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Effects of oral lubrication on satiety, satiation and salivary biomarkers in model foods: A pilot study

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Abstract

With a dramatic increase in overweight and population with obesity over the last decades, there is an imminent need to tackle this issue using novel strategies. Addressing obesity issues by generating satiety in food to reduce energy intake has been one of those prominent strategies and often textural interventions have been used to generate satiety, specifically in short-term trials. This study aimed to investigate the role of preloads varying in their oral lubrication properties on appetite sensations, food intake, salivary friction and concentration of salivary biomarkers (proteins, α-amylase and mucins) in collected human saliva (n=17 healthy participants). The preloads were model foods (flavored hydrogels) either high or low in their lubricating properties, assessed both by instrumental and sensorial measurements. The results showed that hunger and desire to eat decreased immediately after preload and remained decreased for 10 and 20 min, respectively, after preload in the high lubricating condition compared to control (all $p < 0.05$). Fullness increased immediately after preload and remained increased for 10 and 20 min, respectively, after preload in high lubricating condition compared to control ($p < 0.05$). However, after controlling the values for baseline, such significant effect of the intervention did not exist anymore. Only the effect of time is observed. Consuming high lubricating hydrogels showed no effect on food intake and salivary biomarkers in this pilot study. Salivary lubrication correlated with feeling of fullness. Considering the issue of large time-interval (30 min) between preload and next meal in this study, it is worthwhile investigating the immediate effects of oral lubrication on appetite control, food intake and salivary biomarkers.

Keywords: Hydrogels; Friction; Appetite; Food intake; Amylase; Saliva

1. Introduction

It is well known that the prevalence of obesity has increased dramatically over the last decade (WHO, 2018) and has been associated with chronic non-communicable diseases (McMillan, Sattar, & McArdle, 2006; Rexrode, et al., 1998; Steppan, et al., 2001) that could have significant morbidity and mortality consequences. It is widely agreed that the overconsumption of food (above energy needs for body size) contributes to the high prevalence of obesity (Public Health England, 2018). Therefore, exerting some control over food consumption is a priority for weight management and the prevention of obesity and therefore, achieving satiety and satiation through food textural design is one of the promising nutritional strategies.

Satiation describes within-meal inhibition and can be said to determine meal size and bring a particular eating episode to an end, whereas satiety is known to be associated with the inter-meal period, through the suppression of hunger and the inhibition of further eating (Blundell, 2010; Blundell, et al., 2010; Blundell, Rogers, & Hill, 1987). Satiety can be evaluated through psychological, behavioural and physiological procedures (Gibbons, Hopkins, Beaulieu, Oustric, & Blundell, 2019). Psychological measurements include perceived visual appetite ratings (such as hunger, fullness, desire to eat, prospective food consumption), whilst physiological measures mainly involve, changes in gastrointestinal biomarkers such as stomach dynamics or peptide hormones in blood, although changes in saliva may also be of significance (Gibbons, et al., 2019; Harthoorn, et al., 2007).

One promising approach to gain satiety and in turn reduce food intake is to consider 'food texture' manipulation during designing of the food application. A recent systematic review and meta-analysis has revealed that foods with higher textural characteristics (solid, high viscous, high lubricity and heterogeneous) have an effect on both satiation and satiety by suppressing appetite and reducing food intake (Stribiţcaia, Evans, Gibbons, Blundell, & Sarkar, 2020a). In recent years, there has been increased interests from researchers in understanding the role of structural/ textural complexity of food, specifically through the development of model foods such as hydrogels in generating satiety. The construct of food structural/ textural complexity offers quite a new concept in oral processing and satiety research field (Krop, Hetherington, Miquel, & Sarkar, 2019a; Krop, et al., 2018; Larsen, Tang,

Ferguson, & James, 2016; Tang, Larsen, Ferguson, & James, 2016). Textural/ structural complexity of food is often defined by the degree of heterogeneity or inhomogeneity in a food, where the food or the intervention product includes some additive materials (*e.g.* hydrogels with sunflower/poppy seeds or alginate beads), which distinguishes it from a control product which has a homogenous texture lacking any inclusions (Stribiţcaia, et al., 2020a). To date, a limited number of studies has investigated the effect of structural/ textural complexity of food on satiety, however, these studies suggest that a higher structural complexity of food may lead to a reduced subsequent food intake and suppressed appetite (Krop, et al., 2019a; Larsen, et al., 2016; Tang, et al., 2016)

Among the textural complexity, effect of oral lubrication on satiety has thus far attracted very limited attention in literature. Krop et al. (2019a) studied the effects of oral lubrication on satiety in a snack trial setting and it was concluded that snack intake was reduced by 32% following consumption of a low chewing/ high lubricating gel. The mechanism by which lubrication influence food intake is hypothesized to be associated with mouth coating and thereby extending the oro-sensory exposure time leading eventually to a significant reduction in food intake. In other words, high lubricating gels coated oral surfaces better as compared to gels with low lubricating properties, which resulted in reduced food intake in a previous proof of concept snack trial (Krop, Hetherington, Holmes, Miquel, & Sarkar, 2019b; Krop, et al., 2019a). However, the exact mechanisms of oral lubrication on both physiological and psychological aspects of eating remains elusive in literature. Therefore, the mechanism by which lubrication plays a role in food intake and appetite control remains to be investigated in formal experimental trials.

An innovative way to explain the link between food texture and satiety and satiation is to consider salivary biomarkers, which are important contributors to oral lubrication. Some initial observations have shown an association between salivary biomarkers (*e.g.* α-amylase) and perceived satiety and subsequent food intake (Harthoorn, 2008; Harthoorn, et al., 2007). For instance, it has been found that the level of α-amylase increased significantly after a starch based custard preload and *ad libitum* meal (Harthoorn, 2008). Salivary amylase helps in food digestion during oral processing by hydrolysing starch into maltose (Zakowski & Bruns, 1985) and it has been proposed that the concentration of salivary α-amylase may influence directly the hunger levels. For instance, in people with lower

concentration of α-amylase, the digestion of carbohydrates will be slow, and this would lead to a presence of hunger for a longer period of time resulting in greater food intake before achieving satiety (Moreno-Padilla, Maldonado-Montero, Enguix-Armada, & Reyes del Paso, 2020). In addition, a link between macronutrient composition of foods and saliva characteristics has been reported. For example, intake of fatty food was reflected in a fatty acid profile of the collected saliva (Actis, Perovic, Defago, Beccacece, & Eynard, 2005). Likewise, carbohydrate intake showed an antioxidant capacity and increased amylase activity in the collected saliva (Méjean, et al., 2015). Also, the secretion of α-amylase has been reported to be dependent on the diet (Perry, et al., 2007). Taken together, these studies suggest that the composition of saliva is dependent on the type of food consumed. However, in these studies the focus was on food macronutrient composition with no direct or indirect link to lubricity of food. Consequently, there is lack of studies showing the independent effects of oral lubricity, and specifically any studies on model food such as hydrogels on satiety, whilst controlling for the macronutrient or energy composition of food.

Interestingly, it is known that salivary proteins such as mucin (MUC5B) and other low molecular weight proteins contribute to the salivary composition and influence lubrication behaviour (Hopkins, et al., 2020; Humphrey & Williamson, 2001; Sarkar, Kanti, Gulotta, Murray, & Zhang, 2017a; Sarkar, Xu, & Lee, 2019a; Sarkar, Ye, & Singh, 2017b). However, how consumption of a high lubricating gel affects the tribological properties of the human saliva and MUC5B or protein content and how such change (if any) in salivary lubrication affects satiety remains largely unknown. It can be hypothesized that eating a high lubricating food might increase the lubricating properties of saliva and keep the oral surfaces moistened and coated for longer duration. This in turn may lead to appetite suppression and lower subsequent food intake. To date, no studies have reported effects of food texture on satiety and satiation while considering the tribology properties of saliva when consuming a high lubricating versus a low lubricating model food. It is therefore appropriate to examine the relationship between the lubricating behaviour (based on higher concentration of proteins or MUC5B or mechanically measured tribological properties) of saliva on consumption of lubricating preloads and its influence on appetite control and food intake.

Therefore, the aim of the present study was to examine the effects of hydrogels as model foods varying in their oral lubrication properties on satiety, satiation as well as concentration of salivary biomarkers and frictional properties of collected saliva. These effects were evaluated through appetite ratings, subsequent food intake, measurement of frictional properties of saliva and measurement of concentration of salivary biomarkers at specific time points before and after the ingestion of preloads. The main objectives were to: 1) examine whether a systematic model food design with higher lubricity would lower subsequent food intake and suppress appetite in a meal-trial; 2) understand the changes in lubricity of saliva after ingesting hydrogels with different oral lubrication properties; and 3) investigate the effect of hydrogel lubricity on salivary biomarkers, such as MUC5B, proteins and α-amylase. It is hypothesised that ingesting hydrogels possessing higher lubrication properties would lead to 1) a lower energy intake and suppressed appetite ratings; 2) higher levels of lubrication properties of the saliva; and 3) higher concentration in certain salivary biomarkers such as MUC5B and protein and a potential correlation between the salivary biomarkers, salivary lubricating properties, food intake as well as perceived appetite ratings. The strategic objective is to demonstrate whether or not structural/ textural complexity in terms of oral lubricity of food can affect both psychological and physiological aspects of eating behaviour in a meal setting.

