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LEMON JUICE, BUT NOT TEA, REDUCES THE GLYCEMIC RESPONSE TO BREAD IN HEALTHY VOLUNTEERS: A RANDOMIZED CROSSOVER TRIAL

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ABSTRACT

Purpose The inhibition of enzymes that hydrolyze starch during digestion could constitute an opportunity to slow down the release, and ultimately the uptake, of starch-derived glucose. Simple dietary approaches consisting in pairing starch-rich foods with beverages that have the capacity to inhibit such enzymes could be an effective and easily-implementable strategy. The objective of this work was to test the impact of black tea and lemon juice on the glycemic response to bread and subsequent energy intake in healthy adults.

Methods A randomized crossover study was conducted with equal portions of bread (100 g) and 250 ml of water, black tea or lemon juice. Capillary blood glucose concentrations were monitored during 180 min using the finger-prick method. *Ad libitum* energy intake was assessed 3 hours later.

Results Tea had no effect on the glycemic response. Lemon juice significantly lowered the mean blood glucose concentration peak by 30% ($p < 0.01$) and delayed it more than 35 min (78 vs. 41 min with water, $p < 0.0001$). None of the tested beverages had an effect on *ad libitum* energy intake.

Conclusion These results are in agreement with previous *in vitro* studies showing that lowering the pH of a meal can slow down starch digestion through premature inhibition of salivary α -amylase. Furthermore, the effect of lemon juice was similar to what has been repeatedly observed with vinegar and other acidic foods. Including acidic beverages or foods in starchy-meals thus appears to be a simple and effective strategy to reduce their glycemic impact.

Keywords: glycemic index, satiety, vinegar, acidity, salivary α -amylase, starch

Abbreviations: AUC - Area Under the Curve; BMI - Body Mass Index; GI - Glycemic Index; HSA – Human salivary α -amylase; VAS - Visual Analogue Scale

INTRODUCTION

Starch is exclusively made up of glucose residues and, as starch-rich foods supply up to 50% of our energy intake [1], it plays a major role in the postprandial glycemic responses elicited by our diets. The rate at which starch in a given food is hydrolyzed *in vitro* is positively correlated with the glycemic response elicited by that food [2-4]. *In vivo*, starch digestion is initiated in the mouth by human salivary α -amylase (HSA) [5] and continues in the stomach [5,6] until the increasing gastric acidity inactivates HSA, between pH 3.0 and 3.8 [7,8]. Pancreatic α -amylase and brush border enzymes then complete starch digestion in the small intestine, where glucose is finally absorbed [6].

Slowing down starch digestion through the inhibition of digestive amylases and/or glucosidases can slow down the uptake of starch-derived glucose, thereby lowering the glycemic response to starch-rich meals. A practical example is the administration of acarbose in the treatment of type 2 diabetes [9]. A question that remains open is whether pairing starch-rich meals with foods or beverages capable of exerting analogous inhibitory effects could also be an effective approach to lower their glycemic impact.

Using a dynamic *in vitro* digestion system, we have previously shown that HSA can hydrolyze a significant proportion of starch from bread (\approx 50%) and pasta (\approx 15-20%) into oligosaccharides before being inactivated by the increasing gastric acidity [7,10]. We have also shown that pairing lemon juice with the same meals was an effective means to interrupt the amylolytic activity of saliva at the gastric stage through early acid inhibition of HSA [11,12]. In our view, this pH-mediated mechanism could explain, at least in part, the 20-50% reduction of the glycemic response to starch-rich meals that have been acidified by the addition of vinegar [13-21], pickled foods [22], pomegranate juice [23], or by acid bread fermentation [24-29]. Besides pH, other properties of foods and beverages, such as their polyphenol content, may also inhibit digestive amylases and glucosidases. Indeed, subsequent work carried out in our lab has also demonstrated that tea can inhibit salivary and pancreatic amylases by up to 60% in enzymatic assays [12], similarly to what has been reported by other research teams [30-34]. Therefore, we hypothesized that both tea and lemon juice could lower the glycemic response to a starch-rich meal. To the best of our knowledge, the effect of lemon juice has not yet

been studied, and the effect of tea is a current matter of debate because different results have been found *in vitro* [30-34] and *in vivo* [35,36].

In addition to an attenuated glycemic response, another potential benefit of slowing down starch digestion is a prolonged satiety and thus lower energy intake at the next meal. Research in this field has not been conclusive [37] and, interestingly, literature reviews published in the same year have reached contrary conclusions [38,39]. Indeed, it can be difficult to conceive test meals that elicit distinct glycemic responses while controlling for all the possible confounding factors that may affect satiety. The results of these studies can often be biased due to a lack of control of all the variables [40].

The main objective of this work was to determine the effect of drinking lemon juice or black tea with a starch-rich food (bread) on postprandial plasma glucose concentrations, and on subsequent energy intake in healthy humans.

MATERIALS AND METHODS

SUBJECTS

Adults (aged 18-60 years), men and non-pregnant women, with body mass index (BMI) 18-25 kg/m² and in good health were eligible for this study. Exclusion criteria included allergies to any of the ingredients in the test meals, high alcohol consumption, intensive exercising habits, having started/stopped smoking in the previous 3 months, history of eating disorders, current or past diagnosis of diabetes or other medical conditions affecting gastrointestinal function and/or nutrient absorption, as well as the use of anticoagulant drugs, and/or any other medication known to influence food consumption, appetite and/or glucose metabolism.

Subjects were not aware of the real objectives of the study, but they were informed that this research aimed at better understanding the digestion of starch-rich meals when ingested in combination with commonly consumed beverages. All volunteers gave their written informed consent after being provided with oral and written information about the aims and protocol of the study.

This study was one part of a 2-branch investigation. The other part of the study was conducted with the same test meals but with different participants and different measurements were performed. The participant characteristics, study details and results presented in this publication are for this part of the study only. The research protocol has been approved by the ethics committee Lyon Sud-Est IV. This study was carried out in accordance with the declaration of Helsinki (1997) and has been registered at Clinicaltrials.gov (NCT03265392).

