concentrated CHO beverage. Several factors are known to influence the GE rate of a beverage, with volume of the ingested beverage considered the most important (32). When the initial volume of an ingested beverage is equal between trials, such as in the present study, both the energy density and osmolality must be considered as possible reasons explaining the early differences in GE rate between POLY and ENCAP (20). It has been demonstrated that the presence of glucose in the duodenum will reduce the subsequent GE rate of 400 mL of physiological saline (34), suggesting that the duodenum plays an important role in regulating GE. Therefore, should any delay in the diffusion of CHO out of the hydrogel occur once in the duodenum, it is likely that the full energy density of the solution will not be detected, resulting in an augmented GE rate. This is also important considering the duodenum length is rather short (i.e. <30 cm, (35)) and thus, any solution will have a short transit time through the duodenum lumen. In addition, any polymer that is encapsulated would not be hydrolyzed as quickly resulting in a lower osmolality in the intestinal lumen, possibly influencing GE (36). What is unknown, however, is the relative contributions of these factors on the augmented rate of GE when sodium alginate has been added to a CHO beverage and future studies should investigate the precise mechanism of this enhanced GE. As expected, there was a strong correlation in $t_{1/2}$ between MON and POLY (r=0.8) but, notably, there was a weak, non-significant correlation when the $t_{1/2}$ of MON or POLY when compared to ENCAP (r=0.2 and -0.1, respectively), further highlighting the notion that a CHO beverage containing sodium alginate and pectin is handled differently during the early phase of GE. Despite a significantly faster early phase of emptying with ENCAP after 40 min, both ENCAP and POLY had a similar GE emptying rate for the remaining 50 min,

suggesting the effect of ENCAP may affect only the early phase of GE. ENCAP contained a very small amount of extra sodium (i.e. 0.06 g) compared to MON and POLY due to the addition of sodium alginate. This difference is unlikely to have impacted on GE due to the small quantity of additional sodium and the fact that sodium alginate, being a resistant CHO, will only be digested in the lower parts of the intestine.

The osmolality of the gastric aspirate declined in all drinks with the osmolality of MON significantly higher than POLY over the study period and ENCAP up to 80 min after ingestion of the test drinks. There were no differences in the rate of decline in osmolality of the gastric aspirate between POLY and ENCAP. This suggests that the emptying of CHO in ENCAP was of a similar pattern to POLY, rather than emptying a large proportion of the hydrogel at once. Considering that a sodium alginate hydrogel is a porous gel with only large molecules “trapped”, the process of repeatedly aspirating the gastric contents through the small holes of the nasogastric tube will have added a mechanical stress upon the hydrogel, increasing the surface area and increasing the rate of diffusion of CHO out of the hydrogel in the stomach. This is likely to have affected the osmolality of the gastric aspirate sample as osmolality was measured 1-2 hrs after it was aspirated from the stomach and the CHO within the hydrogel may have diffused out of the hydrogel. Therefore, the osmolality of the gastric aspirate may not reflect precisely what is encountered by the osmoreceptors in the intestine. A primary assumption of the method to measure GE used in this study is that the phenol red dye added to the stomach at each timepoint is evenly distributed, an assumption which may be violated if the phenol red dye cannot mix evenly across the hydrogel. Upon the completion of the 90 min period, all the remaining fluid in the stomach was removed, with its volume measured and compared with the calculated volume using the phenol red dye. This comparison indicated that the calculated volume and the actual volume aspirated from the

309 stomach at the end of the 90 min was similar, in all trials, suggesting the phenol red dye was
310 mixed uniformly in each drink.
311
312 Despite emptying well over half the total ingested volume (mean 307 mL) in 30 min, ENCAP
313 did not demonstrate an earlier elevation in serum glucose or insulin concentration compared
314 with POLY which emptied significantly smaller volume (mean 196 mL) in the same time
315 period. This can be illustrated by comparing POLY and ENCAP at 20 min in serum glucose
316 (6.7 ± 1.4 vs 5.5 ± 0.9 mmol·L^{-1}, respectively), serum NEFA (0.4 ± 0.1 vs 0.5 ± 0.1 mmol·L^{-1},
317 respectively) and serum insulin (83.6 ± 38.6 vs 65.5 ± 16.1 μIU·mL^{-1}, respectively). These
318 comparisons reveal that the modest differences between POLY and ENCAP were within the
319 sample variances observed. These secondary outcome measures obtained under resting
320 conditions could provide some insight into whether any increase in emptying of substrate into
321 the intestine actually leads to alterations in substrate delivery to the circulation. From our
322 data, it would appear that despite a faster early emptying rate with ENCAP (i.e. greater
323 carbohydrate delivery to the intestine) there was no difference in circulating serum glucose
324 concentration at the time points assessed. This could reflect a delay in the dissolution of the
325 hydrogel and subsequent reduced rate of appearance of the carbohydrate in the circulation, or
326 it could reflect a difference in glucose rate of disappearance (storage or oxidation) between
327 trials. This finding has striking similarity with a study by Leiper et al., in which it was
328 demonstrated that a 500 mL CHO beverage which formed a viscous gel (containing 78%
329 amylopectin and 22% amylose) emptied significantly faster than an isoenergetic CHO
330 beverage (containing 15% glucose syrup, 13% disaccharides and 72% higher
331 polysaccharides) (37). Despite a significantly faster GE, there were no differences in serum
332 glucose or insulin. The authors theorized that the increased viscosity of the gel beverage
333 increased the time for the CHO to reach the hydrolytic enzymes and subsequently decreased