2. Methods

2.1. Participants

We recruited healthy male and female participants between 18 and 55 years old. The participants were recruited from staffs and students of the University of Leeds. Subjects were excluded if they were smokers, had oral infections/diseases/ problems in chewing and swallowing, had a chronic or acute health condition that could affect the ability to sense, eat, digest or absorb food, were using prescribed or non-prescribed medication that could interfere with their ability to sense, eat, digest or absorb food; were pregnant or lactating, had a food allergy or intolerance, were on a special diet or taking protein/ fibre supplements, were not able to tolerate food gels, had underweight (BMI <18.5 kg/m^2), overweight or obesity (BMI $\geq$ 25 kg/m^2), had blood-borne diseases. The study was approved by University of Leeds

Faculty Research Committee (MEEC 18-049). Sample size was calculated with GPower3.1. As the manipulation of this study involved novel food *i.e.* favoured hydrogels, there was not enough information in the literature in terms of the expected size effect. Therefore, the power analysis was a priori one, and it has been done to determine the number of participants needed for a small effect size (f=0.25) across all four outcomes. As such, according to GPower calculation, 24 participants are required to identify a small effect size (f = 0.25, α = 0.05 and 1-β = 0.80) across 3 groups (low lubricating, high lubricating and control) with 4 outcome (appetite ratings, food intake, salivary biomarkers and lubricity of saliva), with outcomes varying from 3 to 5 measurements. However, due to a UK lockdown related to the COVID-19 pandemic, data were collected and analysed for 17 participants.

2.2. Design

The study (registered at ClinicalTrials.gov as NCT04240795) was an acute, single-blinded, randomized, counterbalanced, within-subject designed cross-over trial. Recruitment poster were placed across the University of Leeds campus and emails were sent to students and staffs. Further, interested participants were emailed an information package (participant information sheet, eligible criteria, Three Factor Eating Questionnaire (TFEQ) and the link to online health screening questionnaire) in which they were informed that the aim of the study was to investigate the acceptance, pleasantness and taste perception of food gels with different textural attributes. At the end of the study, participants were debriefed and informed about the exact purpose of the study. The study took place at the University of Leeds, UK, School of Food Science and Nutrition, between November 2019 and end of March 2020. Participants gave their written informed consent before taking part in the study (ethics approved by University of Leeds (MEEC-16-046)) and received shopping vouchers of £20 value as compensation for their time.

2.3. Session procedure

Before taking part into the study, participants were first screened for eligibility criteria using an online health screening questionnaire. They were also tested for restrained eating using TFEQ. A total of 34

participants was screened, of which 17 were included in the study for data analysis (13 did not meet the inclusion criteria, 2 withdrew from the study and 2 did not finish the study due to the COVID-19 associated lockdown). Each participant was asked to come to the laboratory on three different occasions with 3-7 days of wash out period in between each session. Participants were instructed to fast for 11 h (from 10.00 pm) and to refrain from drinking except water for 24 h before each session. Alcohol was prohibited. Each session lasted 3.5-4 h. Participants were required to come to the laboratory at 8.45 am.

In the first session, weight and height were measured. Body weight was measured to the nearest 0.1 kg after voiding (Seca 763, Seca Birmingham, UK) and height was measured to the nearest 0.5 cm using a portable stadiometer (Seca Portable height measure, Leicester, UK). A schematic overview of the study protocol is presented in Fig.1a. Participants provided baseline appetite ratings on a 100-mm visual analogue scale (VAS). After that, at 9.00 am, they were given a fixed amount of breakfast which consisted of muesli (Neal's Yard Muesli Base), raisins (Neal's Yard Raisins), sultanas (Neal's Yard Sultanas), honey (Sainsbury's Runny Honey) with yogurt (Yeo Valley Natural Yoghurt) purchased from a local supermarket and 150 g of water. The total allocation was 250 kcal for females and 350 kcal for males in order to standardise the appetite levels for all the participants before consuming the preloads (flavoured hydrogels). Participants were required to eat all of the breakfast. Participants then rated their appetite on visual analogue rating scales (VAS hunger, fullness, desire to eat, prospective food consumption and thirst) at every 30 minutes for the next 2.5 h and whole unstimulated saliva was collected at 3 time points, 90 min after breakfast, pre- and post-preload.

After that, they were given the preload: hydrogels differing in lubricity, or water (control). After consuming the preload, appetite ratings were recorded by the participants at three time points (every 10 min for a duration of 30 min). Whole unstimulated saliva was collected after breakfast, immediately after consuming the hydrogels as well as after 10 min of resting period. An *ad libitum* lunch was offered 30 min after ingesting the preload followed by the final appetite ratings. The *ad libitum* lunch consisted of vegetarian chilli (Stagg Low Fat Vegetable Chilli, Danish Crown Ltd., Manchester, UK) and rice (Microwave Rice Basmati, Sainsbury's Supermarkets Ltd., London, UK) and strawberry yogurt (Yeo Valley, Blagdon, UK) purchased from a local supermarket and *ad libitum* water. Participants were

provided with 770.4 kcal/ 845 grams of chilli and 778.2kcal/ 525 grams of yoghurt. They were asked to eat until a comfortable level of fullness.

2.4. Preload characterisation

The preload consisted of watermelon-flavoured hydrogels (see Figs. 1b and 1c) that were selected based on their difference in textural attributes (measured sensorially) and oral lubrication properties (measured instrumentally) as described previously (Stribiţcaia, Krop, Lewin, Holmes, & Sarkar, 2020b). Briefly, the hydrogels were cut in heart shape and each participant received a total amount of 30 g of each hydrogel or control (water) on different testing days. The difference in sensorial and oral lubrication attributes was achieved by mixing the same gelling agents but structuring differently. One type of hydrogel contained a mixture of κ-carrageenan (κC) and sodium alginate (NaA) (see Fig.1b), whilst the other hydrogel was layered containing κC and alginate, with the latter in the form of calcium alginate-based spherical beads (CaA) of 1800 μm and consisted of three layers: top and bottom layers were pure κC and the middle layer contained CaA beads (see Fig. 1c).

The concentration was the same for both hydrogels: κC + NaA hydrogel (1.67 wt% κC and 0.33 wt% NaA) and κC + CaA hydrogel (1.67 wt% κC and 0.33 wt% CaA). Based on instrumental (see Fig. 2a for tribological *i.e.* friction measurement) and sensorial analyses (see Fig. 2b), the κC + NaA hydrogel was characterised as pasty (Fig. 2b) and was high in oral lubrication properties (Fig. 2a) (*i.e.* low in friction), and this hydrogel is referred to as high lubricating hydrogel (HL) hereafter. On the other hand, the κC + CaA hydrogel was characterised as sensorially hard (Fig. 2b) and the inclusion (CaA beads) resulted in high frictional properties (Fig. 2a) and consequently low in oral lubrication properties, therefore is referred to as low lubricating hydrogel (LL), hereafter. The instrumental characteristics of the hydrogels were determined by performing tribology analysis using a Mini Traction Machine (MTM2) tribometer (PC Instruments, London, UK), and the ratings of the sensorial attributes were obtained by performing intensity ratings with 60 untrained participants, details have been provided previously (Stribiţcaia, et al., 2020b). The hydrogels were flavoured with food-grade watermelon aroma (Special Ingredients Ltd, Chesterfield, UK), coloured with food-grade watermelon food colouring

(AmeriColor Corp., Placentia, California USA) and sweetened with stevia granulated sweetener from a local supermarket (Leeds, UK) to increase acceptability of these model foods by the consumers without addition of any calorific sugar. Water was provided to the participants as a control. The water also contained the watermelon flavour, colour and sweetness to match the flavour profile and intensity of sweetness of the hydrogels.

2.5. Study measures

2.5.1. Appetite ratings

Participants rated their appetite at eleven different time points using a 100-mm VAS scale, which has been shown to be valid and reliable for appetite research (Flint, Raben, Blundell, & Astrup, 2000; Stubbs, et al., 2000). The scales anchored from 'not at all' to 'extremely' were administered at: -5, 0, 30, 60, 90, 120, 150, 160, 170, 180, 210 min on each testing day (see Fig.1a). The participants rated hunger, fullness, desire to eat, prospective food consumption and thirst. Additional scales contained questions concerning nausea and the mood – contentedness and mental alertness. In addition, participants rated the palatability and the acceptability of the hydrogels and control. The time point of 150 min is referred to as 'pre-preload', 160 min to as 'post-preload', 170 min to as '10 min after preload' and 180 min to as '20 min after preload' throughout the text.