TEST MEALS

Three starch-rich meals with similar macronutrient composition were compared. Each test meal consisted of four slices (100 g) of crustless, wheat sandwich bread (Harry's 100% mie nature, Barilla S.A.S, Boulogne-Billancourt, France) and 250 ml of either spring water (Mont Roucous, Lacauze-les-bains, France), tea, or lemon juice. Tea (Lipton yellow label black tea, Unilever France, Rueil Malmaison, France) was freshly brewed (1% w/v) with spring water at a temperature of 100 °C and was infused for 15 min before the tea

bag was removed. Lemon juice was prepared by mixing 125 ml of commercial lemon juice (Lazy lemon, Polenghi, Milan, Italy) with 125 ml of spring water. The energy and nutrient content of the meals (estimated from the products' labels) are presented in Table 1. The pH of lemon juice was 2.3 and the pH of tea was 6.7.

STUDY DESIGN

A randomized crossover design was used. All subjects consumed the three test meals for breakfast in a random sequence on three different days. A software-generated list of 25 sets of treatment (test-meal) sequences was created prior to onset of participant recruitment by an investigator with no clinical involvement in the trial. Participants were then allocated to a treatment sequence according to recruitment order. It is known that insulin sensitivity [41], appetite control and eating behavior [42] can vary during the menstrual cycle in healthy women. Additionally, variations in stress levels of both men and women can influence the glucose metabolism [43] and the production of HSA [44,45]. To try to minimize any biases possibly induced by these factors, participants were asked to choose the dates of the three study sessions over four consecutive days. The study visits took place at the sensory analysis department of our research unit (UMR GMPPA, INRA, AgroParisTech, Université Paris-Saclay, 78850, Thiverval-Grignon, France).

An overview of the procedures carried out on each study day is provided in Fig.1. Participants were asked to refrain from excessive alcohol consumption and intensive physical activity the day before the study sessions. The test meals were consumed as breakfast after an overnight fast. Participants were instructed to eat their last meal at least 10 h before the study session and to refrain from ingesting anything but water until breakfast. All subjects were provided with a check-list to verify compliance with pre-session requirements, a kit to monitor capillary blood glucose and breakfast. They were asked to start answering the check-list and make two baseline blood glucose measurements between 9h00 and 9h15. Breakfast was to be consumed at a steady pace, within 12 to 15 min, between 9h15 and 9h30. Subjects were then asked to measure blood glucose again 15, 30, 45, 60, 90, 120, 150 and 180 min after the breakfast start time. Apart from that, they were free to maintain their usual routines in the mornings of the study, but were asked not to consume anything between the breakfast and the *ad libitum* lunch. Lunch was consumed at the study site between 12h30 and

13h00. The *ad libitum* meal consisted of a store-bought ready-to-eat rice salad with tuna and vegetables (Riz à la Provençale au thon & basilic, Bonduelle, Villeneuve-d'Ascq, France) and a 250 ml bottle of spring water (Mont Roucous, Lacaune-les-Bains, Brance). According to the label, 100 g of the *ad libitum* meal provided 132 kcal (553 kJ), 4.5 g of fat, 18 g of carbohydrates (1.5 g of sugars) and 4 g of protein. Participants were provided with a single serving of the *ad libitum* meal weighing 1200 g, and were instructed to drink all the water served and eat until they were comfortably full. In order to prevent social influences that could impact food intake, the *ad libitum* meal was consumed in individual cabins and all participants were instructed to stay in the room for a minimum of 30 min, even if they finished the meal earlier. To rule out a potential saturation effect due to repeated consumptions of the same meal, palatability ratings (pleasantness, visual appeal, smell, taste and aftertaste) were completed immediately after consumption. Participants were asked to answer validated visual analogue scale (VAS) questionnaires [46] consisting of 100 mm lines with descriptors anchored at each end describing the extremes (e.g. “how would you rate the smell of this meal? Not good at all/ Extremely good”). Each characteristic was rated by placing a mark across each line on paper, and participants were not able to refer to their previous ratings when completing the VAS.

MEASUREMENTS

Each participant self-monitored her/his capillary blood glucose with the finger-prick method, using the capillary blood glucose monitoring kit provided by the research team. The kit was composed of single-use Accu-Chek Safe-T-Pro plus lancets, an Accu-Chek Performa glucose meter and corresponding test strips (all from Roche Diabetes Care France, Meylan, France). Capillary blood glucose measured with the Accu-chek Performa glucose meter, corresponded to plasma concentration. Participants performed two repeated measurements at baseline and single measurements at all other time points. They reported the meal consumption time as well as the blood glucose concentrations and measurement times in a standard form provided by the research team. Blood glucose concentrations and measurement times were confirmed with the data stored in the memory of the glucose meters. Time-response curves were constructed and the

areas under the glucose response curves (AUC) (mmol · min/L) were calculated using the trapezoidal rule with fasting values as baseline and truncated at zero. Negative areas (below the fasting baseline value) were ignored. Peak values (mmol/L) and time-to-peak (min) were also determined.

The amount eaten (g) during lunch was determined by weighing the *ad libitum* meal before and after consumption. The nutritional information provided by the manufacturer was used as reference to calculate the energy (kJ) consumed. Analysis of the VAS questionnaires was conducted by measuring the horizontal distance from the left-hand end of the line to the mark indicated by the participant.

STATISTICAL ANALYSIS

The number of subjects was determined using power calculations on the basis of previous work [47]. A total of 15 volunteers was necessary to detect a minimum difference of 260 kJ in energy intake with $\alpha = 0.05$ and $\beta = 0.1$ and assuming SD = 200 kJ. To allow for a 20% dropout rate, 18 participants needed to be recruited.

