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Processing apples to puree or juice speeds gastric emptying and reduces postprandial intestinal volumes and satiety in healthy adults

--Manuscript Draft--

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Abstract:	ABSTRACT: Background: Whole apples produce greater satiety than processed apples, but the underlying mechanisms remain unclear. Objective: Our aim was to assess the intragastric processing of apple preparations and the associated small and large bowel contents using MRI. Methods: An open label , 3-way crossover, randomized, controlled trial. 18 healthy adults [mean ± SD age, 25±4y; BMI(kg/m ^{2}), 22.7±3.5] underwent serial MR scans on 3 occasions separated by 7d, after consumption of isocaloric (178 kcal) portions of either whole apples, apple puree or apple juice. Gastric emptying, small bowel water content (SBWC)(Primary endpoint), were measured at baseline and at 45 minutes intervals (0 - 270 min) post meal ingestion. Fullness and satiety were also assessed at each time point. Treatment effects between groups were analyzed using ANOVA. Results: Gastric emptying half-time (GE t_{50}) was greater ($P < 0.0001$) after participants consumed whole apple (mean ± SEM), 65 (3.3) min) compared with when they consumed apple puree (41(2.8) min) or apple juice (38 (2.9) min), times which did not differ. Postprandial AUC (135 - 270 min) SBWC was also greater for whole apples than puree ($P = 0.025$) and juice ($P = 0.0004$) but juice and puree did not differ. AUC for fullness and satiety (0 – 270 min) post-ingestion was also greater ($P = 0.002$ and 0.004 respectively) for whole apple compared to juice but juice and puree did not differ. Conclusions: Gastric emptying is slower after whole apples consumption causing greater sensation of fullness and satiety than puree or juice in healthy adults. Whole apples increased small bowel and colonic contents during the later phase of the study which may be relevant for subsequent food consumption. This study was registered at clinicaltrials.gov as NCT 03714464.
Additional Information:	

Question	Response
Has this manuscript been previously submitted?	No
Designated Alternate Author	Shanti Krisnasamy
Please select a collection option from the list below:	Obesity and Metabolism Research Articles
Has this manuscript been deposited on a preprint server?	No
Author Comments:	redirected from Am J Nutrition We have uploaded a document stating the changes we made in response to their comments They did not send for review

8th of June 2020,

Dear Dr Tome,

RE: JN-2019-1474R3 manuscript entitled Processing apples to puree or juice speeds gastric emptying and reduces postprandial intestinal volumes and satiety in healthy adults.

We would like to thank you and your reviewers for effort in reviewing our manuscript and for your comments and suggestions to improve the manuscript.

We hope your find our responses satisfactory and that our manuscript is now acceptable for publication.

Best wishes,

Robin Spiller on behalf of all the authors

RE: JN-2019-1474R2
Processing apples to puree or juice speeds gastric emptying and reduces postprandial intestinal volumes and satiety in healthy adults

Dear Editor,

Many thanks for you and your reviewers for their considerable effort . We believe the manuscript is now much clearer and hope is it now acceptable.

Editor

Stat test results could be added on figures. Or the lack of stat test results on figures should be justified.

Response: The statistical test results have been added to figures and removed from legends for all figures. We have also added a brief description of the main findings to the legend for easier reading.

Please use L · min for AUC units, i.e. the · should be raised.

Response: These have been corrected for table 3,4 and 5.

Image files

Figure panels should be the same height and/or width and should line up vertically and/or horizontally along the Y and X axes; revise e.g. figure 6; e.g.:
A B C
or
A
B
C

Response: Figure 6 panels are now lined up vertically.

Layout for figure 7:
A B C
D E

All aspects of all printed figures (fonts, points, bolding, etc.) should be in proportion so that they will be legible when printed in 1-column (< 9 cm) width
or for some complex, multi-panel figures, 2-column width.
REDUCED text and symbols should be 6-8 points; enlarge ORIGINAL text and symbols as needed.

All lines and symbols must be easily distinguished from one another (data and key).
It may be helpful to use variable lines; e.g. - - - - _._._. etc.
Several continuous line functions should be revised.

Response: The layout for figure 7 has been changed as requested. All images are now in actual size as requested.
All aspects of printed figures are now in proportion.
The lines and have symbols have also been revised for all figures. A solid line is used for whole apple, ……. is for puree and _._. is for juice.

TITLE PAGE

a) Title: Processing apples to puree or juice speeds gastric emptying and reduces postprandial intestinal volumes and satiety in healthy adults

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Processed apples and GI postprandial responses

i) List of abbreviations

AUC: Area under the curve; GI: Gastrointestinal; GE t_{50}: Gastric emptying half-time; GE RateT$_{50:}$ Gastric emptying half-rate; GLP-1: Glucagon-like peptide -1; MoE: Ministry of Education; MRI: Magnetic Resonance Imaging; NDDC: Nottingham Digestive Diseases Centre; ppm: parts per million, PYY: Peptide tyrosine tyrosine; RM-ANOVA: Repeated measures analysis of variance; SBWC: Small bowel water content; SCFA: Short chain fatty acids; SEM: Standard error of the mean; SENSE: Sensitivity encoding; SD: Standard deviation; SoM: School of Medicine; TE: Echo Time; TR: Repetition time; VAS: Visual analogue scale

m. Disclaimers

The views and opinions expressed herein are those of the authors and do not necessarily reflect those of the NIHR, NHS or the Department of Health. The corresponding author had final responsibility for the decision to submit for publication.

ABSTRACT:

Background: Whole apples produce greater satiety than processed apples, but the underlying mechanisms remain unclear.

Objective: Our aim was to assess the intragastric processing of apple preparations and the associated small and large bowel contents using MRI.

Methods: An open label , 3-way crossover, randomized, controlled trial. 18 healthy adults [mean ± SD age, 25±4y; BMI(kg/m^2), 22.7±3.5] underwent serial MR scans on 3 occasions separated by 7d, after consumption of isocaloric (178 kcal) portions of either whole apples, apple puree or apple juice. Gastric emptying, small bowel water content (SBWC)(Primary endpoint), were measured at baseline and at 45 minutes intervals (0 - 270 min) post meal ingestion. Fullness and satiety were also assessed at each time point. Treatment effects between groups were analyzed using ANOVA.

Results: Gastric emptying half-time (GE t_{50}) was greater ($P < 0.0001$) after participants consumed whole apple (mean ± SEM), 65 (3.3) min) compared with when they consumed apple puree (41(2.8) min) or apple juice (38 (2.9) min), times which did not differ. Postprandial AUC (135 - 270 min) SBWC was also greater for whole apples than puree ($P = 0.025$) and juice ($P = 0.0004$) but juice and puree did not differ. AUC for fullness and satiety (0 – 270 min) post-ingestion was also greater ($P = 0.002$ and 0.004 respectively) for whole apple compared to juice but juice and puree did not differ.

Conclusions: Gastric emptying is slower after whole apples consumption causing greater sensation of fullness and satiety than puree or juice in healthy adults. Whole apples increased small bowel and colonic contents during the later phase of the study which may be relevant for subsequent food consumption. This study was registered at clinicaltrials.gov as NCT 03714464.

Keywords: gastric emptying, small bowel water, magnetic resonance imaging, appetite, satiety, fullness, apples, healthy adults

Introduction

Reducing energy intake by enhancing satiety is an important part of weight reducing strategies. Whole fruit is well recognized to enhance satiety and its consumption to be associated with reduced risk of obesity (1). A pioneer and often quoted paper showed that consumption of whole apples reduced hunger and enhanced satiety compared to both apple puree and apple juice. Furthermore, blood sugar showed a striking rebound fall after juice, an effect not seen with apples indicating a slower absorption of carbohydrate (2). A more recent study showed that whole apples not only induced greater fullness but also reduced subsequent *ad libitum* food consumption (3). Possible mechanisms of increased satiety after fruit ingestion include lower energy density and greater viscosity of the meal. Additionally, slower gastric emptying, as well as the impact of slower ingestion rate and increased oral processing by greater chewing may also contribute to satiety (4)(5).Two studies using magnetic resonance imaging (MRI) to image the stomach have shown that both increasing viscosity and increasing calorie content prolong gastric emptying, the calorie load being the main determinant (6). Both studies agreed that viscosity increases perception of fullness which is proportional to the gastric volume. This effect has been referred to as “phantom calories” which may be useful in lowering energy intake (7). The rate of eating is also important at least in so-called “linear eaters”, in whom slower eating leads to greater satiety. When attempting to explain how the physical form of food affecting satiety it is important to not only assess changes in gastric emptying but also the changes in the small intestine and colon since there are important feedback mechanisms whereby intestinal signals alter gastric emptying and satiety even after the meal has left the stomach (8).

Studying the gastric emptying of an apple meal requires a technique which can assess both liquid, solid and gaseous gastric contents, something MRI is well equipped to do.