2.5.2. Energy intake

Ad libitum foods and beverages were accurately weighed (to the nearest 0.1g) prior to being served to participants, and were re-weighed after the participants finished eating in order to determine the amount of food and beverage consumed by each participant. Energy intake (EI) at each meal was calculated. For completeness in reporting, first, the energy intake was calculated (the number of grams of carbohydrate, protein and fat was multiplied by 3.75, 4 and 9, respectively) for rice and vegetable alone and this was referred to as 'main course' and then for yogurt alone referred to as 'dessert' throughout the text. Then, a total EI was calculated for both rice with vegetable and yogurt, and is referred to as 'combined' meal.

2.5.3. Tribology of human saliva

As illustrated in Fig. 1a, saliva was collected at three time points. Participants were asked to spit into a pre-cooled tube till they felt comfortable. The collected saliva from each participants at three different time points were centrifuged for 5 min at at 4000 × *g* and the precipitate containing cell debris was discarded. Approximately, 3 mL of the supernatant was made up to the volume to 10 mL using pre-chilled 20 mM phosphate buffer (pH 7) (*i.e.* 16 vol% unstimulated whole human saliva) (Hopkins, et al., 2020) and was stored at 4 ºC for tribology analysis within the same day using ball-on-disc tribological set up in a Mini Traction Machine (MTM2, PCS Instruments, London, UK) and three separate aliquots (250 µL each) were stored at −20 °C until further use for total protein, *α*-amylase and MUC5B assays, respectively.

Tribology was performed to determine the lubrication properties of saliva after breakfast, before and after preload (Saliva 1-Saliva 3, respectively). Commercially available polydimethylsiloxane (PDMS) ball (diameter of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) and disc (diameter of 46 mm, thickness of 4 mm, MTM ball Slygard 184, 50 Duro, PCS Instruments, London, UK) were used as surfaces to mimic palate and tongue, respectively for the oral tribology measurements (surface roughness of the PDMS tribopairs, R_a < 50 nm). The saliva supernatant (9 mL) was loaded into the minipot equipped with the PDMS ball and disc, where these tribopairs were rotated at different speeds to create a relative motion between the surface of the ball and the disc, resulting in a slide-to-roll ratio (SRR) of 50 %, and the temperature was maintained at 37 °C, simulating oral procedures. The entrainment speed was calculated as the average velocity of the two contacting surfaces (*i.e.* ball and disc) and reduced from 300 to 1 mm/s to simulate tongue movement, and friction forces were measured at a load of 2 N with a maximum of 343 kPa of Hertzian contact pressure (Sarkar, Andablo-Reyes, Bryant, Dowson, & Neville, 2019b). Friction forces in presence of saliva collected at different time points and after consuming preloads or controls were compared at boundary (BL, speed of 0.05 m s^{-1}) and mixed (ML, speed of 0.5 m s^{-1}, 0.1 m s^{-1}) lubrication regimes (Stribiţcaia, et al., 2020b).

2.5.4. Biochemical assays of salivary biomarkers

Supernatants (*i.e.* 50 vol% unstimulated whole human saliva) collected in 250 μL aliquots were assayed for total protein using Pierce BCA Protein Assay Kit (Pierce, Fisher Scientific, Loughborough, UK) and the results were compared to a standard curve generated using bovine serum albumin (BSA). Salivary mucin (MUC5B) was analyzed using human MUC-5B ELISA Kit (OKEH02841, Aviva Systems Biology, Insight Biotechnology, Wembley, UK). Salimetrics α-amylase kit (Stratech, Ely, UK) was used to measure salivary α-amylase enzyme activity. The biochemical assays were run in duplicate and absorbance values recorded using Tecan Spark 10 M microplate reader (Tecan, Reading, UK). Results were expressed as Units/mg protein for amylase, ng/ mg protein for MUC5B and μg/mL for protein.

2.6. Statistical analysis

Data are presented as mean and standard deviations (SDs) in the text and tables, and means and SEMs in the figures. All statistical analyses were performed using SPSS (IBM® SPSS® Statistics, v25, SPSS Inc, Chicago, USA). Differences between conditions were tested by repeated measures ANOVA for appetite ratings at relevant time points, overall appetite ratings, food intake, salivary biomarkers, and lubricating capacity of human saliva after ingesting the preloads. The differences in palatability of the preload, nausea, mental alertness and content mood were also assessed by repeated measures ANOVA. 3 × 5 level factorial repeated measure ANOVA was used to examine the main effect of the intervention condition (LL, HL, Control), time (pre-preload, post-preload, 10 min, 20 min after preload and after lunch) and condition*time interaction on appetite ratings. Analysis of appetite ratings were also compared after controlling for baseline ratings using the analysis of difference from baseline. As the food (hydrogels) in this study was novel, there is sufficient uncertainty about the immediate post-gel experience to make conclusion based on analysis controlled for baseline only. Therefore, appetite results from both with and without controlled for baseline analysis are reported. Where the assumption of sphericity had been violated, indicated by Mauchly's test, Greenhouse-Greisser corrected tests are reported. Statistical significant differences were calculated by Bonferroni corrected post-hoc t-tests and

was set at $\alpha < 0.05$ level. Pearson correlations were performed to assess the relationship between appetite ratings, food intake, tribology of saliva and concentration of biomarkers (protein, α-amylase). Data for appetite ratings, overall appetite scores and food intake were analysed for all 17 participants. For salivary biomarkers data were analysed for α-amylase and protein on all 17 participants, however, for mucin data were analysed on 9 participants due to the negative values or values out of standard range on the rest of the participants. To check the outliers, the Explore function in SPSS was used, with the IQR (interquartile range) multiplier approach (Tukey, 1977). Where the values from the end of the box plot were more than 3 IQR's (also, labelled as 'extreme') and where denoted with an asterisk (*),the data were treated as outliers and excluded further from the analysis (Hoaglin & Iglewicz, 1987). For tribology, there were complete datasets for 7 participants only. After removing the outliers (n=3), the salivary tribological analysis was completed on 4 participants only. Therefore Pearson's correlation was also analysed on data from 4 participants. Data were plotted using the software Origin® (OriginPro 2018; OriginLab Corporation, Northampton MA, USA).

3. Results

3.1. Participants' characteristics

A total of 17 participants (6 males/ 11females) completed the study, see the characteristics in Table 1. The age of the participants ranged from 20 to 29 years. Their BMI was 22.6 ± 2.9 kg/m², with 15 participants in the healthy range and two with overweight (both 26.7 kg/m²); all 17 were included in the analysis. The TFEQ analysis revealed that 4 participants had a high restraint score (between 14-18).

3.2. Appetite ratings

Descriptive data for appetite ratings between preloads at relevant time points (fasting, pre-/post-preload, 10, 20 min after preload and after lunch) are given in Table 2. There was no significant difference between groups for fasting for all appetite ratings and for pre-preload time, whereas an increased fullness was noticed in HL (high lubricating) versus LL (low lubricating condition) and vs Control (see Table 2). Palatability was measured on a 100-mm VAS scale immediately after preload in

terms of texture, sweetness and flavour. The only difference noted was between LL and Control in terms of sweetness ($p < 0.005$) where Control was perceived sweeter than LL. In terms of texture and flavour, there was no significant difference between preloads (see Supplementary Table S1). In the following sections, we focussed on the appetite rating differences.

Hunger

There was no main effect of intervention $F(2,32) = 1.83$ ($p = 0.18$) but there was a main effect of time $F(2.17, 34.82) = 94.02$ ($p = 0.000$) and intervention*time interaction on hunger $F(8,128) = 2.13$ ($p = 0.024$) (Table 2). A post-hoc pairwise comparison test revealed that hunger significantly decreased in HL condition versus Control ($p < 0.05$) and was significantly lower post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, the key effect was confirmed. There was a main effect of intervention*time interaction on hunger $F(8,128) = 2.13$ ($p = 0.024$). However, the effect of intervention alone does not exist anymore. Only the effect of time is noted. A post-hoc pairwise comparison test revealed that hunger significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Fullness

There was an effect of intervention $F(2,32) = 8.01$ ($p = 0.002$) and time $F(1.73, 27.76) = 53.77$ ($p = 0.000$), but no effect of intervention*time interaction on fullness $F(8,128) = 1.53$ ($p > 0.05$) (Table 2). A post-hoc pairwise comparison test showed that fullness significantly increased in HL condition versus Control ($p < 0.05$) and was significantly higher post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, an effect of time on fullness $F(1.73, 27.76)=53.77$ ($p=0.000$) was noticed. A post-hoc pairwise comparison test showed that that fullness significantly increased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Desire to eat

For desire to eat, there was an effect of time only $F(2.37, 37.92) = 78.53$ ($p = 0.000$), and no effect of intervention $F(2,32) = 2.18$ ($p > 0.05$) or intervention*time interaction $F(4.41, 70.54) =1.51$ ($p>0.05$)

(Table 2). Post-hoc pairwise comparison test revealed that desire to eat was significantly lower post-preload, 10 min after preload and *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, an effect of time on desire to eat F(2.37, 37.92)=78.53 ($p=0.000$) was observed. A post-hoc pairwise comparison test showed that desire to eat ratings significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Prospective food consumption