Normality of the data and of the residuals was checked using the Shapiro-Wilk test and homoscedasticity was checked using the Bartlett's test. When normality and homoscedasticity were both verified (glucose peaks and AUCs in Table 3, VAS scores and *ad libitum* energy intake), single factor ANOVAs were performed to compare the effect of the test meals containing tea or lemon juice to that of the control meal (water). To control for possible biases due to the repeated consumption of the *ad libitum* meal, the effects of the day on which the study was performed on (1) palatability ratings and (2) energy intake were also investigated. When normality and/or homoscedasticity were not verified (data of Figure 3, and time-to-peak data in Table 3), Friedman's test (a non-parametric method) was used instead. When statistically significant differences were detected, *post-hoc* analysis was conducted using one-tailed Dunnett's test with the water meal as control. Statistically significant effects were accepted at the 95% level. Data are presented as means \pm SEMs unless otherwise specified. All statistical analyses were performed in R (version 3.5.1).

RESULTS

PARTICIPANTS' CHARACTERISTICS

A total of 18 subjects (7 men and 11 women) were recruited (between January 2019 and June 2019) and completed the study (Fig. 2). Participants' characteristics are presented in Table 2. Their mean age was 32.8 ± 10.4 (range 20-55) and all subjects had normal BMIs ($23.0 \pm 1.8 \text{ kg/m}^2$) and normal fasting glucose ($5.0 \pm 0.06 \text{ mmol/L}$). All of them were healthy, with no history of diabetes and did not use medications known to affect energy intake, appetite, gastrointestinal function nor blood glucose, as confirmed by means of a full medical history and examination. All data from one participant has been excluded due to failure to comply with the pre-session requirements. Another participant failed to attend one of the *ad libitum* meals, therefore the corresponding data (palatability ratings and energy intake) was excluded from the *ad libitum* meal data set and associated analyses.

POSTPRANDIAL BLOOD GLUCOSE RESPONSES

Postprandial blood glucose responses elicited by the water, tea and lemon juice meals are presented in Fig. 3. Data refers to 17 participants. There were no significant differences in baseline blood glucose concentrations between the three test-meals ($p = 0.87$), nor between water and tea. However, consumption of bread with lemon juice elicited a lower blood glucose response than with water, with statistically significant differences from $t = 15$ to $t = 45 \text{ min}$.

The glycemic index (GI, calculated from the $\text{AUC}_{0-120 \text{ min}}$ and using the bread + water meal as a reference), peak blood glucose concentrations, time-to-peak, and mean AUCs (0-90 min and 0-180 min) are presented in Table 3, together with the equivalent changes as percentage of the control experiment (water meal). Again, no differences were found for any of these parameters between the water and tea meals ($0.64 \geq p \leq 0.86$). Despite no statistically significant difference in the glycemic index ($p = 0.10$), lemon juice significantly lowered the mean peak blood glucose concentration by 33% ($p < 0.01$). Additionally, it significantly ($p < 0.0001$) delayed the time to reach the peak blood glucose concentration by more than 30 min on average (78 vs. 41 min). There were no

significant differences in the AUC over the 3-hour monitoring period ($p = 0.52$). It can, nonetheless, be noted that a comprehensive analysis considering different time points showed that the AUC of the lemon juice meal was significantly lower during the first 90 min ($p = 0.04$), hence suggesting a delayed, rather than an incomplete, absorption of glucose from the meal.

AD LIBITUM MEAL

An *ad libitum* meal was served 3h after the test meal to investigate whether there was any effect of the test-meals on subsequent energy intake. To rule out a potential saturation effect, due to repeated consumptions of the same meal, participants were asked to evaluate this meal by rating 5 palatability-related attributes using VAS questionnaires during each study session.

The palatability results are presented in Fig. 4. Overall, ratings of pleasantness, visual appeal, smell, taste and aftertaste over the 3 study days ($n=16$) were respectively 6.1 ± 0.4 , 7.1 ± 0.4 , 6.3 ± 0.4 , 6.2 ± 0.5 and 6.1 ± 0.5 ($\bar{x} \pm \text{SEM}$). Single factor ANOVA ($\alpha=0.05$) did not reveal any significant effect of the test meal ($0.27 \leq p \leq 0.97$) nor of the day of the study ($0.76 \leq p \leq 0.91$) on palatability ratings.

Ad libitum energy intake (kJ) is presented in Fig. 5. Data refers to 16 participants. After the water, tea, and lemon juice meals, average intake was 2051 ± 193 , 1764 ± 216 and 2168 ± 182 ($\bar{x} \pm \text{SEM}$), respectively. The effect of the meal ($p = 0.34$) and of the study day ($p = 0.70$) were both statistically insignificant.

DISCUSSION

In spite of a 5% higher carbohydrate content in the lemon juice meal (Table 1), consumption of lemon juice with bread led to a significant delay (> 30 min, $p < 0.01$) and reduction (33%, $p = 0.03$) of the peak postprandial blood glucose concentration. The glycemic index also tended to be, on average, 23% lower (though this was not statistically significant, with $p = 0.10$). Altogether, these results are in agreement with the 20–50% lower glycemic response observed in numerous other clinical trials on the effects of vinegar, pickled foods, pomegranate juice, and acid bread fermentation [13–29]. This reinforces the reasoning presented in the introduction, describing the key role of the low pH of these drinks and foods in the underlying mechanism. According to our previous results and interpretation, the phenomenon that is most likely responsible for this effect is the premature inhibition of HSA during gastric digestion [7]. Based on *in vitro* digestion experiments, we have previously observed that HSA can remain active for a long period in simulated gastric conditions, hydrolyzing an important proportion of starch from bread ($\approx 50\%$) and pasta ($\approx 15\text{--}20\%$) into oligosaccharides, before it is inactivated by the increasing acidity of gastric contents [7,10]. Therefore, acidic foods and meals should lower the contribution of HSA to starch digestion by a premature acid-induced inactivation of this enzyme. This is indeed what we have observed during physiologically-based dynamic *in vitro* digestions: lemon juice completely interrupted the hydrolysis of starch from bread and pasta into oligosaccharides by HSA during the gastric phase [11,12]. *In vivo*, as a consequence of this early interruption of starch hydrolysis in the stomach, the chyme emptied into the duodenum would have contained a higher proportion of non-hydrolyzed starch, what, in turn, would have delayed the subsequent digestive steps and, ultimately, the absorption of starch-derived glucose. Naturally, one cannot rule out the potential contribution of other factors. Acidic foods or drinks could reduce the gastric emptying rate, for instance. The polyphenol content of lemon juice could also play a role, though it is unlikely this could explain our results since we have previously observed that it largely loses its capacity to inhibit digestive amylases at neutral pH [12]. More precisely, results of *in vitro* enzymatic tests revealed that the inhibitory capacity of pH-neutralized lemon juice was nearly twice lower than that of the studied black tea [12]. The citrate and acetate, respectively in citric acid