334 the speed at which intestinal absorption occurred. This theory is supported by research
335 suggesting that it is the rate of hydrolysis of CHO which predominately influences the
336 subsequent absorption and blood glucose concentration elevation (38). Thus, the lack of
337 difference in glucose and insulin response suggests that hydrolysis and subsequent absorption
338 of the carbohydrates within the test solutions was not different despite faster early emptying
339 rate in the ENCAP trial. While the formulation of the gel in the study by Leiper et al and the
340 gel within the current study are different, the lack of differences in blood metabolites may be
341 caused by similar mechanisms, such as an increased viscosity and subsequent increased time
342 to reach hydrolytic enzymes, or CHO remaining within the gel, delaying absorption. Future
343 studies should consider investigating the intestinal perfusion of POLY and ENCAP to
344 determine the effect of ENCAP on intestinal receptors/transporters, without the influence of
345 GE. The time required for complete dissipation of the hydrogel at intestinal pH is not known,
346 however, our own pilot studies indicate that the gel will have completely dissipated within
347 approximately 30 min when placed into a buffer solution maintained at a pH of ~6.4 (i.e. the
348 approximate pH of the duodenum). Therefore, it is possible there remains a portion of the
349 CHO "trapped" within the hydrogel as it passes through the duodenum, avoiding receptors
350 and/or transporters.
351
352 All but one participant (PPT4, grey solid line with black open squares) had emptying
353 characteristics similar to the average values presented in Fig 1 (i.e. GE rate slowest for MON,
354 followed by POLY with ENCAP fastest). This one participant emptied both POLY and
355 ENCAP at similar rates ($t_{1/2}$ of 37 and 39 min, respectively). It is also notable that the
356 participant who had the second fastest ENCAP $t_{1/2}$ of 15 min (PPT8, grey dashed line with
357 grey open squares), had a significantly delayed serum glucose and insulin peak (peaks at 50
358 min) following ENCAP in comparison with MON and POLY (MON: 20 and 30 min and

359 POLY: 20 and 20 min, respectively). These differences in the response to ENCAP may be the
360 result of the well-known individual variation in the GE response to an ingested beverage (39)
361 but also an additional variation in stomach and intestinal pH affecting the formation and
362 dissolution of the hydrogel resulting in a greater variation in availability of CHO for
363 intestinal absorption.
364
365 The daily individual variation in $t_{1/2}$, measured using the double sampling method has been
366 shown to be 29.1% (39). The difference in $t_{1/2}$ between MON and POLY is less than this
367 (27%, Table 2) and therefore daily variation could be a major contributing factor to the
368 difference in the GE rate observed between these two drinks. On the other hand, differences
369 in $t_{1/2}$ between MON and ENCAP (59%) and POLY and ENCAP (43%) exceed daily
370 variation and are therefore, likely caused by the differences in beverage composition, altering
371 the GE rate. Despite the $t_{1/2}$ between MON and POLY being statistically significantly
372 different, the volume of test drink in the stomach did not differ at any timepoint throughout
373 the experimental period (Fig 1), however, it is worth noting that this study is underpowered to
374 detect differences in the GE rate between MON and POLY over time.
375
376 The participants recruited in this study were habitually active but not trained athletes and
377 therefore may have a lower density of sodium-dependent glucose transporter 1 (SGLT-1)
378 than their trained counterparts due to the higher daily CHO intake associated with athletic
379 training (40). While the density of SGLT-1 transporters will have implications for the rate of
380 intestinal absorption of CHO and subsequent exogenous CHO oxidation rate, there is
381 currently limited evidence on the effect of CHO training on GE rate during exercise, although
382 training status has been shown to have little effect on GE during exercise (41). Therefore, the
383 similar pattern of emptying shown by all participants (with the exception of one participant

emptying all three experimental drinks at similar rates), suggest the response may be similar across a range of habitual CHO intake rates. That said, the reader should be cautioned against extrapolating the results of this study to an exercising athlete, with the present results indicating CHO beverages containing additional sodium alginate and pectin are handled differently in the intestine than more traditional CHO beverages, at least at rest.