Studies using MRI have shown how the viscosity and calorie content interact to determine the rate of emptying (9) and that the physical form of a meal influences "gastric sieving". This allows for a faster fall in gastric volume in mixed liquid / solid meals, as the liquid is preferentially emptied. However, when the liquid and solid components are homogenized to form a soup (6), or masticated as with chewing apples then sieving is no longer possible and gastric emptying is delayed.

The aim of this study was to apply MRI to further our understanding of the satiating effects of whole compared to processed apples and in particular to understand how changes in gastric processing alter intestinal contents with influences on subsequent feeding. We also assessed breath hydrogen after the meal since apple fiber may impair carbohydrate absorption in the small bowel and increase its delivery to the colon where it is fermented to hydrogen which is rapidly detected in breath.

Subjects and methods

Study design

This study was an open label, three-way crossover, randomized, controlled trial in healthy adults. It was approved by the Research Ethics Committee of the School of Medicine at the University of Nottingham (A13102015 SoM NDDC SPMMRC) and this trial was registered at ClinicalTrials.gov (NCT03714464). The procedures carried out were in accordance with the ethical standards of University of Nottingham, and in accordance with the principles of the Declaration of Helsinki revised in 2013.

Study setting

The study was carried out at the Queens Medical Centre and Sir Peter Mansfield Imaging Centre, University of Nottingham between March to August 2016. Healthy participants were recruited using advertisements across Nottingham University campuses, via social media and through the departmental database of research volunteers.

Eligibility

The inclusion criteria for this study were healthy participants above 18 years of age who could read and write in English and who were able to give consent. Participants were screened for eligibility by using the ROME III criteria (10), to exclude those with functional gastrointestinal diseases. Further exclusion criteria included, major gastrointestinal surgery (excluding appendectomy), intake of medication which alters GI transit, excessive alcohol consumption, medical condition requiring special diet, changes in dietary habits in the last 6 months, aversion to the test product, contraindications to having an MRI scan and a weight of more than 120kg to avoid overloading the scanner bed motors.

Study Visit

Participants were asked to avoid food and drink from 22:00 h the previous day, with only a small cup of water (50 mL) being permitted on waking on the morning of the study, they avoided alcohol, caffeine, and strenuous exercise for the previous 18 hours. In addition, they avoided beans, pulses, lentils, and excessively spicy foods for 24 hours before the study to reduce the inter-individual variability in colonic contents. On the study day, prior to the baseline breath test, participants were asked to rinse their mouths using standard non-alcoholic mouthwash (**Figure 1**). The study days were seven days apart for each participant.

Study day protocol

Participants completed a symptom and satiety questionnaire, followed by a baseline fasting breath test using a portable hand-held breath H2 meter (Gastro+ Gastrolyzer, Bedfond Scientific, Kent UK) and a baseline MRI scan. They then received a test product which was either whole apples or apple puree or apple juice and completed consumption within 20 minutes. Although most subjects consumed the juice within 5-10 minutes, it took them around 15 to 20 minutes to consume both puree and apples. This was followed by a second breath test, completion of the symptom and satiety questionnaires and an MRI scan. The same sequence of interventions was repeated every 45 minutes until 270 minutes postprandial. At the end of the scans, participants were offered a light packed lunch and water as refreshments and were then discharged**.**

Questionnaires

Subjects completed an appetite questionnaire to record hunger, satiety, fullness, prospective consumption and desire to eat (11)(12)(13) as well as their gastrointestinal symptoms (14) using a 0 to 100 mm visual analogue scale (VAS).

Test apple products

The apple variety chosen for the study was “Pink Lady” sourced from a supermarket chain (JS Sainsbury Limited UK). The core was removed for all 3 test products with the skin left intact for whole apples and apple puree. All test products were isocaloric (178kcal) and isovolumetric (600mL). On the study day, whole apples were served with 173mL water, apple puree with 224mL water and apple juice with 260mL water to obtain a final volume of 600mL. Apart from the whole apples which were purchased on the day of the study, the other two apple products were prepared at Campden BRI Ltd (Chipping

Campden, UK), in a facility accredited to International Standard ISO(IEC) by the United Kingdom Accreditation Service (UKAS). The whole apples were cored and cut at the MRI center in a sterile environment providing 350g per portion, fresh for each study day. At the processing facility, apple puree was made in a single batch with whole apples cut, cored and steamed for 20 minutes, before being pureed. Meanwhile apple juice was prepared by draining cored apples for 2 minutes before immersing in an ascorbic acid solution to prevent oxidization. It was then milled and pressed. The resultant juice was collected in a sterilized container under the fruit press, with the pulp retained within a clean pressing cloth. This process was repeated until 85% of the apples were juiced. Both the puree and juice were filled into vacuum sealed cans and then pasteurized in a tank of water at 100°C for 10 minutes.

The nutrient composition analysis of the test products was carried out by Campden BRI Ltd, Gloucester, UK. The analysis of the test products per edible portion is shown in **Table 1**.

Sample size

There are no comparable studies on which to base a power calculation so this must be regarded as a pilot study; however, a previous study (15) showed that small bowel water content (SBWC) area under the curve (AUC) 135-270 minutes was 34.8 (21) mL after mannitol ingestion (n = 18 healthy volunteers). Thus, it was calculated that with α = 0.05 and a power of 80%, it would be possible to detect a 42% change in SBWC between groups using 18 participants. While it is not clear what the minimal clinically significant change is, this is less than the 50% increase in SBWC we observed in a separate study when giving 2 large kiwifruit (16).

A total of 26 participants were recruited for this study and met eligibility criteria, however, 8 withdrew from the study after their first visit. 3 were unable to finish the test product and 5 were unable to commit to further attendance. 18 participants completed all study visits and were subsequently included in the analysis. Their age range was between 18 to 35 years (mean ± SD of 25 ± 4 years) with a body mass index of 22.7 ± 3.5 kg/m^2. Participants were mostly female (78%), non-smokers (87%), with the largest ethnic group being white Caucasians (39%). (**Table 2**).

MRI data acquisition

Based on our validated MRI protocol, images were acquired using a whole-body 1.5T scanner (Achieva, Philips Medical System, Best, The Netherlands). Volunteers were scanned in the supine position (head-first) in the scanner, with a 16-element Sensitivity encoding (SENSE), (Philips Medical System) torso coil wrapped around the abdomen. Details of the MRI sequences used are provided as "**Supplemental method**".

In brief, gastric emptying was measured using a balanced gradient echo sequence which acquired 50 contiguous axial slices during 1 expiration breath hold of 16.5 s. The imaging sequence was able to yield good contrast between stomach content and surrounding organs. Small bowel water content was assessed from images acquired using a coronal single-shot turbo spin echo sequence. This sequence produces high-intensity signals from the liquid regions of the body. SBWC was estimated from these images using our previously validated method (17). Colonic volume and gas were determined using a coronal dual-echo gradient echo sequence which enhances the contrast between the colon and other organs and allows easy assessment of colonic gas.

Depending on the sequence, each image set was acquired with an expiration breath-hold of between 13 and 24 seconds, monitored using a respiratory belt. Each period of imaging lasted around 10 minutes, after which participants were able to sit comfortably outside the scanner in the adjacent volunteer lounge. They were advised to sit upright, in order to minimize any posture effect on digestion of test product.

Data analysis

Gastric content volume was measured from a balanced turbo field echo (bTFE) using Analyze® software (Mayo Clinic, Rochester, MN, USA). This was done semi-automatically using a thresholding technique to define (on each image slice) the region surrounding both the meal and around the gas within the stomach (18). These regions across all the slices gave a total sum of meal and gas volume, corresponding to the total gastric volume. Gastric emptying was determined by measuring gastric half emptying time (GE t_{50}) and half emptying rate (GE $Rate_{50}$) using a previously described equation utilizing MATLAB version 2016b (The MathWorks Inc, United States).

The SBWC was measured using in-house software previously validated as published (19). Colonic volume and colonic gas were measured using Analyze 9 software (Biomedical Imaging Resource, Mayo Foundation, Rochester, MN.). The measurements followed a similar protocol to gastric volume, whereby regions of interest were manually traced on each image slice around the ascending, transverse, and descending segments of the colon as previously reported (20).

Gastric emptying is usually rapid during the early phase of emptying, influenced by meal volume (21), returning to baseline by 135 min as previously shown (22). Therefore, total observation time for gastric contents volume, small bowel water and breath hydrogen in this study was divided into an early phase (0-135min) and late phase (135-270min). Meanwhile, appetite scores and symptoms scores were assessed from 0-270 minutes.