For prospective food consumption there was an effect of intervention F(2,32) = 4.55 ($p = 0.018$) and an effect of time F(1.69, 27.13) = 91.72 ($p = 0.000$), but no effect of intervention*time interaction F(8,128) = 1.11 ($p > 0.05$) (Table 2). Post-hoc pairwise comparison test showed that prospective food consumption significantly decreased in HL condition compared to Control one ($p < 0.05$) and was significantly lower post-preload, 10 min after preload and *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, an effect of time on prospective food consumption F(1.69, 27.13)=91.72 ($p=0.000$) was sen. A post-hoc pairwise comparison test showed that it prospective food consumption ratings significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Thirst

Thirst had the same pattern as desire to eat, there was an effect of time only F(4,64) = 41.93 ($p = 0.000$), and no effect of intervention F(2,32) = 0.014 ($p > 0.05$) or intervention*time interaction F(8,128) = 0.328 ($p > 0.05$) (Table 2). Post-hoc pairwise comparison test revealed that thirst significantly decreased only after *ad libitum* lunch ($p<0.05$) compared to pre-preload, post-preload, 10 and 20 min after preload ($p > 0.05$). After controlling for baseline ratings, again, an effect of time on thirst F(4,64)=41.93 ($p=0.000$) was noticed. A post-hoc pairwise comparison test showed that thirst significantly decreased only after *ad libitum* lunch ($p < 0.05$) compared to pre-preload, post-preload, 10 and 20 min after preload ($p=0.000$).

To check if there was any significant difference in overall appetite suppression (OAS) the following equation was used:

$$\text{OAS} = \frac{Hunger + PFC + (100 - Fullness)}{3} \quad (1)$$

There was a significant difference (see Fig. 4) immediately after preload (post-preload) between HL (48 ± 21) and Control (61 ± 20) conditions ($p = 0.05$), 10 min after preload between HL (52 ± 22) and Control (64 ± 21) conditions ($p = 0.000$), and between LL (53 ± 18) and Control (64 ± 21) ($p = 0.016$). Also, at 20 min after preload, there has been noted a significant difference between HL (61 ± 20) and Control (69 ± 18) conditions ($p = 0.039$). These results corroborate with the ones discussed above with respect to Table 2. There was no significant difference of AUC between groups for all appetite ratings (see Supplementary Table S2).

After controlling for baseline ratings, the overall appetite scores showed an effect of time $F(1.69,27.14)=100.07$, $p=0.00$ and an effect of intervention*time interaction $F(8,128)=2.38$, $p=0.02$. A post-hoc pairwise comparison test revealed that overall appetite scores significantly decreased post-preload, 10 min after preload and after *ad libitum* lunch ($p < 0.05$) compared to pre-preload and 20 min after preload ($p > 0.05$).

Appetite ratings for all the eleven time points (from fasting until after *ad libitum* food intake (after lunch), including breakfast as well) are illustrated in Fig. 3. A clear pattern can be noted where hunger (Fig. 3a), desire to eat (Fig. 3c), prospective food consumption (Fig. 3d) and thirst (Fig. 3e) decreased immediately after breakfast, increased before preload (almost 2.5 h after breakfast), decreased immediately after the intervention and returned to the baseline/ fasting level 20 min after preload. As expected, a contrasting pattern could be noted for fullness too (Fig. 3b), where it (fullness) increased immediately after breakfast, decreased before preload (almost 2.5 h after breakfast), increased immediately after intervention and finally returned to baseline levels 20 min after preload.

Due to the novelty of the model foods used in the current study as preloads (hydrogels), we assessed for the feelings of nausea, as well as for the mood of participants after ingesting the preloads. Therefore, three more measurements were taken (on a 100 mm VAS scale): nausea (Fig. 3f), content (how content participants felt at each time point during each study session) (Fig. 3g) and mental alert

(how mentally alert participants felt at each time point during each study session) (Fig. 3h). There was no significant main effect of intervention/preload, time point or intervention*time interaction in terms of nausea and mental alertness. However, a significant effect of intervention $F(1.44, 23.08) = 5.621$ ($p = 0.017$), time $F(1.21, 19.40) = 17.91$ ($p = 0.000$) and no effect of intervention*time interaction $F(3.53, 56.54) = 2.254$ ($p = 0.082$) was noted for contentedness (how content participants felt at each time point during each study session). Post-hoc pairwise comparison test revealed that participants felt more content after eating HL preload compared to LL and Control ($p = 0.027$) immediately after preload, 10 and 20 min thereafter ($p < 0.05$). It is unknown why this occurred. One explanation could be that participants liked more HL preload in comparison with LL and Control, however, there was no significant difference in preloads palatability (see Supplementary Table S1).

3.3. Energy intake

For *ad libitum* energy intake at lunch (see Fig. 5), there was no statistical difference for main course, dessert, and combined meal between interventions – HL, LL and Control. Therefore, the total amount of food participants consumed was almost the same in all three conditions. The same was noticed for water, no significant difference between groups in the water intake.

3.4. Lubrication properties of collected saliva

To check if there were differences in lubrication properties of saliva between conditions before and after the intervention, tribological measurements were performed on the collected saliva. There was no significant difference in the lubrication properties of saliva expressed through friction of coefficient between interventions/preloads (HL, LL and Control) before preload (see Fig. 6a) and after preload (see Fig. 6b). See Supplementary Table S3a and S3b for the descriptive data showing no difference in the lubrication properties after consuming HL, LL or Control (after breakfast data were excluded from analysis due to its irrelevance to the study).

3.5. Salivary biomarkers

A total concentration of protein (see Fig. 7a), *α*-amylase (see Fig. 7b) and MUC5B (see Fig. 7c) were assessed for each condition at two time points: before and after preload (after breakfast data were excluded from analysis due to its irrelevance to this study). There was no significant differences in any salivary biomarkers (protein, *α*-amylase and MUC5B) between interventions (see Fig. 7a-7c). Surprisingly, an increase in total MUC5B was noted in LL compared to HL and Control condition after intervention, however this was not statistically significant (see Fig. 7c). For total protein, there was no effect of time $F(1,16) = 4.21$ ($p = 0.057$), intervention $F(2,32) = 0.623$ ($p = 0.543$) or intervention*time interaction $F(2,23) = 0.751$ ($p = 0.480$). The same was noted for *α*-amylase and MUC5B. There was no effect of time $F(1,16) = 0.550$ ($p = 0.469$), intervention $F(2,32) = 2.46$ ($p = 0.101$) or intervention*time interaction on salivary *α*-amylase concentration $F(1.42, 22.78) = 2.40$ ($p = 0.126$). Also, there was no effect of time $F(1,8) = 0.356$ ($p = 0.567$), intervention $F(1.03, 8.28) = 2.12$ ($p = 0.182$) or intervention*time interaction on salivary MUC5B concentration $F(1.01, 8.12) = 1.45$ ($p = 0.263$). These data suggest no effect of consuming non-calorific model-food differing in its lubrication properties on salivary biomarkers such as α-amylase, protein and MUC5B.

3.6. Pearson's correlation

To examine whether the changes after preload in appetite ratings, energy intake and tribological properties of saliva were related to salivary biomarkers, we performed Pearson's correlation between the aforementioned parameters for all interventions (HL, LL and Control) (see Supplementary Table S4). Statistical association was noted between dessert (yogurt) and protein activity ($r = 0.985$; $p = 0.025$). Also, there was a statistical association between friction coefficient (tribology) of saliva and fullness ($r = -0.991$; $p = 0.009$, $r = -0.995$; $p = 0.005$) meaning that the lower the friction coefficient (which means higher lubricating properties of saliva), the higher the feeling of fullness which in line with our hypothesis. In the rest, there was no relation between appetite ratings, energy intake, tribological properties of saliva and salivary biomarkers (see Supplementary Table S4).

4. Discussion

In this article, we investigated the effect of oral lubricity on appetite control, food intake and salivary biomarkers using model foods *i.e.* hydrogels varying in their lubricating properties. Additionally, we explored the lubrication properties of human saliva after eating the hydrogels, as well as the relation between oral lubricity and appetite, food intake and salivary biomarkers. With regard to appetite ratings, an effect of HL (high lubricating hydrogels) on reducing hunger, desire to eat and prospective food consumption as well as increase in fullness was observed as compared to Control (water) immediately after ingestion, and 10 and 20 min thereafter. Although HL lowered appetite ratings such as hunger, desire to eat and prospective food consumption as well as increased the fullness ratings as compared to LL, difference between HL (high lubricating) and LL (low lubricating) hydrogels on appetite was not significant. These findings suggest there was no effect of high lubricity versus low lubricity conditions on subjective appetite sensations in this study, however, there was an effect of HL (high lubricating hydrogels) condition compared to the Control. This is the first study to show an effect of oral lubricity on appetite sensations on a meal setting.