There are several limitations in this study, firstly, the present study is underpowered to detect differences in the GE rate over time and therefore the potential for a type two error has increased, particularly when comparing MON and POLY. Secondly, the procedure of swallowing the tube is unpleasant and not tolerable by everyone and therefore, the participants recruited within this study may not wholly represent the general population. Similarly, the repeated aspiration of the stomach contents through the nasogastric tube may have affected the structure and possibly function of the hydrogel. Therefore, future studies should consider using alternative methods such as the indirect ^{13}C-acetate breath test or magnetic resonance imaging to measure gastric emptying as well as utilizing deuterium oxide to asses water flux across the intestine, without influencing the hydrogel. In addition, future studies should consider measuring plasma volume as the ultimate fate of the ingested fluids is unclear, and it is unknown if the hydrogel affects intestinal absorption of fluids during exercise. The present study was also performed at rest with a single 500 mL bolus of the beverage instilled into the participant's stomach which is in stark contrast to the repeated ingestion of smaller volumes that typically occurs with *ad libitum* ingestion during exercise. Although an enhanced GE during exercise may not be beneficial *per se*, it may be indicative of differential sensing of energy density/osmolality in the intestine which may reduce some of the symptoms of GI distress. Therefore, it is advisable that future studies investigating the

efficacy of sodium alginate in a CHO beverage utilize drinking strategies similar to that observed during competition.

In conclusion, the present study demonstrates that the addition of a sodium alginate hydrogel to a concentrated CHO beverage will significantly enhance the GE rate in comparison with an isoenergetic beverage during the early, rapid phase of GE. It has also been demonstrated that dramatically increasing the osmolality of a concentrated CHO beverage only had a minor (i.e. smaller than daily variation) effect on GE rate. Despite a significant difference in the early GE rate between POLY and ENCAP, there were no subsequent differences in circulating serum glucose, NEFAs or insulin concentration. The lack of any differences in these blood parameters may suggest a delayed absorption with ENCAP, possibly negating any potential metabolic advantages with the enhanced GE rate during exercise. Whether the addition of sodium alginate and pectin to a CHO beverage prevents GI distress and improve performance during exercise remains to be investigated, preferably with randomized controlled trials.

Acknowledgements:

The authors would like to thank Dr John Leiper for his assistance in data collection throughout the study. This study was supported by a grant from Maurten AB and the Sub2 Foundation. All drinks were provided by Maurten AB. The results of this study do not constitute endorsement by ACSM. Results of this study are presented clearly, honestly, and without fabrication, falsification or inappropriate data manipulation.

Conflict of interest:

One of the authors, YP, is the founding member of the Sub2 project (www.sub2hrs.com); The Sub2 project is affiliated to a non-trading company (Athlome Limited, UK) that is minor (<1.1%) shareholder of Maurten AB. Author SS is a PhD student funded partly by the Sub2 project and Maurten AB. Authors SS and SG have received one travel grant each. This research is funded partly from a research grant from the Sub2 project and Maurten AB. None of the authors are paid consultants or have ownership of any patents linked to the present research.

Appendix

Figure 1. Volume of each test drink remaining in the stomach following the ingestion of each experimental drink. Panels illustrate the average response followed by the individual responses to each experimental drink. n = 8. Monomer: glucose, fructose and sodium chloride (MON); Polymer: maltodextrin, fructose and sodium chloride (POLY); and Encapsulated polymer: maltodextrin, fructose, sodium chloride, sodium alginate and pectin (ENCAP). b denotes significant difference between MON and encapsulated ENCAP ($P<0.05$) and c denotes significant difference between POLY and ENCAP ($P<0.05$).

Figure 2. Serum glucose (a), NEFA (b) and insulin (c) concentration. n = 7. Monomer: glucose, fructose and sodium chloride (MON); Polymer: maltodextrin, fructose and sodium chloride (POLY); and Encapsulated polymer: maltodextrin, fructose, sodium chloride, sodium alginate and pectin (ENCAP).