Gastric emptying has a major effect on SBWC during the early phase, while pancreatic and biliary secretions together with absorption and rate of transit to the colon, influence small bowel volume during the late phase. Area under the curve (AUC) was calculated using the trapezoidal rule.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism for Windows, Version 7.0 (Graph Pad Software, La Jolla California USA). Data was assessed for normality using the Shapiro Wilk test. Normally distributed endpoints were analyzed using parametric methods. Baseline characteristics of the participants were expressed as means ± SD and categorical variables were expressed as percentages. Gastric volume, gastric GE t_{50}, GER_{50}, SBWC, colonic volume, colonic gas, breath hydrogen, subjective ratings of appetite and symptoms were reported as mean ± (SEM). A 2-factor repeated measures (RM) ANOVA was used to determine the main effects of test product, time and the test product x time interaction. GE t_{50} , GER_{50}, AUC for gastric content, SBWC, colonic volumes, colonic gas, breath hydrogen, appetite scores and symptom scores divided into early phase (0-135 minutes) and late phase (135-270 minutes) were analyzed using one factor RM ANOVA. Regional colonic volumes were also analyzed at 270 minutes using -1 factor RM ANOVA. When interaction effect or treatment effect were significant Tukey's honestly significant difference test for multiple comparisons were performed. Differences were considered significant if $P < 0.05$.

Results

Gastric Emptying

The MRI images clearly showed the differences in physical form of the 3 meals, with the apple meal showing a heterogenous mixture of whole apples, liquid and gas (**Figure 2**). Serial imaging after ingestion showed a rapid decrease from the initial peak in gastric contents volume in all three groups after ingestion of the test product with gastric emptying being slower for whole apples compared to the puree and juice (**Figure 3**), . 2-factor RM ANOVA of postprandial gastric volume showed significant effect of time ($P < 0.0001$) and test product ($P <0.0001$) and a significant interaction between time and test product, (P-Interaction, < 0.0001). Post-hoc test showed gastric emptying was slower for whole apple compared to puree ($P < 0.0001$), whole apple compared to juice ($P < 0.0001$), and for puree compared to juice ($P = 0.009$).

GE t_{50} for whole apples (65 (3.3) min) was slower than puree (41 (2.8) min) and juice (38 (2.9) min), 1-factor RM ANOVA, $P < 0.0001$ (**Figure 4A**). Post-hoc tests showed significant difference in emptying time between whole apples versus puree ($P < 0.0001$) and whole apples versus juice ($P < 0.0001$) but not between puree versus juice ($P = 0.761$).

GER_{50} were lower for whole apples (3 (0.2) mL/min) compared to puree (4.9 (0.3) mL/min) and juice (5.3 (0.4) mL/min), 1-factor RM ANOVA , $P < 0.0001$. (**Figure 4B**). Post-hoc test showed significant lower rate of emptying between whole apples and puree ($P = 0.0001$) and whole apples compared to juice ($P = 0.0003$) but not between puree versus juice ($P = 0.671$).

Small Bowel Water Content

SBWC for all three test apple products rose to a peak between 0 and 45 minutes and then steadily declined for the juice and puree but rose again in the late phase (135-270 minutes) for the whole apple meal only. 2-factor ANOVA showed a significant effect of time ($P < 0.0001$) and test product ($P = 0.02$) with a significant time x test product interaction ($P < 0.0001$). Post hoc comparison showed apples were associated with significantly greater values than juice ($P = 0.015$), other comparisons were not significant. (**Figure 5**). A significant effect of test product ($P < 0.0005$) was also found for postprandial SBWC AUC in the late phase (135-270 minutes) but not the early phase (**Table 3**).

Colonic Volume

Colonic volume analyses (**Table 3**) were performed on a data set of 16 participants, as 2 participants could not be segmented owing to poor quality images as a result of excessive movement during scanning. During the late phase, colonic volume rose after both whole apples and puree while after juice they remained unchanged **(Figure 6).** 2-factor ANOVA showed a significant effect of time ($P < 0.0001$), the test product effect was not significant but there was a significant interaction effect between time x test product ($P < 0.0001$). Post hoc comparisons showed no significant differences between products.

However, given the significant time effect we considered separately the early and late phase. 1-factor ANOVA showed no significant differences in AUC between the test products in either phase (Table 3).

Colonic volumes were divided into the different colonic regions namely ascending, transverse and descending colon. When ascending colon volumes at 270 min were compared [whole apple:263 (14.7) mL versus apple puree: 278 (17.3) mL versus apple juice: 213 (19.1) mL] using 1-factor ANOVA there was a significant difference in volumes between all meals with P = 0.034. Post hoc analysis using Tukey’s test showed colonic volumes after apple puree were significantly greater than after apple juice, P = 0.036. However, there were no significant differences for either transverse or descending colon volumes.

Colonic Gas

Colonic gas volumes were low initially and fell during the study. 2-factor ANOVA showed a significant time effect (P < 0.0001) but there were no differences between test products and no interaction.

Breath Hydrogen

Breath hydrogen were low initially and rose by only a small amount in the whole apples group at 45 min but thereafter declined steadily. It fluctuated in the other groups with low levels in both phases. (**Figure 6C**). 2-factor ANOVA showed no effect of time nor test product.

Appetite Scores

Participants appetite ratings on hunger, desire to eat and prospective consumption increased steadily over time. The reverse was observed for fullness and satiety scores, which declined steadily (**Figure 7B and 7C**). Appetite scores were analyzed from 0-270 min by 2-factor RM ANOVA. There were no significant differences between meals at baseline for all appetite scores. There was a significant time effect for all appetite scores

(P < 0.0001). AUC was calculated for scores post ingestion from 0-270 minutes. Fullness AUC was significantly greater after whole apples compared to apple juice (P = 0.002). Likewise, satiety AUC was significantly greater after whole apples compared to apple juice (P = 0.004). There was no significant effect for other appetite scores (**Table 5**).

Symptom scores

There were no differences in baseline scores for symptoms except bloating, which was significant with P = 0.048. There was a significant time effect for gas (P = 0.015) and bloating respectively (P < 0.0001). AUC for symptom scores (**Table 5**) were also analyzed from 0-270 min. Overall, the symptom ratings were low for this group of healthy adults, (**Figure 8**) therefore no significant interaction was observed between the groups for AUC symptom scores.

Discussion

We have shown that the physical form of fruit alters its intragastric processing and that whole apples were associated with significantly higher satiety scores compared to juice. We used MRI to assess gastric processing of the different forms of apple which allowed us to assess both gastric emptying as well as small bowel and colonic contents. When compared with scintigraphy MRI gives much more detail about the breakdown of solids and a longer time for 50% emptying because it also includes gastric secretions (18). It has been used to show effects of the physical form of food and fat on gastric emptying. The present study found whole apples had a significantly slower gastric emptying as assessed by GE t_{50} when compared to puree and liquid. Although calorie content is the main determinant of gastric emptying (23)(24)(25), control is not perfect and when calorie density is reduced or viscosity increased as with the whole apples, emptying is slower. This delay in gastric emptying was associated with an increased AUC of fullness between 0-270 minutes for whole apples compared to juice.

The masticated apples and puree have a greater viscosity than juice which is likely to play a role by increasing fullness. An MRI study on varying viscosity and nutrient content of a locust bean gum meal showed that fullness linearly correlated with total gastric volume and at equal volumes. Fullness was higher for high viscosity meals compared to low viscosity meals (6), a finding which has been confirmed by others (7).The effect of viscosity on satiety is also seen with liquid meals, (22) an effect which likely depends on prior experience and beliefs about the greater satiating effects of solids (26).

Satiety depends on more than just fullness, with significant contributions from rates of consumption, duration and force of chewing required as well as textural complexity (5)(27). Subjects took longer to consume apples and chewed for longer which is known to enhance satiation (4). Furthermore whole apples provide greater textural complexity than either puree or juice which is known to enhance satiation even after taking account of lower energy density and more prolonged time of ingestion (28).

This was similar to the Haber study (2), in which depectinized juice emptied faster and led to a drop in plasma glucose and a reduction in satiety. Whole apples with the pectin intact, may have also increased satiety by impeding nutrient absorption in the upper small bowel, leading to their delivery to the PYY and GLP-1 secreting enteroendocrine cells in the distal small intestine (28).

The initial increase in SBWC for all test products is related to the arrival of the meal chyme, followed by a fall due to nutrient-driven fluid absorption as the simple sugars such as fructose, and glucose are absorbed (29). Later, after the stomach had emptied and the simple sugars had been absorbed, the pectins and more whole apple components in the small bowel determine the small bowel content. We found the whole apples increased SBWC significantly at this time compared to both puree and liquid. **(Table 3).**

A similar pattern has been observed with a rice pudding and coarse bran meal, which showed an initial fall in volume post feeding due to rapid sugar absorption; this was followed by a rise in volume to a plateau associated with the rice and indigestible bran component (29). We hypothesized that the bran effect was due to the physical effect of bran particles exerting shear forces on the mucosa which are known to stimulate secretion (30) .The increase in SBWC in the current study could be due to similar effects of the indigestible fiber in the whole apples. Meanwhile, the SBWC following juice consumption was lower compared to that of puree (**Figure 5**) in the later phase. This was most likely because both pectin and insoluble fiber were removed during cold pressing, while the pectins present in whole apples and puree trapped fluid in the distal small bowel as we have shown for ispaghula (31).