In a previous study by Krop et al. (2019a) employing hydrogels differing in their lubricating properties on appetite ratings in a snack trial there was reported no difference in appetite ratings. A potential explanation of inconsistency in outcomes between these two studies could be the study design in terms of appetite measurements. Krop et al. (2019a) measured appetite at lesser number of time points than our study. For instance, they rated the appetite before, immediately after preload and after *ad libitum* snack, whereas in the current study appetite was rated on two more time points after preload. Therefore, we showed the dynamic of appetite over a period of 30 min after ingesting the preloads differing in their lubricating properties, and a significant suppression of appetite in HL condition compared to Control was noted. It is also noteworthy that appetite sensations returned to their initial level after 20 min after ingesting the preload. This suggests that the lubricity may have a small effect on appetite sensations.

It is worth pointing that the energy intake was similar in all the three conditions HL, LL and Control. These findings are not in agreement with other studies dealing with textural complexity (Krop,

et al., 2019a; Larsen, et al., 2016; Tang, et al., 2016). For instance, Krop et al. (2019a) demonstrated that the snack intake was lowered in high lubricating hydrogels as compared to low lubricating hydrogels. To explain the inconsistency in results, the following factors should be taken into account. Firstly, literature shows that the longer the time between the intervention and the next meal, the weaker is the effect of preload on subsequent food intake (Blundell, et al., 2010; Rolls, et al., 1991; Stribiţcaia, et al., 2020a). Secondly, the energy density of the preload plays a role too. Studies that had the preload with a low energy density had a shorter time interval between intervention and next meal (Krop, et al., 2019a; Larsen, et al., 2016; Stribiţcaia, et al., 2020a; Tang, et al., 2016). As such, the preload in our study was free of energy density and macronutrients, and the time between the preload and *ad libitum* next meal was of 30 min. Whereas other studies with a reduced energy density of the preload had a reduced time to the next meal *i.e.* 10 min after preload (Tang, et al., 2016) or immediately after preload (Krop, et al., 2019a). Thus, the long-time interval between the preload and *ad libitum* lunch in our study may have diminished the effect of lubricity on food intake.

Interestingly, appetite ratings returned to the baseline 20 min after the intervention, which means that the appetite sensations were the same in all three conditions before serving the *ad libitum* lunch. This also may explain the lack of significant differences between conditions regarding food intake that was associated with the time interval between the intervention and the *ad libitum* lunch. Therefore, we can infer that effect of lubricity of hydrogels on food intake is time dependent *i.e.* the 30 min time between the preload and the next meal in this study might be too long to show an effect on food intake. As such, it may imply a very short time effect or an immediate effect on the subsequent food intake as it was seen in a similar recent study (Krop, et al., 2019a). Thus, for future research addressing the role of lubricity on subsequent food intake, the time between preload and next *ad libitum* meal should be short or even immediately after preload. Also, it would be of high interest to investigate the effect of oral lubricity on satiation. Therefore an *ad libitum* intake design of the model food differing in their lubricating properties would add better understanding on this matter.

With regard to lubricating properties of saliva after ingesting the preload, we could not detect any significant differences between interventions *i.e.* saliva had the same level of lubricity regardless the preload varying in lubricity. In other words, this means that the lubricating properties of the

hydrogels did not translate into physiologically detectable increase or decrease in lubrication property of the saliva after consumption of the preload. It is known that lubricating properties of saliva depend on the presence of salivary proteins such as mucins (Aguirre, et al., 1989; Hahn Berg, Lindh, & Arnebrant, 2004), statherins (Douglas, et al., 1991; Hahn Berg, et al., 2004), α-amylase (Aguirre, et al., 1989) and others. In fact, no differences between the interventions were observed regarding the presence of proteins, α-amylase and mucin in saliva. Therefore, this may explain the lack of significant difference in lubricating properties of saliva after preload. Likewise, this suggests a potential correlation between the presence of salivary biomarkers and lubricating properties of saliva as was observed by (Hopkins, et al., 2020). An important factor to consider while interpreting lubricating properties of saliva, is the inter-individual variation. It is worth noting that the variations among individuals were very large irrespective of the conditions and time to detect any noticeable difference.

In terms of salivary biomarkers, there was no significant differences in protein, α-amylase and MUC5B concentration in saliva between interventions. Interestingly, a trend could be noted where the total concentration of protein in saliva seemed to slightly decrease after preload compared to before preload in all three conditions (HL, LL and Control). One might argue that this is linked to the fact that although unstimulated saliva was collected, it was stimulated enough by the preload resulting in lowering in protein concentration (Al-Manei, Almotairy, Bostanci, Kumar, & Grigoriadis, 2020). Although not significant, an increase of total α-amylase concentration in saliva in HL and LL condition compared to Control was noted, which might suggest that there could be some association between external lubricity of preloads and the α-amylase activity. However, these results must be interpreted with caution, as we did not detect any significant statistical difference. It is known that α-amylase secretion is initiated more in the presence of starch or after ingesting starch-based food (Froehlich, Pangborn, & Whitaker, 1987) and α-amylase is often used as an objective measure of satiety in starch based food, such as starch-based custard (Harthoorn, 2008).

The total MUC5B concentration slightly decreased after preload in HL and Control condition, but increased in LL condition, though not significant. It might be linked to the fact that the hydrogels (HL) was lubricating enough that it did not require intrinsic lubrication salivary mucins. However, interpreting such data in lack of statistical significance can be challenging as MUC5B levels might be

affected by the degree of stimulation by the hydrogels, age of the participants and time of the day of the intervention *etc.* (Helmerhorst & Oppenheim, 2007; Mariscal, et al., 2019). Overall, it can be inferred that subtle changes in lubricity of samples might not alter the biochemical components of saliva. Factors such as macronutrients, energy density of the food (Harthoorn, 2008) might play an important role in the physiological aspect of satiety and satiation, and thus are worthwhile to explore in conjunction with lubricity in future research**.**

4.1. Limitations and strengths

A limitation of this study is the sample size, it was smaller than planned due to the pandemic, which influenced the results. Measuring saliva after breakfast did not give us any relevant information, therefore, future studies should focus on two or three time points of saliva collection after preload with one immediately after consuming the preload. Change in statistical analysis (controlling baseline values of the appetite ratings and removing the outliers) that has not been initially planned, is another limitation of this study. Nevertheless, a clear strength of this study is the measurements of saliva in terms of tribological aspects and biomarkers. This is first study that has attempted to link food texture (from an oral lubrication perspective) to satiety together with salivary biomarkers, as well as lubricating properties of saliva, which presents a feasible approach to connect psychological aspects of appetite to physiological aspects of salivary properties. Also, using a within-subject design gives a strong edge to the current study as many, if not, most satiety trials are conducted with repeated measures designs where each of the participants acts as their own control (Gibbons, et al., 2019).

5. Conclusion

In summary, when data are not controlled for baseline, model food (hydrogels) with higher lubricating properties suppressed appetite ratings compared to water, and such effect is small. However, after controlling the data for baseline, such effect does not exist anymore. Therefore, the results should be interpreted with caution. No effect of lubricity on food intake and salivary biomarkers was found, which might be associated with the subtle change in lubrication between the preloads or the long time

between the intervention and the measurement. Therefore, future research should reduce the time between preload and next *ad libitum* meal in order to demonstrate the immediate effect of lubricity on satiety and satiation. In addition, studies should also employ energy density and macronutrients/real food as opposed to non-calorific hydrogels to understand the combinatorial effect of calorie and lubricity to be closer to real food and test the effects on satiety.

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Table 1. Participant's characteristics[1].

Characteristics	Values	Units
Male/ female	6/ 11	
Age	25.4 ± 2.7	years
Weight	63.9 ± 11.3	kg
Height	1.67 ± 0.06	m
BMI	22.6 ± 2.9	kg/m^2
TFEQ Restrain	10.9 ± 4.5	
TFEQ Disinhibition	9.2 ± 4.5	
TFEQ Hunger	7.9 ± 6	

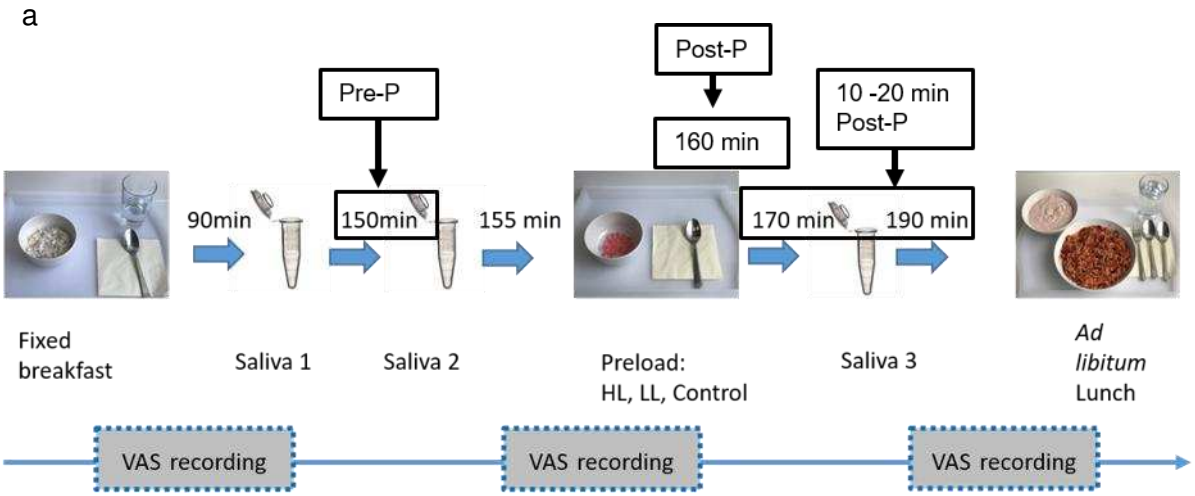
[1]Values are means ± SDs (n=17). TFEQ, Three Factors Eating Questionnaire; BMI, Body Mass Index

Table 2. Appetite ratings (mm) at relevant time points for subjects eating HL (high lubricating), LL (low lubricating) or Control preloads, n=17 (means ± SD).