(lemon juice) and in acetic acid (vinegar or sourdough bread), are also important intermediaries in cellular metabolic pathways, which could influence the glycemic response indirectly. In fact, there has been extensive debate on other potential mechanisms, and until very recently [48], the pH inhibition of HSA is an hypothesis that has been hardly addressed or was not even considered [49-52]. Nonetheless, important hints supporting our interpretation can be found in the literature. For example, the pH of common breads is in the range of 5.6-6, whereas that of acid breads eliciting a reduced glycemic response was typically between 3.9 and 4.6 [17,25,27,28]. Additionally, the effect of vinegar on the glycemic response was lost when it was (i) replaced with a neutral-pH equivalent [13,15], (ii) not consumed at the same time as the starch-rich food [15] or (iii) when the starchy food was substituted with dextrose [15]. These cases reflect situations in which HSA would not be inhibited (i), any effect on its activity would be irrelevant due to the mismatched consumption times (ii), or not applicable at all (iii).

The lack of effect observed with tea is in line with previous reports that pairing 300 ml of green tea (3% w/v) with a starch-rich breakfast, or incorporating green tea extract (2% w/w) in a starch-rich food did not exert any effect on postprandial blood glycaemia [35,36]. The tea used in this study was selected after *in vitro* tests showing it could inhibit over 60% of the amylolytic activity of both human saliva and porcine pancreatin in our enzymatic assay conditions, exhibiting the strongest inhibitory capacity out of 12 teas [12]. During dynamic *in vitro* digestions of the same meals as those used in this study, starch digestion with tea was overall similar to that of the control meal (water) during the gastric phase, and was inhibited by only 20% in the intestinal phase [12]. This lower inhibitory effect in more physiological conditions was attributed to the higher dilution of the meal, induced by the digestive secretions. Indeed, it has been observed that below a certain concentration, tea polyphenols and tea extracts may lose their inhibitory capacity, or even enhance the catalytic activity of digestive amylases [53,54]. The lack of effect in our present investigation, as well as in other *in vivo* studies, suggests that one would have to consume an unreasonably high quantity of tea to reproduce the magnitude of the effects observed *in vitro*.

With regards to the energy intake 3 hours after the test meal, no effect was observed. The aforementioned studies investigating the effect of low pH meals did not study

energy intake, but satiety perceptions analyzed through visual analogue scales in four of them led to inconclusive results. Two studies reported an increased satiety [18,24], and two others found no effect [26,27]. The European Food Safety Agency states that a GI reduction of at least 45 units can be used for an increased satiety claim for carbohydrates with a low glycemic index [55]. It is therefore possible that a greater reduction of the glycemic impact would have been necessary to observe an effect. But, as previously reported, it is also highly plausible that lowering the GI *per se* is not a good indicator of an increased satiety, and that the effects observed in other studies are due to other factors [40].

To the best of the author's knowledge, this is the first human study addressing the effect of lemon juice on the glycemic response to a starch-rich meal. Major strengths of our study include the simplicity of the approach, and the fact that statistically significant results could be obtained in spite of an increased amount of sugars in the lemon juice meal. Furthermore, both the non-inclusion of a standardized meal in the previous evening and the semi-free living context (participants were allowed to carry-on their normal routines after breakfast), used in our study, suggest that this dietary approach could be equally effective in a real-life setting. One could also point-out the number of participants (17 subjects), determined by power calculations to detect a statistically significant difference on *ad libitum* energy intake, which is well above the minimum of 10 subjects recommended by the ISO standards in the evaluation of postprandial glucose concentrations [56].

One obvious limitation of our study is that the effect of lemon juice with neutralized pH was not investigated, which could have unambiguously demonstrated the predominant effect of pH as well as the negligible effect of the polyphenol composition. This decision was based on the results of our *in vitro* studies (which, as mentioned above, showed an important loss of the inhibitory capacity of lemon juice at neutral pH) [12] and on the results of *in vivo* studies showing the major role of the pH on the attenuation of the glycemic response observed with vinegar [13,15].

In summary, we have investigated the blood glucose response and energy intake following the consumption of equal portions of bread with either water, tea or lemon juice at breakfast. No effect was observed on energy intake. Tea had no effect on the glycemic response, but the peak blood glucose concentration was significantly lowered

and delayed by lemon juice. Our results confirm that lowering the pH of a starch-rich meal appears to be an effective way to attenuate the glycemic response. It is likely that this commonly observed phenomenon is, at least partly, explained by an early acid-induced inhibition of the amylolytic activity of saliva during gastric digestion. Finally, because of the semi-controlled nature of this study, our results suggest that pairing an acid drink with a starchy meal could be a simple and effective strategy to reduce, or flatten, the glycemic response to starch-rich foods in everyday-life meals.

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CONFLICT OF INTEREST

The authors report no conflict of interest.