Colonic volumes rose steadily after 90 minutes from ingestion of the whole apples and puree meals, likely reflecting the passage of ileal contents into the colon with water trapped by the undigested pectins. By contrast, after juice which had the pectins removed, colonic volume remained steady over the study period. We have previously shown a similar increase in colonic volumes following consumption of the water trapping gelling agent ispaghula, which is widely used as a laxative to soften stool. This traps water in the small bowel, increasing SBWC and colonic volumes associated with an increase in T1, also known as relaxation time which a MRI marker of increased water content (32).

As participants were healthy adults with normal colon physiology and morphology, they experienced very few symptoms except for gas and bloating. These symptoms, observed immediately after feeding, could have been due to intra-abdominal volume increment. Normal colons are able to relax and accommodate increased contents without causing symptoms, as distinct from patients with gastrointestinal functional disorders (33).

The sensation of fullness and satiety was much lower in the later phase of the study (**Figure 7**), when the stomach was mostly empty. However, the observation that high fiber meals which trap water reduce appetite and desire to eat later in the day, suggests that such meals continue to exert effects as they pass down the intestine. Distension of the small intestine after high dose fructose provides sensory input, and the SBWC correlates with symptoms of bloating and abdominal discomfort in irritable bowel syndrome (IBS) patients after fructose ingestion (34). As undigested residue enters the colon, fermentation products of pectins, including short chain fatty acids, signal to the various central receptors which may be important in appetite regulation (35). The lower satiety experienced after juice may reflect the removal of pectins and insoluble fiber in the form of skin during processing, but to prove this would require further larger studies.

Limitations

The subjects were mostly female, and a gender reflection of the irritable bowel syndrome (IBS) population who are being advised on low FODMAP diets in our present clinical setting. Apples contain the FODMAPs fructose and sorbitol, thus the study findings will be relevant to most IBS patients.

MRI is an expensive technique which meant that study duration and number of subjects were accordingly restricted, and although adequate to assess objective MRI endpoints we were probably underpowered to detect the differences in sensations such as satiety.

Limiting the study to 6 hours meant that meal residue had only just entered the colon by the end of the study. More prolonged recording may have been useful to demonstrate significant effects in the colon. Furthermore, demonstration that the increase in satiety translated into reduced *ad libitum* food consumption during the subsequent meal would have added value to the study, though others have already shown this to be true (3). Although all test products were matched for calories and volume, the fiber content differed. The apple juice was prepared by cold pressing, containing only 1.7g of fiber, compared to 6.7g in the whole apple and 8.1g in the puree. This may have also been a factor in reducing the effect of juice on colonic volumes.

In summary, our study suggests that whole fruit is likely to have greater health benefits than processed apples, both on appetite and colonic function. Future, more prolonged studies should combine both MRI and examination of circulating blood peptides such as CCK, PYY and GLP-1 and examine the *ad libitum* consumption of a second meal. This would more clearly define the mechanisms underlying the effect on satiety, and particularly how intestinal effects influence subsequent food consumption - which is pivotal to successful weight reduction.

Acknowledgement

The author's responsibilities were as follows: RCS, LC, SK, and MCEL designed research, SK and SP conducted research, EB, LM and SK processed the test products, RCS, CH and PG provided essential materials, SK, JA, and SP analyzed MRI data, RS, ML and SK wrote the paper, and LC and CH provided feedback. RS had primary responsibility for the final content. All authors read and approved the final manuscript.

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Tables

Table 1

Energy and nutrient composition of test apple products consumed by each healthy adults during three study days[1]

	Test products		
Characteristics	Whole apples	Puree	Juice
Weight, *g*	350	384	338
Volume, *mL*	427	376	340
Total Energy, *kcal*	178	178	178
Carbohydrates, *g*			
Total carbohydrates	56.0	51.5	45.6
Available carbohydrates	49.4	43.4	45.6
Total Sugars	39.9	38.4	43.6
Fiber, *g*	6.7	8.1	1.7

[1] All values are for each edible portion. Available carbohydrates is the sum of soluble sugars and starch digested and absorbed in the small intestine.

[2]173mL water was provided with whole apples, 224mL water with apple puree and 260mL water with apple juice.

Table 2

Selected characteristics of 18 healthy adults participating in apple study[1].

	Value
Age, *y*	25 (4)
Gender, *n (%)*	
Female	14 (78)
Male	4 (22)
Weight, kg ,	62.3 (13)
BMI, (kg/m^2)	22.7 (3.5)
Smoking *n (%)*	
Yes	2 (13)
No	16 (87)
Ethnicity *n (%)*	
White British/European	7 (39)
Black British/Afro-Caribbean	1 (6)
Chinese	3 (17)
Hispanic	5 (28)
Arab	1 (5)
Others (Turkic)	1 (5)
Vegetarian *n (%)*	
Yes	4 (22)
No	14 (78)

[1] Values are mean ± SD and n (%) unless otherwise stated; n=18 for all measures.

Table 3

MRI variables of healthy adults expressed as AUC after consumption of 3 test products[1]

MRI variables (AUC)	**Test products**			
Early phase (0-135 minutes)	Whole apple	Puree	Juice	P
Gastric contents volume, L · min	68.4 (1.9)[a]	51.6 (2.7)[b]	41.9 (2.9)[c]	<0.0001
Small bowel water content, L · min	34.6 (3.9)	42.7 (3.7)	40.3 (5.1)	0.39
Total colonic volume, L · min (n=16)	163.5 (11.3)	167.4 (9.9)	163.9 (13.5)	0.97
Colonic gas, L · min	1.2 (0.3)	2.3 (0.8)	1.8 (0.6)	0.99
Late Phase (135-270 minutes)				
Gastric contents volume, L · min	13.4 (1.4)[a]	8.4 (0.7)[b]	7.3 (0.8)[b]	0.0003
Small bowel water content, L · min	41.7 (5.2)[a]	25.9 (4)[b]	17.5 (2.9)[b]	0.0005
Total colonic volume, L · min (n=16)	177.8 (10.6)	186 (9.9)	161.5 (14.3)	0.33
Colonic gas, L · min	2.8 (0.9)	4.3 (1.3)	3.4 (1.2)	0.66

[1]All values are mean ± SEM unless otherwise stated; n=18 for all measures except total colonic volume and colonic gas; n=16. Tukey's honestly significant difference post hoc analysis was performed for variables with a significant overall effect by 1-factor RM ANOVA. Labelled means in a row without a common letter differ: a>b>c, P<0.05. AUC, area under the curve; RM, repeated measure

Table 4

Breath hydrogen concentration AUC of healthy adults after consumption of 3 test products[1].

Breath hydrogen (AUC)	**Test products**			
	Whole apple	Puree	Juice	P
Early phase (0-135 minutes)				
Breath hydrogen, ppm · min	1565 (298)	1880 (745)	1813 (517)	0.91
Late phase (135 – 270 minutes)				
Breath hydrogen, ppm · min	1032 (241)	2315 (651)	1883 (513)	0.19

[1]All values are mean ± SEM unless otherwise stated; n=18 for all measures. Analysis by 1-factor RM ANOVA. AUC, area under the curve; ppm, parts per million; RM, repeated measure.

Table 5

Appetite and symptom variables of healthy adults expressed as AUC after consumption of 3 test products[1]

Vas scores (AUC)	**Test products**			
Appetite (0-270 minutes)	Whole apple	Puree	Juice	P
Hunger, cm · min	2510 (270)	2744 (280)	3180 (283)	0.23
Fullness, cm · min	2426 (256)[a]	1973 (184)[ab]	1331 (202)[b]	0.003
Satiety, cm · min	2505 (237)[a]	2037 (207)[ab]	1441 (228)[b]	0.006
Desire to eat, cm · min	2595 (310)	2890 (277)	3478 (245)	0.08
Prospective consumption, cm · min	2779 (296)	3187 (211)	3646 (220)	0.05
Symptom (0-270 minutes)				
Gas, cm · min	336 (133)	336 (110)	166 (76.8)	0.45
Bloating, cm · min	290 (136)	330 (105)	144 (61)	0.43
Pain, cm · min	47 (19)	75 (40)	87 (49)	0.74
Diarrhea, cm · min	42 (22)	49 (32)	46 (28)	0.98

[1]All values are mean ± SEM unless otherwise stated; n=18 for all measures. Tukey's honestly significant difference post hoc analysis was performed for variables with a significant overall effect by 1-factor RM ANOVA. Labelled means in a row without a common letter differ: a>b>c, P<0.05. AUC, area under the curve; RM, repeated measure, VAS; visual analogue scale.

Figure Legends

Figure 1

Schematic diagram representing overall study visits including measurements taken at each visit for healthy adults receiving either whole apple, puree or juice (n=18) from baseline (-45 min) up to 270 minutes. Participants were given 173mL water with whole apples, 224mL water with apple puree and 260mL water with apple juice so the final volume was isovolumetric; 600mL for all test products. They had to consume the test products within 20 minutes after their baseline scan. MRI, magnetic resonance imaging; min, minutes.