Figure 3. Osmolality of the gastric aspirate. n=5 Monomer: glucose, fructose and sodium chloride (MON); Polymer: maltodextrin, fructose and sodium chloride (POLY); and

Encapsulated polymer: maltodextrin, fructose, sodium chloride, sodium alginate and pectin (ENCAP). a denotes significant difference between MON and POLY ($P<0.05$), b denotes significant difference between MON and ENCAP ($P<0.05$).

Table 1. Composition of each 500 mL experimental beverage. Monomer: glucose, fructose and sodium chloride (MON); Polymer: maltodextrin, fructose and sodium chloride (POLY); and Encapsulated polymer: maltodextrin, fructose, sodium chloride, sodium alginate and pectin (ENCAP).

Table 2. Comparison of the half emptying time ($t_{1/2}$) between experimental beverages. The $t_{1/2}$ is expressed in min (mean±SD). Monomer: glucose, fructose and sodium chloride (MON); Polymer: maltodextrin, fructose and sodium chloride (POLY); and Encapsulated polymer: maltodextrin, fructose, sodium chloride, sodium alginate and pectin (ENCAP).

References

1. Smith JW, Pascoe DD, Passe DH, et al. Curvilinear dose–response relationship of carbohydrate (0–120 g·h− 1) and performance. Med Sci Sports Exerc. 2013;45(2):336-41.
2. Coyle E, Hagberg J, Hurley B, Martin W, Ehsani A, Holloszy J. Carbohydrate feeding during prolonged strenuous exercise can delay fatigue. Journal of Applied Physiology. 1983;55(1):230-5.
3. King AJ, O'Hara JP, Morrison DJ, Preston T, King RF. Carbohydrate dose influences liver and muscle glycogen oxidation and performance during prolonged exercise. Physiological Reports. 2018;6(1).
4. Maughan R, Bethell L, Leiper J. Effects of ingested fluids on exercise capacity and on cardiovascular and metabolic responses to prolonged exercise in man. Experimental Physiology. 1996;81(5):847-59.
5. Sawka MN, Burke LM, Eichner ER, et al. American College of Sports Medicine position stand. Exercise and fluid replacement. Med Sci Sports Exerc. 2007;39(2):377.
6. Thomas DT, Erdman KA, Burke LM. Position of the Academy of Nutrition and Dietetics, Dietitians of Canada, and the American College of Sports Medicine: Nutrition and athletic performance. Journal of the Academy of Nutrition and Dietetics. 2016;116(3):501-28.
7. Rowlands DS, Houltham S, Musa-Veloso K, Brown F, Paulionis L, Bailey D. Fructose–Glucose Composite Carbohydrates and Endurance Performance: Critical Review and Future Perspectives. Sports Med. 2015;45(11):1561-76.

8. Cheuvront SN, Montain SJ, Sawka MN. Fluid replacement and performance during the marathon. Sports Med. 2007;37(4-5):353-7.
9. Pfeiffer B, Stellingwerff T, Hodgson AB, et al. Nutritional intake and gastrointestinal problems during competitive endurance events. Med Sci Sports Exerc. 2012;44(2):344-51.
10. Wilson PB. Does carbohydrate intake during endurance running improve performance? A critical review. The Journal of Strength & Conditioning Research. 2016;30(12):3539-59.
11. de Oliveira EP, Burini RC. Carbohydrate-dependent, exercise-induced gastrointestinal distress. Nutrients. 2014;6(10):4191-9.
12. Rehrer NJ, van Kemenade M, Meester W, Brouns F, Saris WH. Gastrointestinal complaints in relation to dietary intake in triathletes. International Journal of Sport Nutrition. 1992;2(1):48-59.
13. Maughan R. Optimizing hydration for competitive sport. Optimizing Sport Performance Carmel, IN: Cooper Publishing. 1997:139-83.
14. Vist GE, Maughan RJ. Gastric emptying of ingested solutions in man: effect of beverage glucose concentration. Med Sci Sports Exerc. 1994;26(10):1269-73.
15. Galloway SD, Maughan RJ. The effects of substrate and fluid provision on thermoregulatory and metabolic responses to prolonged exercise in a hot environment. Journal of Sports Sciences. 2000;18(5):339-51.
16. Maughan R, Noakes T. Fluid replacement and exercise stress. Sports Medicine. 1991;12(1):16-31.
17. Galloway S, Maughan RJ. The effects of substrate and fluid provision on thermoregulatory, cardiorespiratory and metabolic responses to prolonged exercise in a cold environment in man. Experimental Physiology. 1998;83(3):419-30.