REFERENCES

1. Stylianopoulos CL (2012) Carbohydrates: Requirements and Dietary Importance. In: Caballero B, Allen LH, Prentice A (eds) Encyclopedia of human nutrition. vol 1, 2nd edn. Academic press, pp 316-321
2. Goñi I, Garcia-Alonso A, Saura-Calixto F (1997) A starch hydrolysis procedure to estimate glycemic index. Nutrition Research 17 (3):427-437. doi:[http://dx.doi.org/10.1016/S0271-5317\(97\)00010-9](http://dx.doi.org/10.1016/S0271-5317(97)00010-9)
3. Granfeldt Y, Hagander B, Björck I (1995) Metabolic responses to starch in oat and wheat products. On the importance of food structure, incomplete gelatinization or presence of viscous dietary fibre. European Journal of Clinical Nutrition 49 (3):189-199
4. Jenkins DJA, Ghafari H, Wolever TMS, Taylor RH, Jenkins AL, Barker HM, Fielden H, Bowling AC (1982) Relationship between rate of digestion of foods and post-prandial glycaemia. Diabetologia 22 (6):450-455
5. Bornhorst GM, Singh RP (2012) Bolus formation and disintegration during digestion of food carbohydrates. Comprehensive Reviews in Food Science and Food Safety 11 (2):101-118
6. Gropper SS, Smith JL (2013) Digestion of Polysaccharides. In: Advanced nutrition and human metabolism. 6th edn. Cengage Learning, United States of America, p 70
7. Freitas D, Le Feunteun S, Panouille M, Souchon I (2018) The important role of salivary alpha-amylase in the gastric digestion of wheat bread starch. Food & function 9 (1):200-208. doi:10.1039/c7fo01484h
8. Fried M, Abramson S, Meyer J (1987) Passage of salivary amylase through the stomach in humans. Digestive diseases and sciences 32 (10):1097-1103
9. Salvatore T, Giugliano D (1996) Pharmacokinetic-pharmacodynamic relationships of Acarbose. Clinical pharmacokinetics 30 (2):94-106
10. Freitas D, Le Feunteun S (2019) Oro-gastro-intestinal digestion of starch in white bread, wheat-based and gluten-free pasta: unveiling the contribution of human salivary α -amylase. Food chemistry 274:566-573
11. Freitas D, Le Feunteun S (2018) Acid induced reduction of the glycaemic response to starch-rich foods: The salivary α -amylase inhibition hypothesis. Food & function 9 (10):5096-5102. doi:10.1039/C8FO01489B
12. Freitas D, Le Feunteun S (2019) Inhibitory effect of black tea, lemon juice, and other beverages on salivary and pancreatic amylases: What impact on bread starch digestion? A dynamic in vitro study. Food Chemistry 297. doi:<https://doi.org/10.1016/j.foodchem.2019.05.159>
13. Brighenti F, Castellani G, Benini L, Casiraghi M, Leopardi E, Crovetti R, Testolin G (1995) Effect of neutralized and native vinegar on blood glucose and acetate responses to a mixed meal in healthy subjects. European journal of clinical nutrition 49 (4):242-247
14. Johnston CS, Buller AJ (2005) Vinegar and peanut products as complementary foods to reduce postprandial glycemia. Journal of the American Dietetic Association 105 (12):1939-1942
15. Johnston CS, Steplewska I, Long CA, Harris LN, Ryals RH (2010) Examination of the antiglycemic properties of vinegar in healthy adults. Annals of Nutrition and Metabolism 56 (1):74-79
16. Leeman M, Östman E, Björck I (2005) Vinegar dressing and cold storage of potatoes lowers postprandial glycaemic and insulinaemic responses in healthy subjects. European journal of clinical nutrition 59 (11):1266
17. Liljeberg H, Björck I (1998) Delayed gastric emptying rate may explain improved glycaemia in healthy subjects to a starchy meal with added vinegar. European Journal of Clinical Nutrition 52 (5):368
18. Östman E, Granfeldt Y, Persson L, Björck I (2005) Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. European journal of clinical nutrition 59 (9):983

19. Johnston CS, Kim CM, Buller AJ (2004) Vinegar improves insulin sensitivity to a high-carbohydrate meal in subjects with insulin resistance or type 2 diabetes. *Diabetes Care* 27 (1):281-282
20. Liatis S, Grammatikou S, Poulia K-A, Perrea D, Makrilakis K, Diakoumopoulou E, Katsilambros N (2010) Vinegar reduces postprandial hyperglycaemia in patients with type II diabetes when added to a high, but not to a low, glycaemic index meal. *European journal of clinical nutrition* 64 (7):727
21. Mitrou P, Raptis AE, Lambadiari V, Boutati E, Petsiou E, Spanoudi F, Papakonstantinou E, Maratou E, Economopoulos T, Dimitriadis G (2010) Vinegar decreases postprandial hyperglycemia in patients with type 1 diabetes. *Diabetes Care* 33 (2):e27-e27
22. Sugiyama M, Tang A, Wakaki Y, Koyama W (2003) Glycemic index of single and mixed meal foods among common Japanese foods with white rice as a reference food. *European journal of clinical nutrition* 57 (6):743
23. Kerimi A, Nyambe-Silavwe H, Gauer JS, Tomás-Barberán FA, Williamson G (2017) Pomegranate juice, but not an extract, confers a lower glycemic response on a high-glycemic index food: randomized, crossover, controlled trials in healthy subjects. *The American journal of clinical nutrition* 106 (6):1384-1393
24. Bo S, Seletto M, Choc A, Ponzo V, Lezo A, Demagistris A, Evangelista A, Ciccone G, Bertolino M, Cassader M (2017) The acute impact of the intake of four types of bread on satiety and blood concentrations of glucose, insulin, free fatty acids, triglyceride and acylated ghrelin. A randomized controlled cross-over trial. *Food research international* 92:40-47
25. Lappi J, Selinheimo E, Schwab U, Katina K, Lehtinen P, Mykkänen H, Kolehmainen M, Poutanen K (2010) Sourdough fermentation of wholemeal wheat bread increases solubility of arabinoxylan and protein and decreases postprandial glucose and insulin responses. *Journal of Cereal Science* 51 (1):152-158
26. Liljeberg H, Björck I (1996) Delayed gastric emptying rate as a potential mechanism for lowered glycemia after eating sourdough bread: studies in humans and rats using test products with added organic acids or an organic salt. *The American journal of clinical nutrition* 64 (6):886-893
27. Liljeberg HG, Lönner CH, Björck IM (1995) Sourdough fermentation or addition of organic acids or corresponding salts to bread improves nutritional properties of starch in healthy humans. *The Journal of nutrition* 125 (6):1503-1511
28. Maioli M, Pes GM, Sanna M, Cherchi S, Dettori M, Manca E, Farris GA (2008) Sourdough-leavened bread improves postprandial glucose and insulin plasma levels in subjects with impaired glucose tolerance. *Acta diabetologica* 45 (2):91-96
29. Scazzina F, Del Rio D, Pellegrini N, Brighenti F (2009) Sourdough bread: Starch digestibility and postprandial glycemic response. *Journal of Cereal Science* 49 (3):419-421
30. Hara Y, Honda M (1990) The inhibition of α -amylase by tea polyphenols. *Agricultural and Biological Chemistry* 54 (8):1939-1945
31. Kashket S, Paolino V (1988) Inhibition of salivary amylase by water-soluble extracts of tea. *Archives of oral biology* 33 (11):845-846
32. Kwon Y-I, Apostolidis E, Shetty K (2008) Inhibitory potential of wine and tea against α -amylase and α -glucosidase for management of hyperglycemia linked to type 2 diabetes. *Journal of Food Biochemistry* 32 (1):15-31
33. Quesille-Villalobos AM, Torrico JS, Ranilla LG (2013) Phenolic compounds, antioxidant capacity, and in vitro α -amylase inhibitory potential of tea infusions (*Camellia sinensis*) commercialized in Chile. *CyTA-Journal of Food* 11 (1):60-67
34. Striegel L, Kang B, Pilkenton SJ, Rychlik M, Apostolidis E (2015) Effect of black tea and black tea pomace polyphenols on α -glucosidase and α -amylase inhibition, relevant to type 2 diabetes prevention. *Frontiers in nutrition* 2:3
35. Josic J, Olsson AT, Wickeberg J, Lindstedt S, Hlebowicz J (2010) Does green tea affect postprandial glucose, insulin and satiety in healthy subjects: a randomized controlled trial. *Nutrition journal* 9 (1):63