Figure 2

Axial MRI images of stomach content of one healthy adult after ingestion of test product; A (whole apple), B (puree) and C (juice) at various time points on 3 separate study days. Image A shows pieces of whole apples, fluid and some intragastric air. Image B is puree with a more homogenous appearance, mainly liquid and a small amount of solid present. Image C is juice, a homogenous liquid. Air, intra gastric air; L, liver; M, test meal; MRI, Magnetic resonance imaging; Sp, spine; S, spleen.

Figure 3

Gastric contents volume of healthy adults from 0 – 270 minutes ingestion of test product; whole apple, puree and juice on 3 separate study days. Arrow depicts time when test product was consumed. Early phase is 0-135 minutes from consumption of test product and late phase is 135-270 minutes from consumption of test product Values are mean ± SEM, n=18 unless otherwise stated. The main effects of time, test product and time x test product (interaction) was examined by 2-factor RM ANOVA. Whole apple emptied significantly slower than puree which in turn emptied slower than juice. Tukey's post hoc comparisons showed significant differences between WA vs P ($P < 0.0001$), WA vs J ($P < 0.0001$) and P vs J ($P = 0.009$); J, juice; P, puree; vs, versus; RM, repeated measures, WA, whole apple.

Figure 4

Half gastric emptying time (GE t_{50}) (A) and Half gastric emptying rate (GER_{50}) (B) from 0 to 270 minutes of healthy adults after ingestion of test product ; whole apple, puree and juice on 3 separate study days. Values are mean ± SEM, n=18 unless otherwise stated. Labeled means without a common letter differ a> b>, $P < 0.001$. The effect of test product was examined by 1-factor RM ANOVA. GE t_{50} was significantly greater and GER_{50} significantly slower for whole apple than puree and juice. Tukey's post hoc comparisons showed significant differences for GE t_{50}; between WA vs P and WA vs J both ($P < 0.0001$) as well as GER50; between WA vs P ($P = 0.0001$) and WA vs J ($P = 0.0003$). J, juice, P; puree; RM, repeated measures vs; versus; WA, whole apple.

Figure 5

Small bowel water content from baseline to 270 minutes of healthy adults after ingestion of test product; whole apple, puree and or juice on 3 separate study days. Arrow depicts time when test product was consumed. Early phase is 0-135 minutes from consumption of test product and late phase is 135-270 minutes from consumption of test product. Values are mean ± SEM, n=18 unless otherwise stated. The main effects of time, test product and time x test product (interaction) was examined by 2-factor RM ANOVA. Whole apple showed a significantly different profile than juice with a rise in the second phase. Tukey's post hoc comparisons showed significant differences between WA vs J (P = 0.015). J; Juice; RM, repeated measures, SBWC, small bowel water content; vs, versus, WA, whole apple.

Figure 6

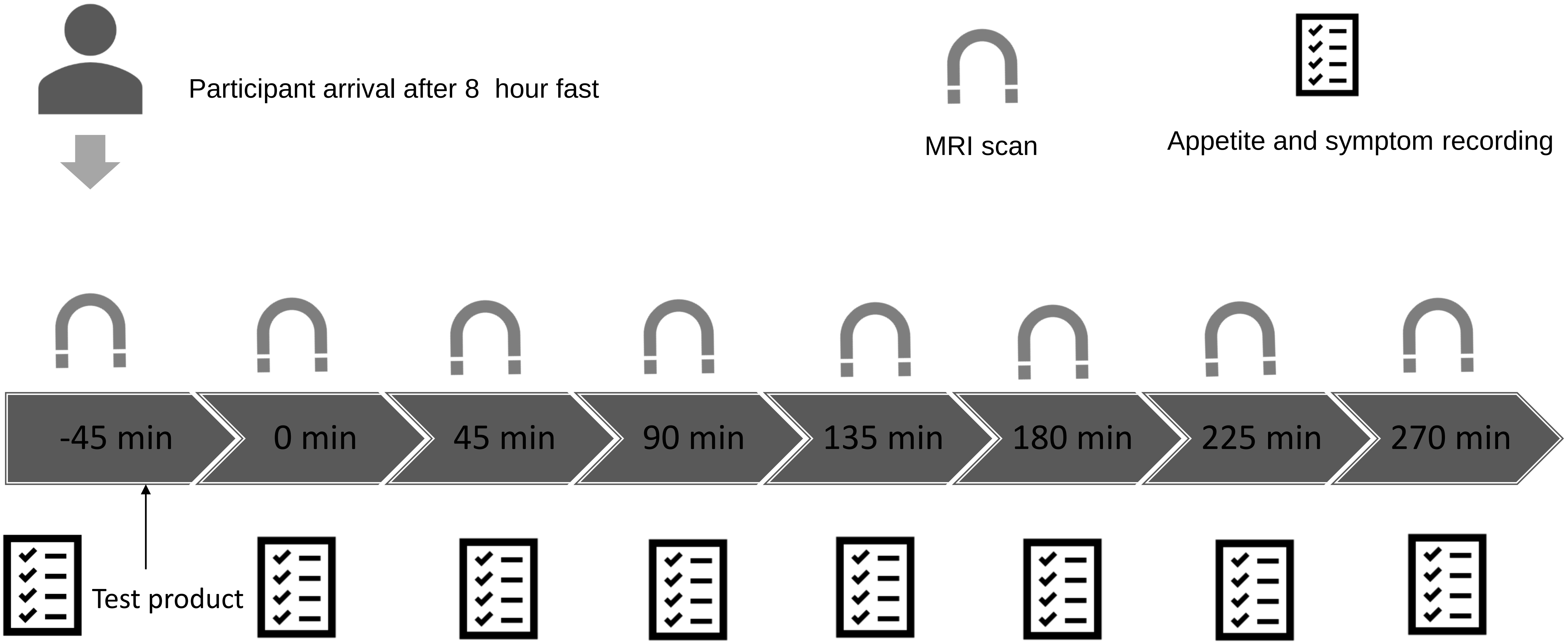
Colonic volume (A), colonic gas (B) and breath hydrogen concentration (C) from baseline to 270 minutes of healthy adults after ingestion of test product; whole apple, puree and or juice on 3 separate study days. Arrow depicts time when test product was consumed. Early phase is 0-135 minutes from consumption of test product and late phase is 135-270 minutes from consumption of test product. Values are mean ± SEM, n = 16 for (A) and (B) and n = 18 for (C). The main effects of time, test product and time x test product (interaction) was examined by 2-factor RM ANOVA. Although whole apple and puree showed a rise in colonic volume towards the end of the study the difference from juice was not significant. Tukey's post hoc comparisons showed no significant difference between products for (A). p.p.m; parts per million; RM, repeated measures.

Figure 7

VAS depicting appetite scores for hunger (A), fullness (B), satiety (C), desire to eat (D) and prospective consumption (E) from baseline to 270 minutes, of healthy adults after ingestion of test product; whole apple, puree and or juice on 3 separate study days. Arrow depicts time when test product was consumed for (A). Values are mean ± SEM, n=18 unless otherwise stated. The main effects of time, test product and time x test product (interaction) was examined by 2-factor RM ANOVA. Whole apple, when compared to juice was associated with significantly greater AUC for fullness and satiety. RM repeated measures; VAS, visual analogue scale.

Figure 8

VAS depicting symptoms scores for gas (A), bloating (B), pain (C) and diarrhea (D) and (E) prospective consumption from baseline to 270 minutes, of healthy adults after ingestion of test product; whole apple, puree and or juice on 3 separate study days. Arrow depicts time when test product was consumed. Values are means ± SEM unless otherwise stated, n=18. VAS, visual analogue scale. The main effects of time, test product and time x test product (interaction) was examined by 2-factor RM ANOVA. While gas and bloating fell over time there were no significant differences between test products. Pain and diarrhea were rarely experienced and the were no significant effects of either time nor test product. RM, repeated measures; VAS, visual analogue scale.