18. Sole C, Noakes T. Faster gastric emptying for glucose-polymer and fructose solutions than for glucose in humans. European Journal of Applied Physiology and Occupational Physiology. 1989;58(6):605-12.
19. Vist GE, Maughan RJ. The effect of osmolality and carbohydrate content on the rate of gastric emptying of liquids in man. The Journal of Physiology. 1995;486(2):523-31.
20. Leiper JB. Fate of ingested fluids: factors affecting gastric emptying and intestinal absorption of beverages in humans. Nutrition reviews. 2015;73:57-72.
21. Shi X, Osterberg KL, Petrie H, Stofan JR, Murray R. Effect of Different Osmolalities, CHO Types, and [CHO] on Gastric Emptying in Humans. Med and Sci in Sport and Exerc. 2017;49(5):1015-21.
22. Lee KY, Mooney DJ. Alginate: properties and biomedical applications. Progress in Polymer Science. 2012;37(1):106-26.
23. Brownlee IA, Seal CJ, Wilcox M, Dettmar PW, Pearson JP. Applications of alginates in food. Alginates: Biology and applications: Springer; 2009. p. 211-28.
24. Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidized alginate and its potential application for tissue engineering. Biotechnology Progress. 2001;17(5):945-50.
25. Sutehall S, Muniz-Pardos B, Bosch AN, Di Gianfrancesco A, Pitsiladis YP. Sports Drinks on the Edge of a New Era. Current Sports Medicine Reports. 2018;17(4):112-6.
26. Ovesen L, Bendtsen F, Tage-Jensen U, Pedersen N, Gram B, Rune S. Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. Gastroenterology. 1986;90(4):958-62.

27. Chavez C. (2017) .This drink hopes to propel elite marathoners to the sub-two hour mark. Sports Ilistrated. Available at: https//www.si.com/edge/2017/03/23/sub-two-hour-marathon-drink-maurten-elite-runners. [Accessed 19.01.19].
28. Hassan M, Hobsley M. Positioning of subject and of nasogastric tube during a gastric secretion study. BMJ. 1970;1(5694):458-60.
29. George JD. New clinical method for measuring the rate of gastric emptying: the double sampling test meal. Gut. 1968;9(2):237-42.
30. Beckers E, Rehrer N, Brouns F, Ten Hoor F, Saris W. Determination of total gastric volume, gastric secretion and residual meal using the double sampling technique of George. Gut. 1988;29(12):1725-9.
31. Faul F, Erdfelder E, Lang A-G, Buchner A. G* Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior research methods. 2007;39(2):175-91.
32. Brouns F, Senden J, Beckers J, Saris WH. Osmolarity does not affect the gastric emptying rate of oral rehydration solutions. Journal of Parenteral and Enteral Nutrition. 1995;19(5):403-6.
33. Noakes TD, Rehrer NJ, Maughan RJ. The importance of volume in regulating gastric emptying. Med Sci Sports Exerc. 1991;23(3):307-13.
34. Brener W, Hendrix TR, McHugh PR. Regulation of the gastric emptying of glucose. Gastroenterology. 1983;85(1):76-82.
35. Hounnou G, Destrieux C, Desme J, Bertrand P, Velut S. Anatomical study of the length of the human intestine. Surgical and radiologic anatomy. 2002;24(5):290-4.
36. Hunt J, Pathak J. The osmotic effects of some simple molecules and ions on gastric emptying. The Journal of Physiology. 1960;154(2):254-69.

568 37. Leiper JB, Aulin KP, Söderlund K. Improved gastric emptying rate in humans of a
569 unique glucose polymer with gel-forming properties. Scandinavian Journal of
570 Gastroenterology. 2000;35(11):1143-9.
571 38. Bornet F, Bizais Y, Des Varannes SB, Pouliquen B, Laval JD, Galmiche J. α-amylase
572 (EC 3.2. 1.1) susceptibility rather than viscosity or gastric emptying rate controls
573 plasma responses to starch in healthy humans. British Journal of Nutrition.
574 1990;63(2):207-20.
575 39. Beckers E, Rehrer NJ, Saris W, Brouns F, Ten FH, Kester A. Daily variation in
576 gastric emptying when using the double sampling technique. Med Sci Sports Exerc.
577 1991;23(10):1210-2.
578 40. Jeukendrup AE. Training the gut for athletes. Sports Med. 2017;47(1):101-10.
579 41. Rehrer N, Beckers E, Brouns F, Ten Hoor F, Saris W. Exercise and training effects on
580 gastric emptying of carbohydrate beverages. Med Sci Sports Exerc. 1989;21(5):540-9.