36. Sapper TN, Mah E, Ahn-Jarvis J, McDonald JD, Chitchumroonchokchai C, Reverri EJ, Vodovotz Y, Bruno RS (2016) A green tea-containing starch confection increases plasma catechins without protecting against postprandial impairments in vascular function in normoglycemic adults. *Food & function* 7 (9):3843-3853
37. Blaak E, Antoine JM, Benton D, Björck I, Bozzetto L, Brouns F, Diamant M, Dye L, Hulshof T, Holst J (2012) Impact of postprandial glycaemia on health and prevention of disease. *obesity reviews* 13 (10):923-984
38. Pawlak D, Ebbeling C, Ludwig D (2002) Should obese patients be counselled to follow a low-glycaemic index diet? Yes. *Obesity reviews* 3 (4):235-243
39. Raben A (2002) Should obese patients be counselled to follow a low-glycaemic index diet? No. *Obesity reviews* 3 (4):245-256
40. Wolever TM, Leung J, Vuksan V, Jenkins AL (2009) Day-to-day variation in glycemic response elicited by white bread is not related to variation in satiety in humans. *Appetite* 52 (3):654-658
41. Pulido JME, Salazar MA (1999) Changes in insulin sensitivity, secretion and glucose effectiveness during menstrual cycle. *Archives of medical research* 30 (1):19-22
42. Dye L, Blundell J (1997) Menstrual cycle and appetite control: implications for weight regulation. *Human reproduction* 12 (6):1142-1151
43. Wing RR, Blair EH, Epstein LH, McDermott MD (1990) Psychological stress and glucose metabolism in obese and normal-weight subjects: A possible mechanism for differences in stress-induced eating. *Health Psychology* 9 (6):693-700. doi:10.1037/0278-6133.9.6.693
44. Nater UM, La Marca R, Florin L, Moses A, Langhans W, Koller MM, Ehlert U (2006) Stress-induced changes in human salivary alpha-amylase activity—associations with adrenergic activity. *Psychoneuroendocrinology* 31 (1):49-58
45. Takai N, Yamaguchi M, Aragaki T, Eto K, Uchihashi K, Nishikawa Y (2004) Effect of psychological stress on the salivary cortisol and amylase levels in healthy young adults. *Archives of oral biology* 49 (12):963-968
46. Flint A, Raben A, Blundell J, Astrup A (2000) Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International journal of obesity* 24 (1):38
47. Arvaniti K, Richard D, Tremblay A (2000) Reproducibility of energy and macronutrient intake and related substrate oxidation rates in a buffet-type meal. *British journal of nutrition* 83 (5):489-495
48. Santos HO, de Moraes WM, da Silva GA, Prestes J, Schoenfeld BJ (2019) Vinegar (acetic acid) intake on glucose metabolism: A narrative review. *Clinical Nutrition ESPEN*
49. Gobbetti M, Rizzello CG, Di Cagno R, De Angelis M (2014) How the sourdough may affect the functional features of leavened baked goods. *Food microbiology* 37:30-40
50. Lim J, Henry CJ, Haldar S (2016) Vinegar as a functional ingredient to improve postprandial glycemic control—human intervention findings and molecular mechanisms. *Molecular nutrition & food research* 60 (8):1837-1849
51. Poutanen K, Flander L, Katina K (2009) Sourdough and cereal fermentation in a nutritional perspective. *Food microbiology* 26 (7):693-699
52. Petsiou EI, Mitrou PI, Raptis SA, Dimitriadis GD (2014) Effect and mechanisms of action of vinegar on glucose metabolism, lipid profile, and body weight. *Nutrition reviews* 72 (10):651-661
53. Tong D-P, Zhu K-X, Guo X-N, Peng W, Zhou H-M (2018) The enhanced inhibition of water extract of black tea under baking treatment on α -amylase and α -glucosidase. *International journal of biological macromolecules* 107:129-136
54. Yang X, Kong F (2016) Effects of tea polyphenols and different teas on pancreatic α -amylase activity in vitro. *LWT-Food Science and Technology* 66:232-238

55. EFSA (2010) Scientific opinion on the substantiation of health claims related to dietary fibre (ID 744, 745, 746, 748, 749, 753, 803, 810, 855, 1415, 1416, 4308, 4330) pursuant to Article 13 (1) of Regulation (EC) No 1924/2006. EFSA journal 8 (10):1735
56. ISO (2010) International Standards Organisation ISO 26642-2010. Food products – determination of the glycaemic index (GI) and recommendation for food classification. International Standards Organisation,