Participant arrival after 8 hour fast
MRI scan
Appetite and symptom recording
-45 min
0 min
45 min
90 min
135 min
180 min
225 min
270 min
Test product

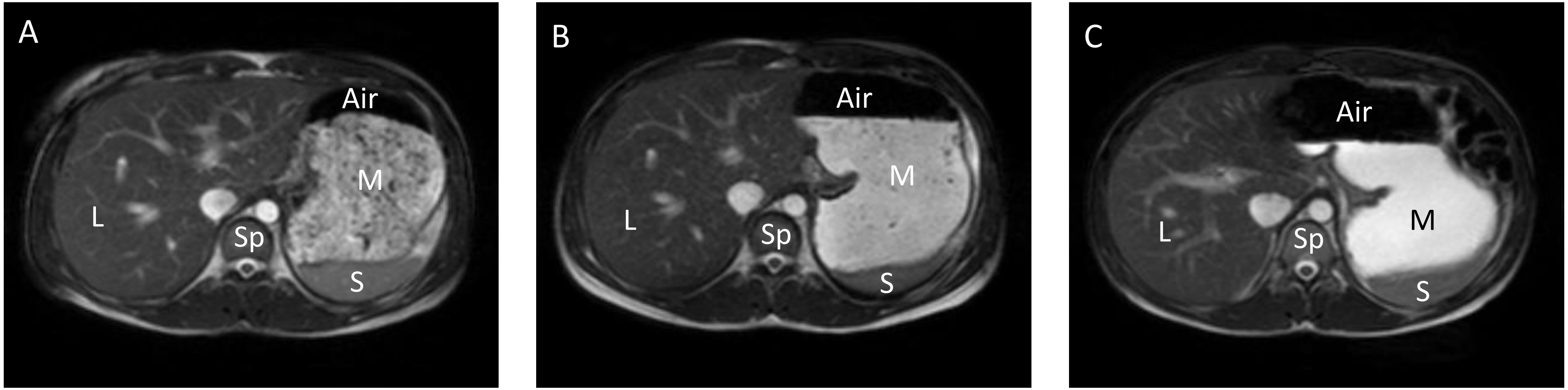

A
Air
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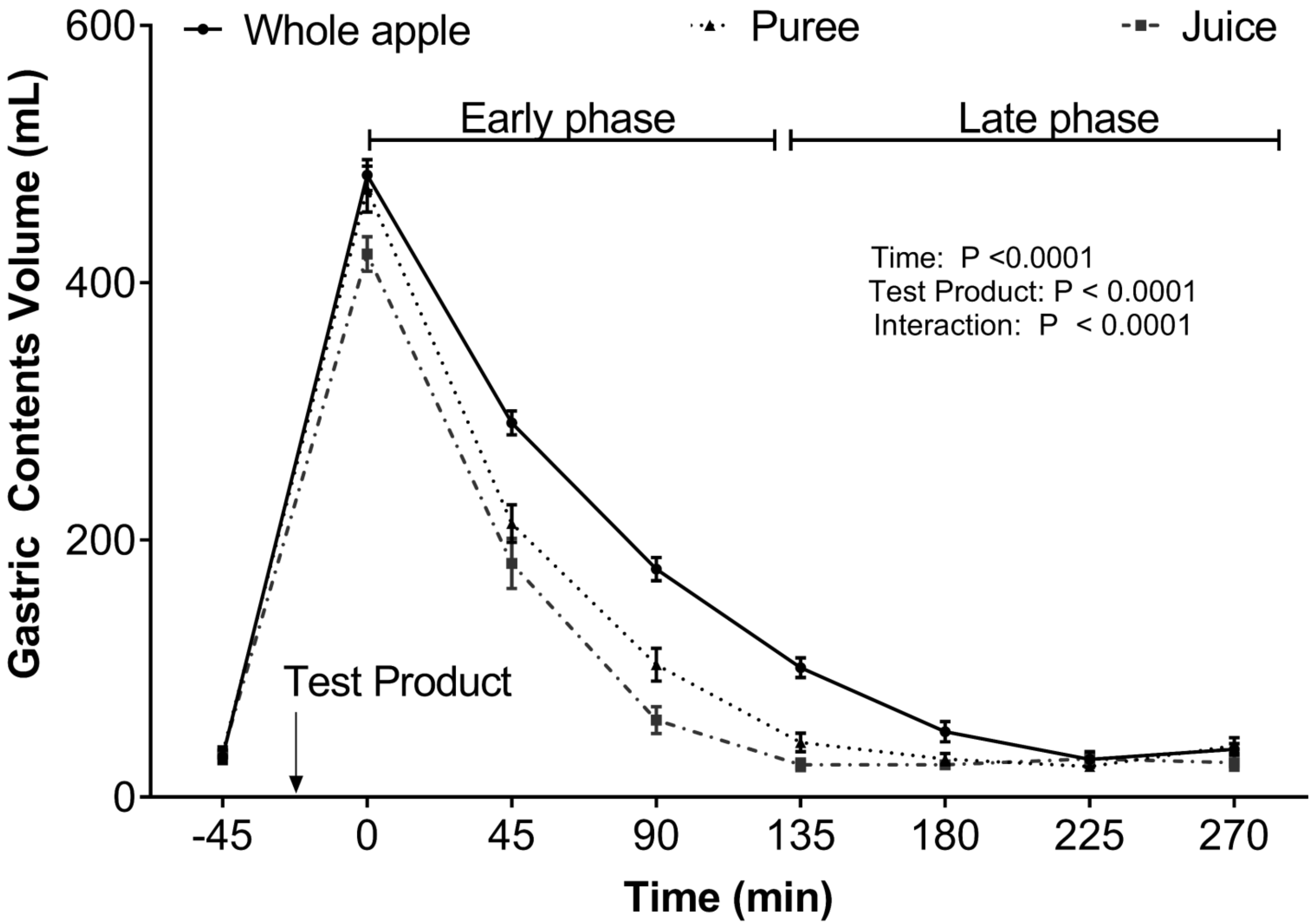
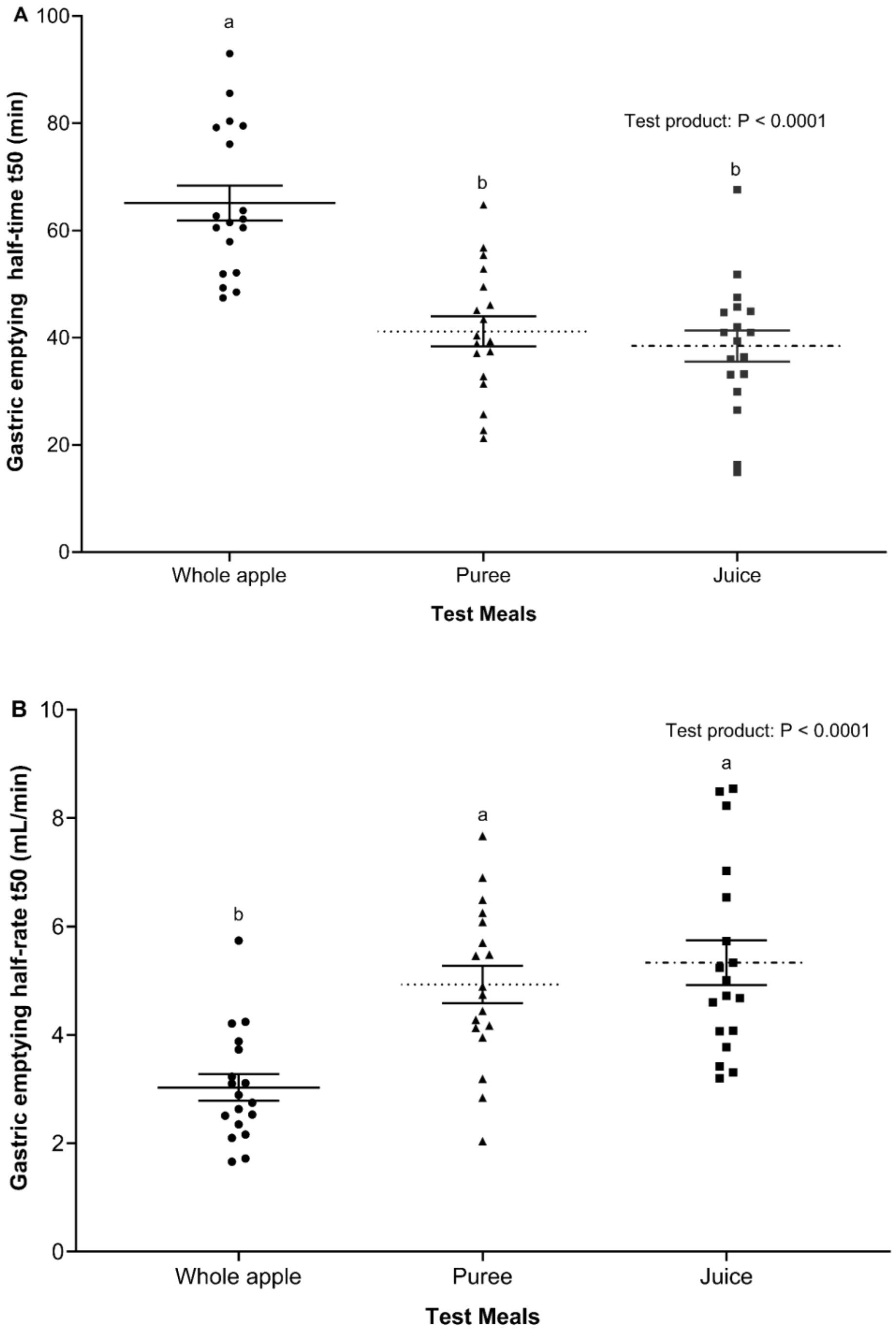
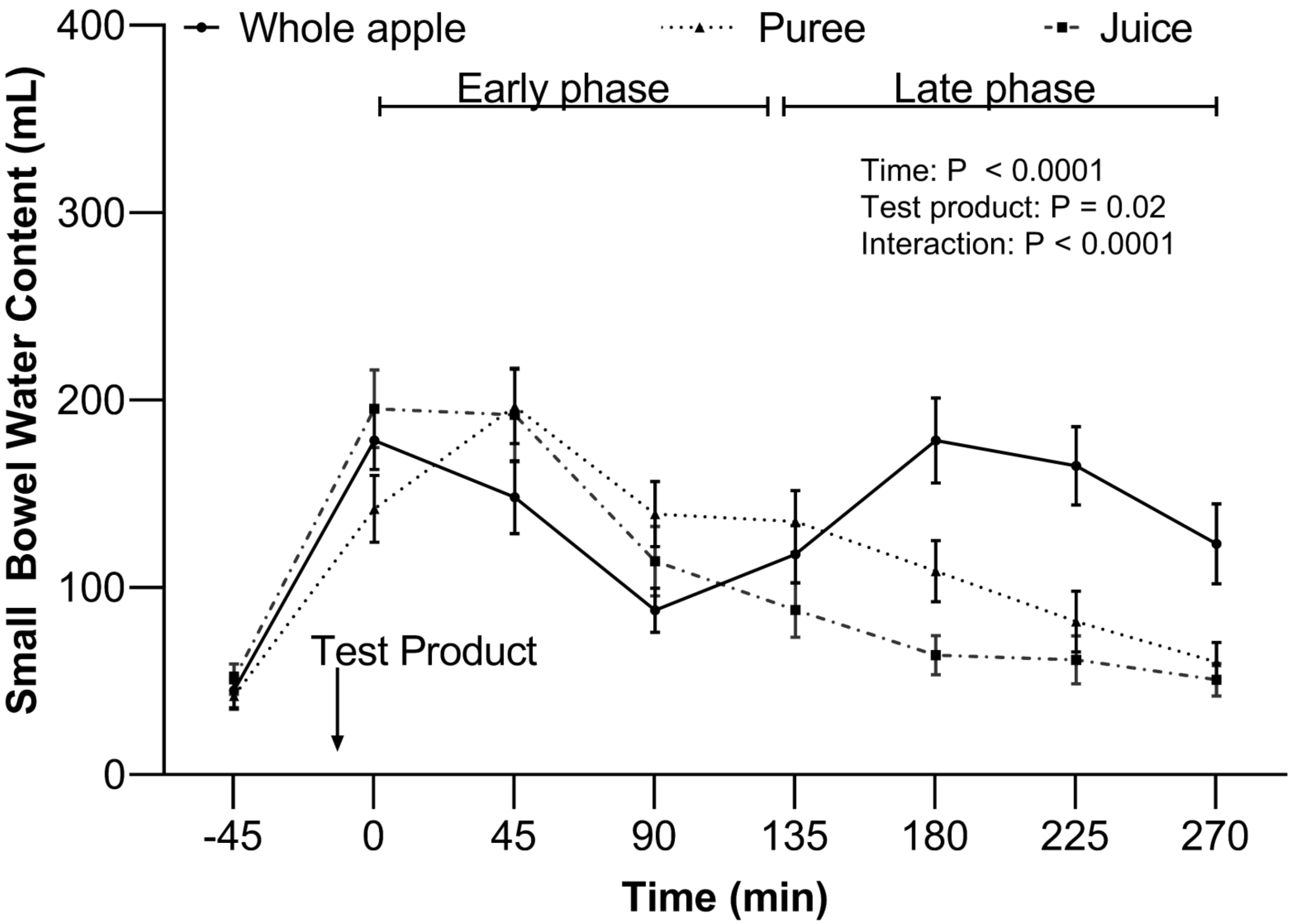
Figure