Appetite ratings	HL	LL	Control	p-value[1]
Hunger				
Fasting	66 ± 23	66 ± 21	64 ± 22	n.s.
Pre-preload	58 ± 21	57 ± 16	60 ± 21	n.s.
Post-preload	42 ± 22^{a}	49 ± 21^{ab}	59 ± 20^{b}	p=0.014
10 min after	47± 27^{a}	48± 23^{ab}	59 ± 22^{b}	p=0.009
20 min after	59 ± 20	62 ± 19	67 ± 18	n.s.
After lunch	3 ± 3	3 ± 5	2 ± 3	n.s.
Fullness				
Fasting	20 ± 19	17 ± 18	16 ± 15	n.s.
Pre-preload	37 ± 23^{a}	29 ± 18^{b}	30 ± 24^{bc}	*p < 0.05*
Post-preload	49 ± 28^{a}	39 ± 24^{ab}	33 ± 24^{b}	*p = 0.004*
10 min after	43 ± 26^{a}	40 ± 24^{ab}	30 ± 22^{b}	*p = 0.004*
20 min after	36 ± 22^{a}	27 ± 16^{ab}	28± 23^{b}	*p = 0.039*
After lunch	90 ± 8	87 ± 20	84 ± 30	n.s.
Desire to eat				
Fasting	69 ± 24	63 ± 24	67 ± 25	n.s.
Pre-preload	61 ± 21	53 ± 20	63 ± 20	n.s.
Post-preload	45 ± 28	48 ± 27	53 ± 23	n.s.
10 min after	50 ± 27^{a}	52 ± 24^{ab}	60 ± 22^{b}	*p = 0.030*
20 min after	59 ± 25	62 ± 22	67 ± 20	n.s.
After lunch	5 ± 7	6 ± 9	5 ± 8	n.s.
Prospective food consumption				
Fasting	67 ± 20	66 ± 16	64 ± 18	n.s.
Pre-preload	60 ± 21	55 ± 19	63 ± 21	n.s.
Post-preload	50 ± 22	48 ± 22	58 ± 22	n.s.
10 min after	53 ± 23^{a}	52 ± 21^{ab}	64 ± 23^{c}	*p < 0.05*
20 min after	60 ± 22	60 ± 21	67 ± 19	n.s.
After lunch	7 ± 8	6 ± 7	9 ± 9	n.s.
Thirst				
Fasting	72 ± 22	65 ± 20	70 ± 22	n.s.
Pre-preload	62 ± 25	61 ± 28	63 ± 23	n.s.
Post-preload	54 ± 27	54 ± 27	50 ± 24	n.s.
10 min after	53 ± 27	50 ± 30	53 ± 23	n.s.
20 min after	61 ± 30	61 ± 33	59 ± 26	n.s.
After lunch	16 ± 21	15 ± 21	18 ± 22	n.s.

[1]A statistical significant difference ($p<0.05$) between the interventions (preloads) is denoted by different letters in superscripts. A non-significant difference ($p > 0.05$) between the interventions is denoted by the letters n.s.

FIGURE 1



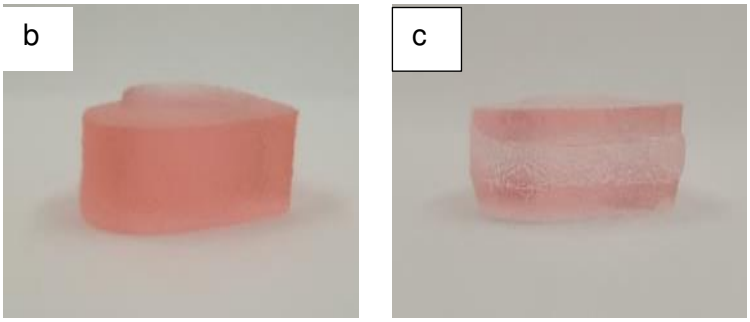


Fig. 1. Overview (a) of the study protocol. First, fasting ratings were taken on a visual analogue (VAS) scale (mm), then a fixed breakfast was provided, and then appetite was rated on a VAS scale (mm) over eleven time points in total. Whole unstimulated saliva was collected on three time points (Saliva 1-Saliva 3). *Ad libitum* lunch was given to the participants 190 min after breakfast or 30 min after the preloads. Preloads represent watermelon-flavoured hydrogels cut in heart shape, (b) κC+NaA - pasty/ high lubricity – HL, and (c) κC + CaA – hard / low lubricity – LL and control was flavoured water. Pre-P=pre-preload, Post-P=post-preload, 10 min Post-P=10 min post-preload, 20 min Post-P=20 min post-preload.