TABLES

Table 1 Energy and nutrient content of the meals. Each meal contained equal amounts of white wheat bread (100 g) and 250 ml of water, tea, or lemon juice

	Water	Tea	Lemon juice
Energy content (kcal)	262.0	262.0	295.8
Energy content (kJ)	1096	1096	1237
Total fat (g)	3.9	3.9	3.9
Total carbohydrate (g)	48.3	48.3	50.8
sugar (g)	6.0	6.0	8.5
Fiber (g)	2.0	2.0	2.0
Total protein (g)	7.5	7.5	8.0

Table 2 Characteristics of the subjects at baseline^a

Age (y)	32.8 ± 10.4 (range 20-55)
Sex, male/female (n)	7/11
Body mass index (kg/m ²)	(23.0 ± 1.8 kg/m ²)
Fasting glucose (mmol/L)	(5.0 ± 0.06 mmol/L)

^a Values are Means ± SD

Table 3 Glycemic Index (GI), peak blood glucose concentration, time-to peak, and incremental postprandial areas under the curve (AUC) from 0 to 90 min and 0 to 180 min, after test-meals composed of 100 g of wheat bread and 250 ml of either water, tea or lemon juice¹

		Water	Tea	Lemon juice
GI ²		100 ± 9	107 ± 12	77 ± 7
	<i>Change %</i> ³	/	+ 8	- 23
Peak	<i>mmol/L</i>	2.7 ± 0.3	2.9 ± 0.2	1.8 ** ± 0.3
	<i>Change %</i> ³	/	+ 7	- 33
Time-to-peak	<i>min</i>	41 ± 4	43 ± 3	78 **** ± 9
	<i>Change %</i> ³	/	+ 5	+ 90
AUC_{0-90 min}	<i>mmol · min/L</i>	145 ± 13	157 ± 18	102 * ± 13
	<i>Change %</i> ³	/	+ 8	- 30
AUC_{0-180 min}	<i>mmol · min/L</i>	212 ± 18	226 ± 26	199 ± 31
	<i>Change %</i> ³	/	+ 8	- 6

¹ all values are $\bar{x} \pm \text{SEM}$. Values within the same row identified with * are significantly different from the water meal. Peak blood glucose concentration ($p < 0.01$) and time-to-peak ($p < 0.0001$) was significantly lower for the lemon juice than for the water and tea meals. AUC_{0-90min} was significantly lower for the lemon juice meal than for the water meal ($p < 0.05$). $N = 17$ healthy adults.

² calculated from AUC (0 to 120 min) and using the bread + water meal as a reference.

³ change as a percentage of the bread + water meal (control).

FIGURES

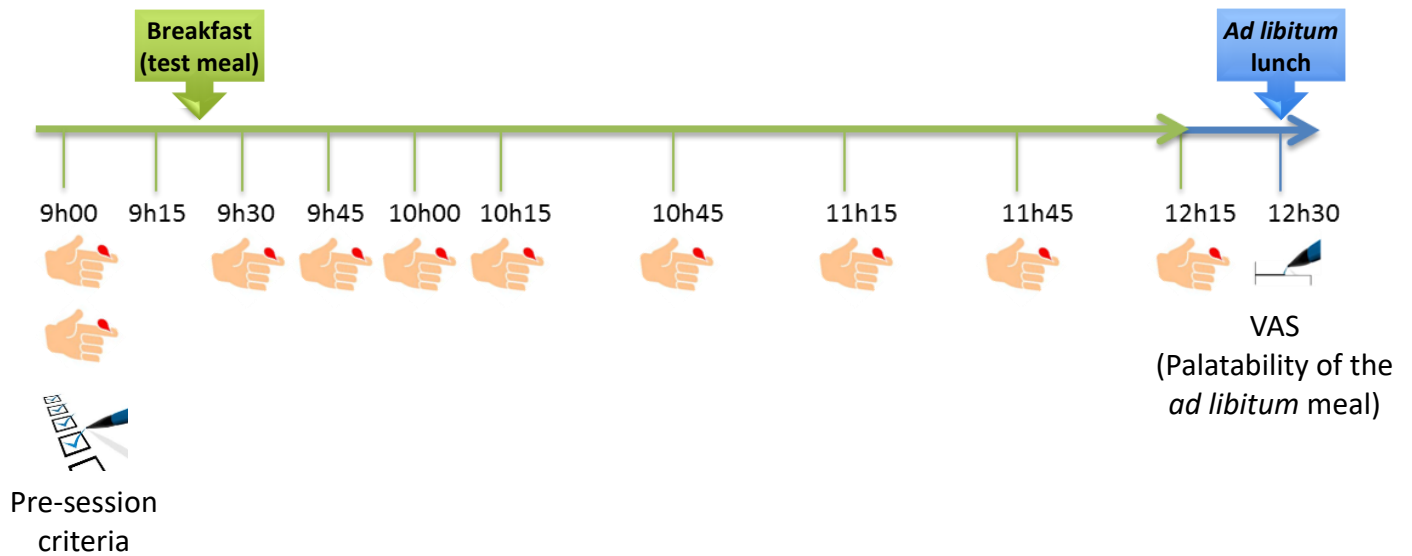


Fig.1 Overview of the study day protocol.