Whole apple
Puree
Juice
Early phase
Late phase
Time: P <0.0001
Test Product: P < 0.0001
Interaction: P < 0.0001
Test Product
Gastric Contents Volume (mL)
600
400
200
0
-45
0
45
90
135
180
225
270
Time (min)

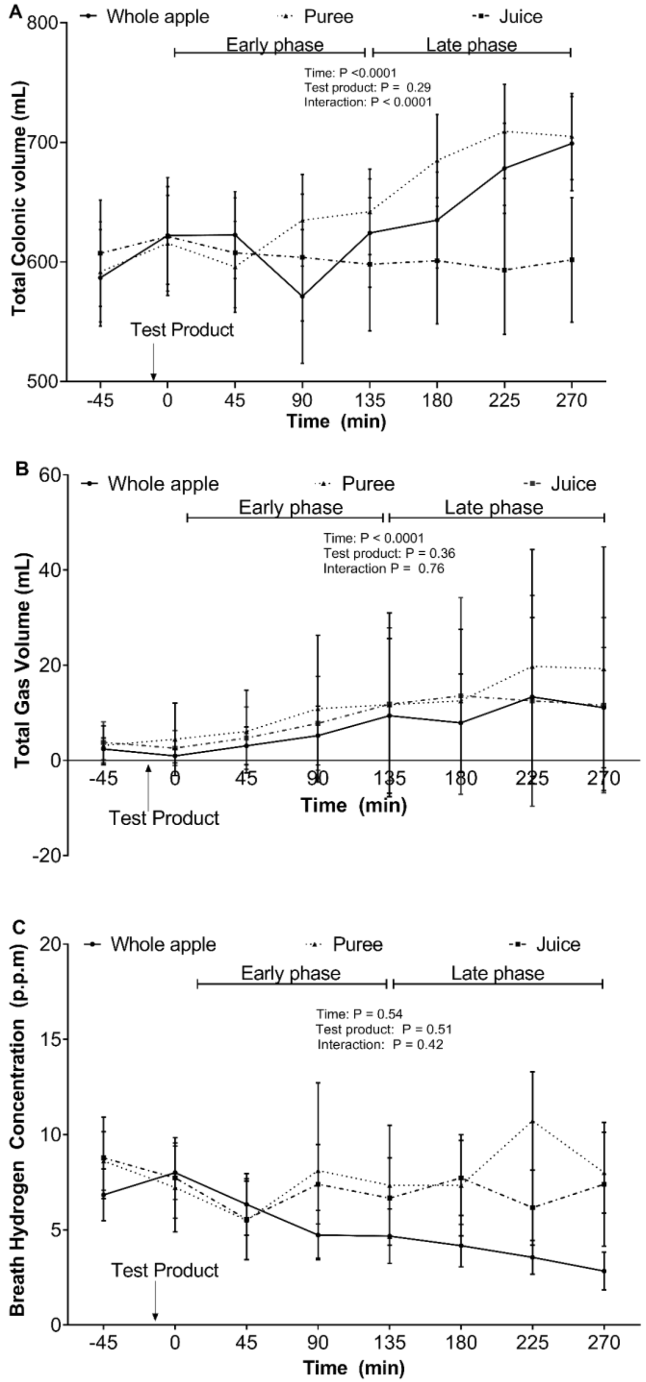
Figure

Click here to access/download;Figure;Fig 4_ GE rate and time.pptx



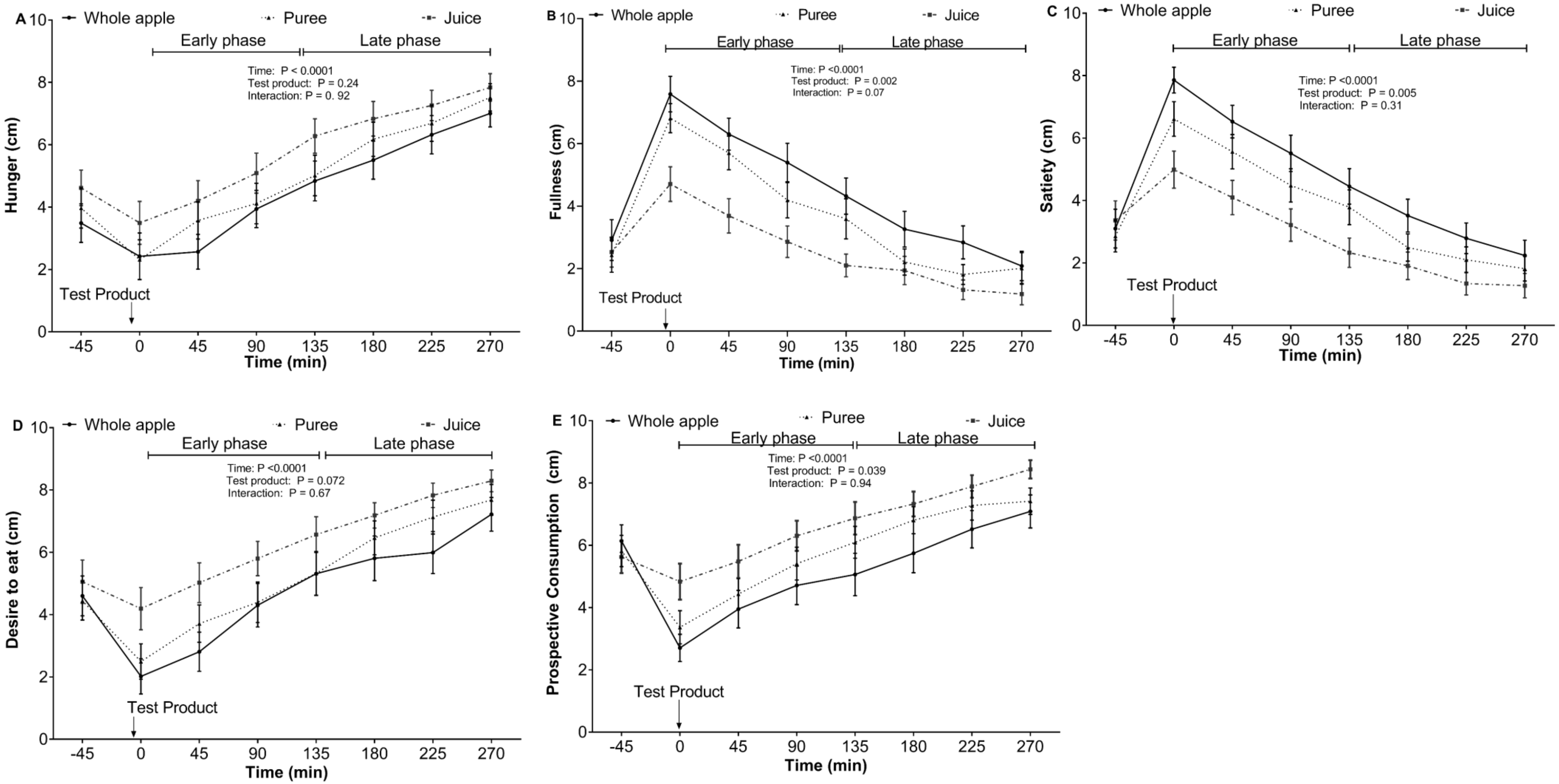


Whole apple
Puree
Juice
Early phase
Late phase
Time: P < 0.0001
Test product: P = 0.02
Interaction: P < 0.0001
Test Product
Small Bowel Water Content (mL)
400
300
200
100
0
-45
0
45
90
135
180
225
270
Time (min)


A
Whole apple
Puree
Juice
Early phase
Late phase
Time: P <0.0001
Test product: P = 0.29
Interaction: P < 0.0001
Total Colonic volume (mL)
800
700
600
500
Test Product
-45
0
45
90
135
180
225
270
Time (min)
B
Whole apple
Puree
Juice
Early phase
Late phase
Time: P < 0.0001
Test product: P = 0.36
Interaction P = 0.76
Total Gas Volume (mL)
60
40
20
0
-20
Test Product
-45
0
45
90
135
180
225
270
Time (min)
C
Whole apple
Puree
Juice
Early phase
Late phase
Time: P = 0.54
Test product: P = 0.51
Interaction: P = 0.42
Breath Hydrogen Concentration (p.p.m)
20
15
10
5
0
Test Product
-45
0
45
90
135
180
225
270
Time (min)

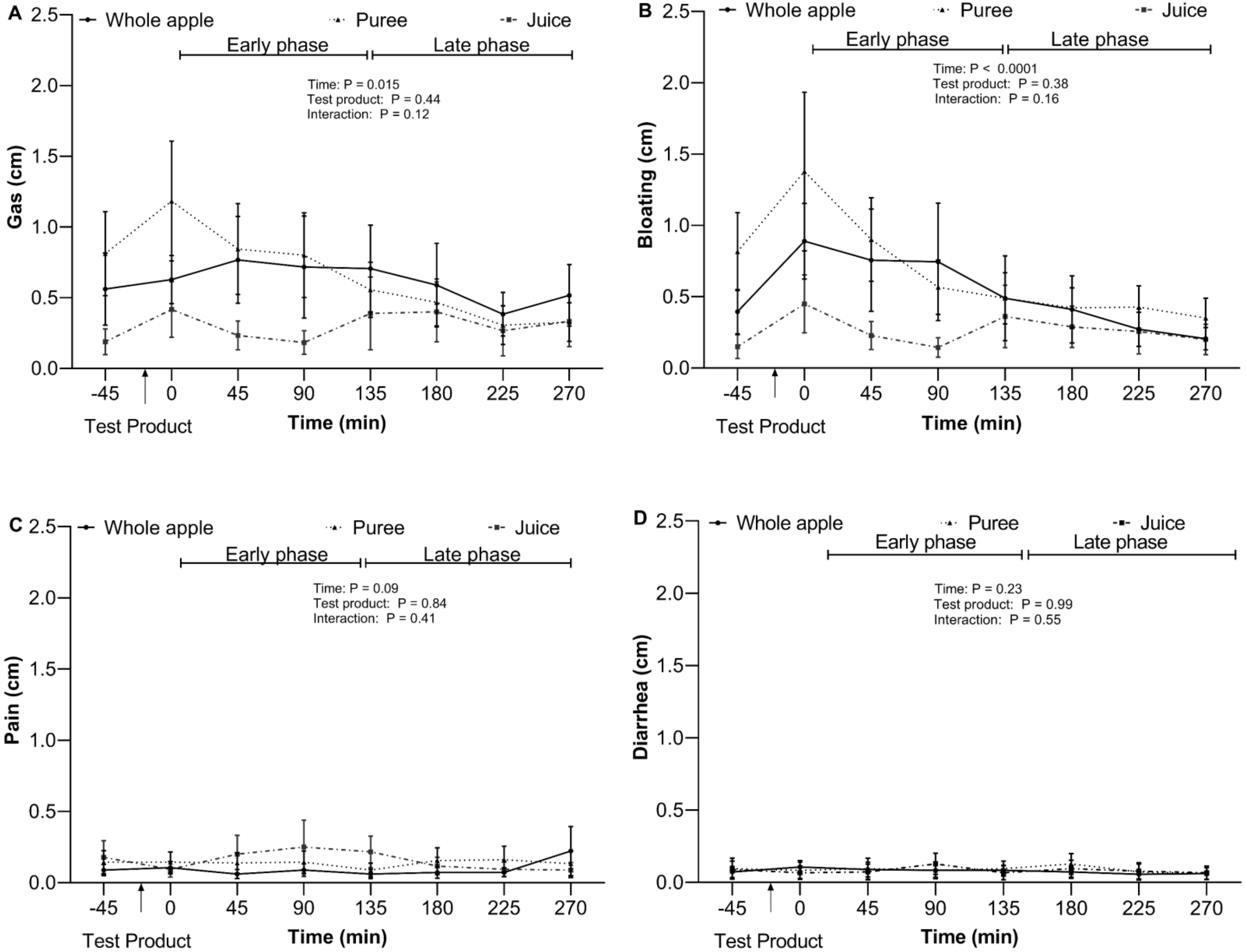
Figure

Click here to access/download;Figure;Fig 7_ Appetite scores.pptx



Figure

Click here to access/download;Figure;Fig 8_Symptom scores.pptx



Processing apples to puree or juice speeds gastric emptying and reduces postprandial intestinal volumes and satiety in healthy volunteers

Krishnasamy S.

Online Supplementary Material