FIGURE 2

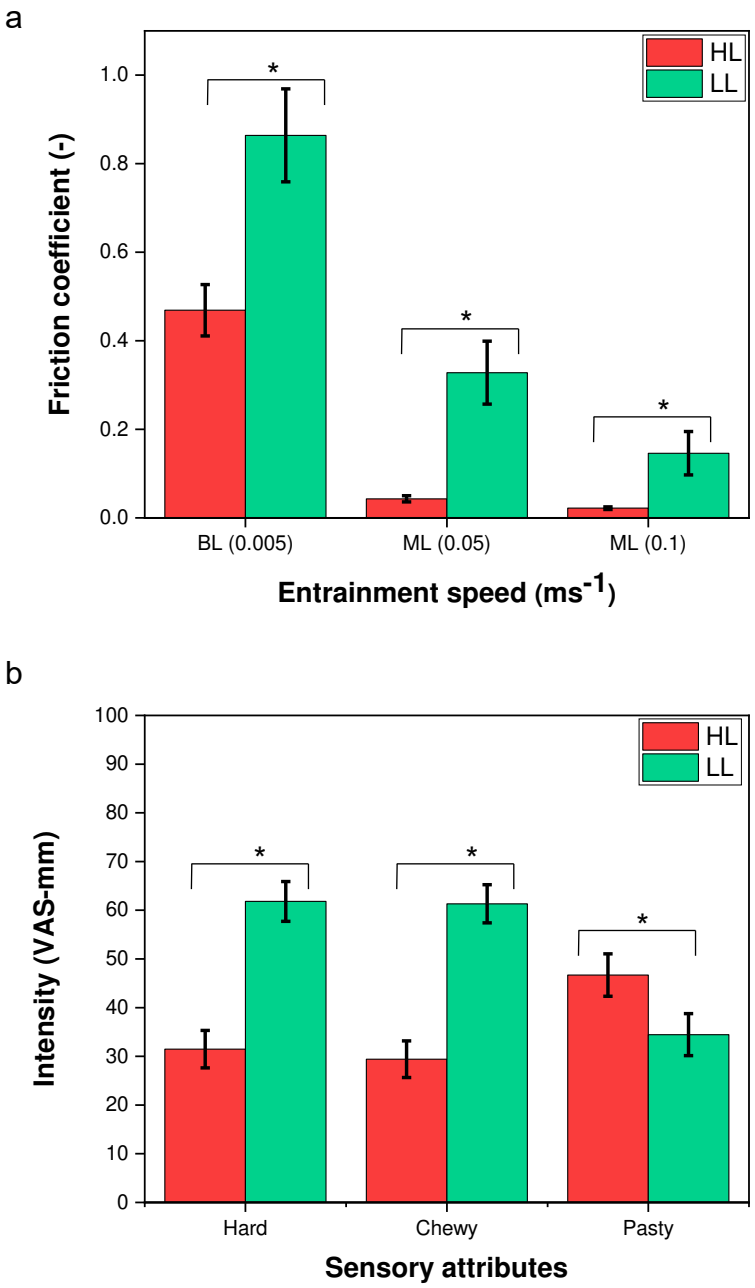
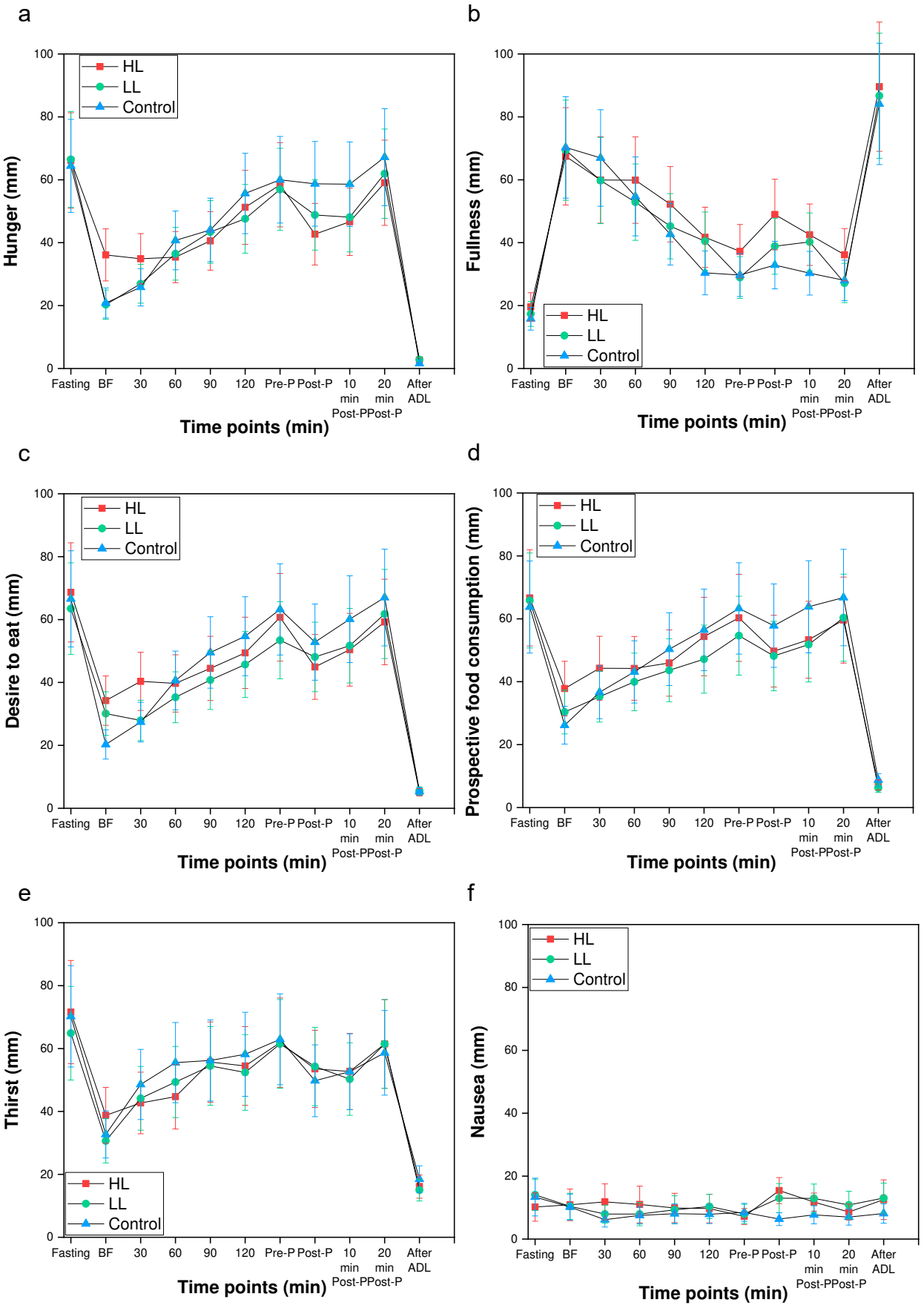


Fig. 2. Instrumental lubricity analysis (a) where data is expressed as friction coefficients at boundary (0.005 m s^{-1} speed) and mixed (0.05 m s^{-1}; 0.1 m s^{-1} at speed) lubrication regimes for the HL (high lubricating) and LL (low lubricating) hydrogels, respectively at various speeds; and sensory analysis (b) including three attributes: hardness, chewiness and pastiness for both HL (high lubricating) and LL (low lubricating) hydrogels. Error bars represent standard error of means (SEMs). The asterisks (*) denote a significant difference between the samples. A lower friction coefficient represents higher lubrication properties of the hydrogels. BL = boundary regime, ML = mixed regime.

FIGURE 3


a
Hunger (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)
b
Fullness (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)
c
Desire to eat (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)
d
Prospective food consumption (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)
e
Thirst (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)
f
Nausea (mm)
HL
LL
Control
Fasting BF 30 60 90 120 Pre-P Post-P 10 min Post-P 20 min Post-P After ADL
Time points (min)

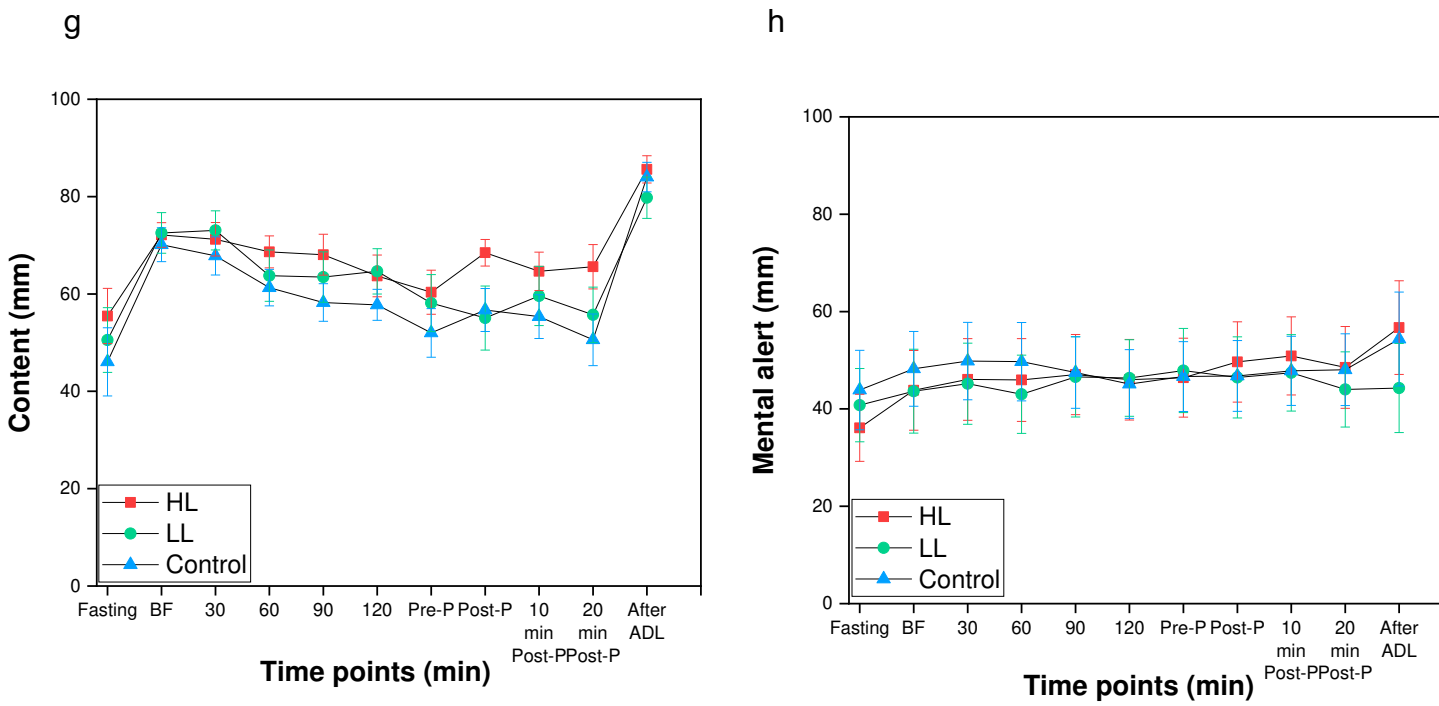


Fig. 3 Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) content, and (h) mental alert (h) over time, from fasting and breakfast (BF) until after *ad libitum* lunch (After ADL) including the relevant time points: pre-preload (Pre-P), post-preload (Post-P), 10 min after preload (10 min Post-P), 20 min after preload (20 min Post-P) during HL (high lubricating), LL (low lubricating) and Control conditions. Values are means and SEMs.