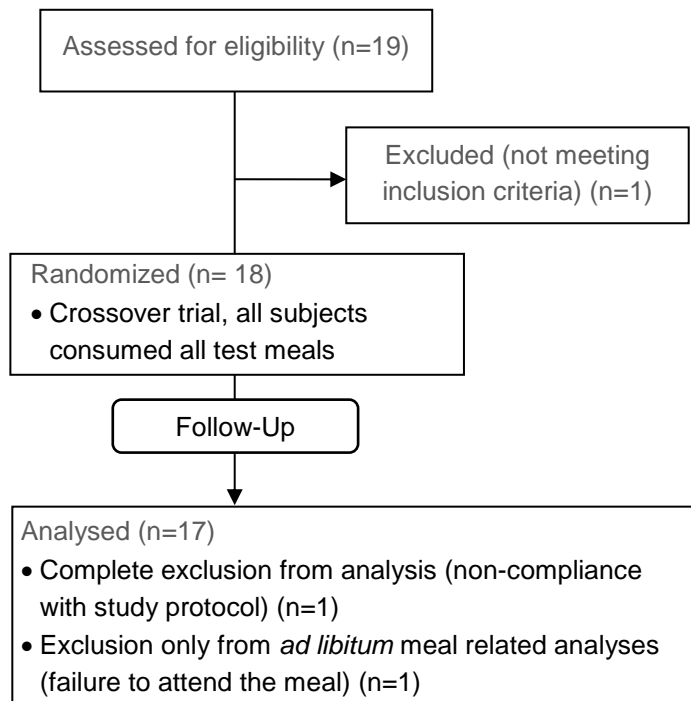


Fig. 2 Diagram of participant flow

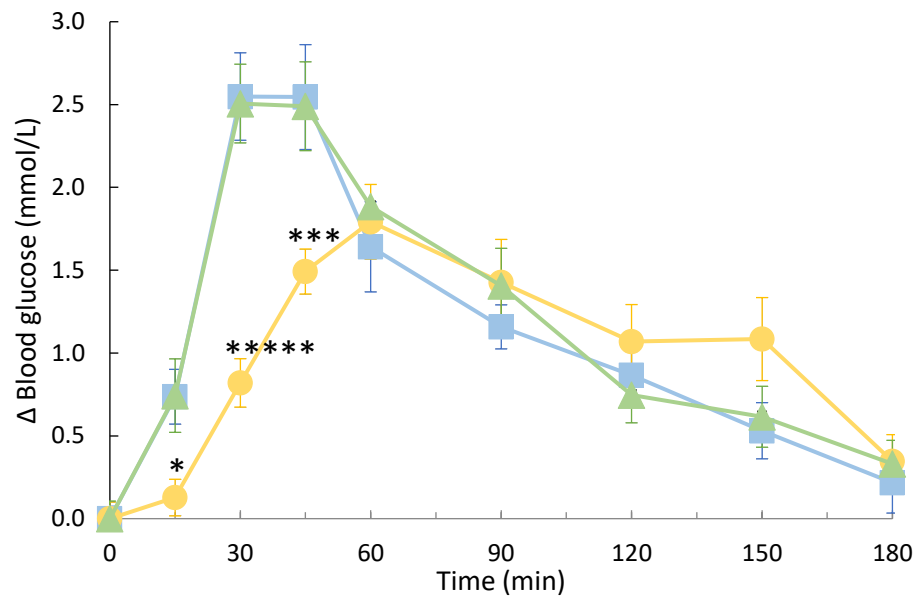


Fig. 3 Postprandial blood glucose responses. Mean (\pm SEM) incremental changes (Δ) in glucose concentrations in response to equal amounts of carbohydrate from a white-wheat-bread consumed with either water (■), tea (▲) or lemon juice (●). Time points at which statistically significant differences were observed (Friedman's test followed by *post hoc* comparisons with one-tailed Dunnett's test where appropriate) are identified by * symbols. Statistically significant differences were observed between the lemon juice and water meals at 15 min ($p = 0.015$), 30 min ($p < 0.00001$) and at 45 min ($p < 0.001$). $n = 17$ healthy adults.

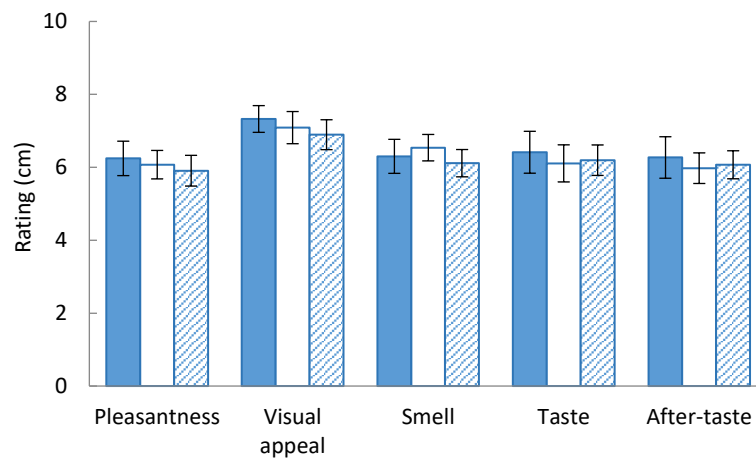


Fig. 4 Palatability of the *ad libitum* meal. Ratings ($n=16$, $\bar{x} \pm \text{SEM}$) of pleasantness, visual appeal, smell, taste and after-taste on study sessions 1 (blue bars), 2 (white bars) and 3 (striped bars).

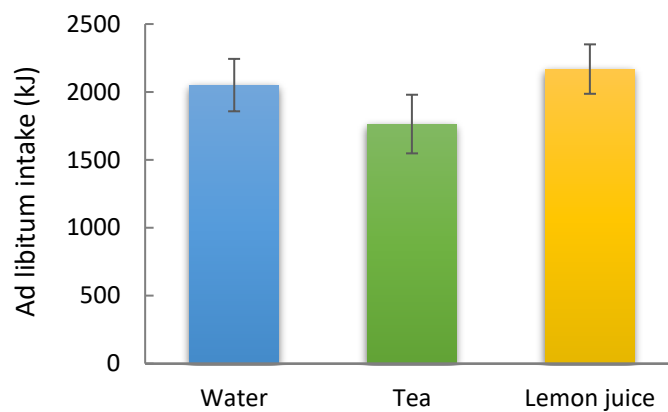


Fig. 5 *Ad libitum* intake. Energy intake ($n=16$, $\bar{x} \pm \text{SEM}$) 3 hours after a test-meal composed of 100 g of wheat bread and 250 ml of either water, tea or lemon juice.