Supplemental Methods for magnetic resonance imaging sequence

Gastric emptying

Gastric emptying was measured using a balanced gradient echo sequence which acquired 50 contiguous axial slices during 1 expiration breath hold of 16.5 s [Repetition time (TR) /Echo Time (TE) = 2.98 / 1.49 ms, flip angle 80° ,256 × 256] reconstructed matrix, reconstructed in-plane resolution 1.56 × 1.56 × 5 mm ^{3}, SENSE 2.0). The imaging sequence was able to yield good contrast between stomach content and surrounding organs.

Small bowel water content

A coronal single-shot turbo spin echo sequence was used to acquire 24 image 'slices' with a single expiration breath hold of 24 s (TR / TE = 8,000 / 320 ms, 512 × 512 reconstructed matrix, voxel size 0.78 × 0.78 × 7 mm ^{3}). This sequence produces high-intensity signals from the liquid regions of the body.

Colonic Volume and Gas

Colonic volume and gas was determined using a coronal dual-echo gradient echo sequence that acquired 24 contiguous slices (1.76×1.76×7 mm ^{3}) with one expiration breath hold of 15 s (TR / TE1 / TE2 = 157 / 2.30 / 4.60 ms, 256 × 256 reconstructed matrix, voxel size 1.76 × 1.76 × 7 mm ^{3}). This sequence allowed simultaneous collection of both in-phase and out-of-phase images, where voxels containing both water and fat are either bright or dark respectively. The out of phase image enhances the contrast between the colon and other organs by giving a black outline to the colon. Combining the two images in both of which colonic gas gives a low signal defines the areas of gas easily.