FIGURE 4

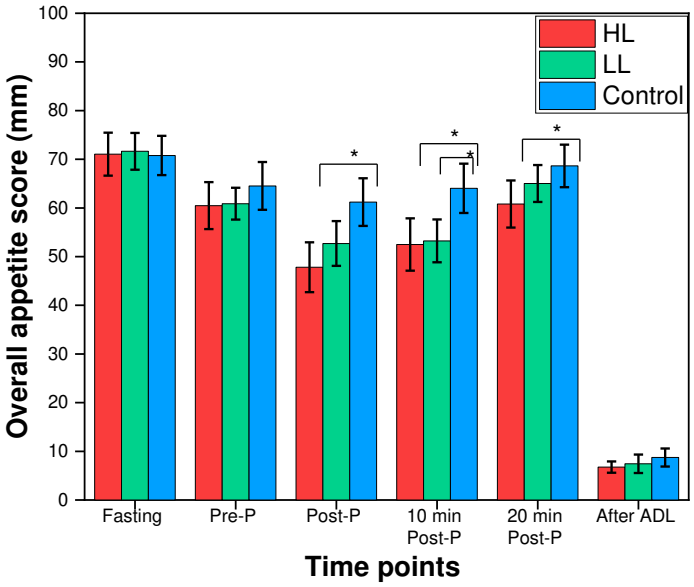


Fig. 4. Overall appetite suppression (mm) for fasting, pre-preload (Pre-P), post-preload (Post-P), 10 min Post-preload (10 min Post-P), 20 min Post-preload (20 min Post-P) and after *ad libitum* lunch (After ADL) for HL (high lubricating), LL (low lubricating) and Control conditions. Values are means and SEMs. The asterisks (*) denote a significant difference between conditions.

FIGURE 5

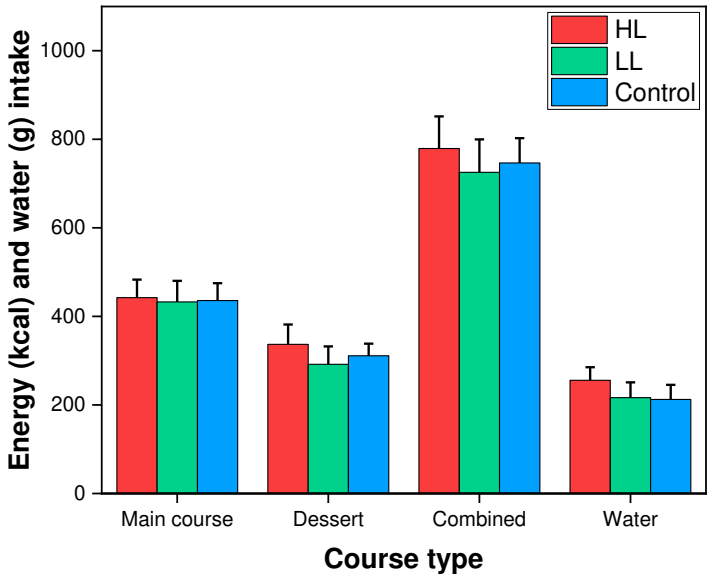


Fig. 5. Energy intake (kcal) and water intake (g) for HL (high lubricating), LL (low lubricating) and Control conditions. Values are means and SEMs.

FIGURE 6

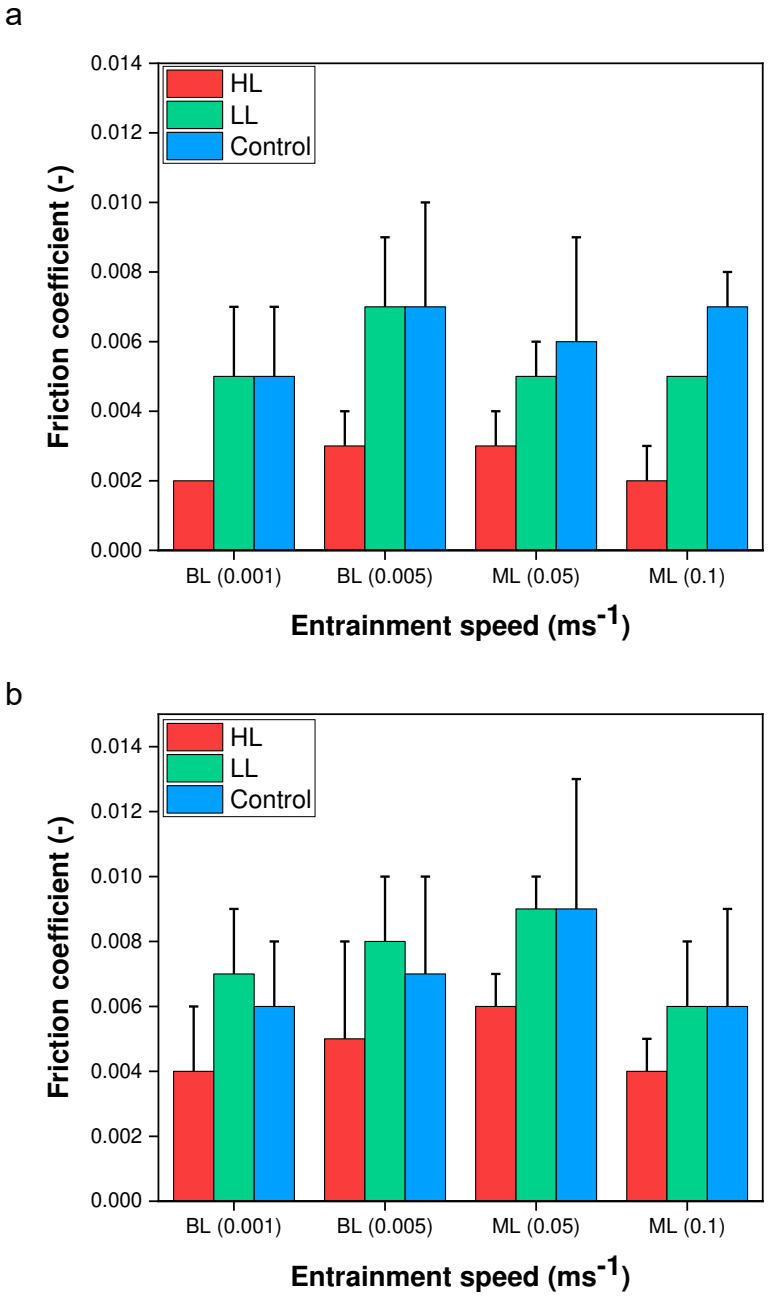
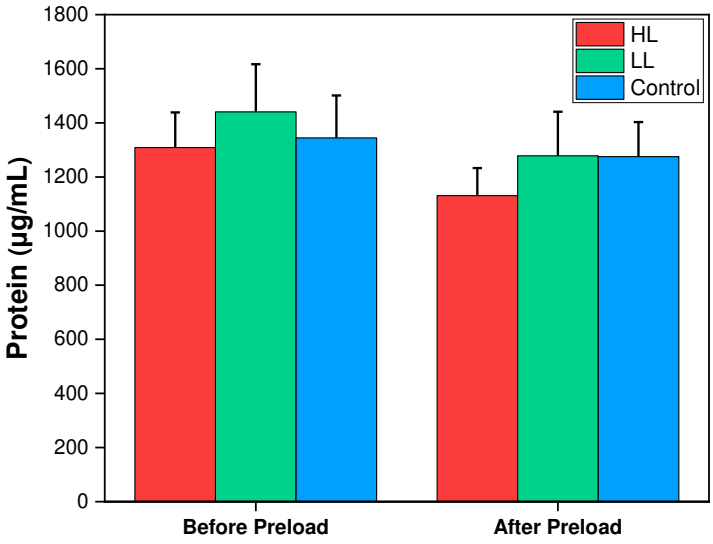


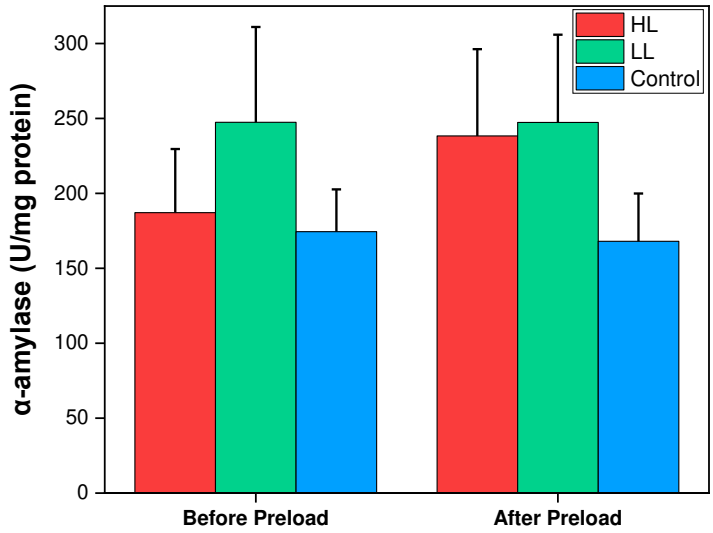
Fig. 6. Friction coefficient of saliva before preload (a) and after preload (b) in all three conditions of HL (high lubricating), LL (low lubricating) and Control at boundary (0.001 m s^{-1}; 0.005 m s^{-1} speed) and mixed (0.05 m s^{-1}; 0.1 m s^{-1} speed) lubrication regimes, n=4 (after removing outliers). Values are mean and error bars of means (SEMs). BL = boundary lubrication regime, ML = mixed lubrication regime. A lower friction coefficient represents higher lubrication performance of saliva.

FIGURE 7

a


Protein (µg/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
HL
LL
Control
Before Preload
After Preload

b


α-amylase (U/mg protein)
300
250
200
150
100
50
0
HL
LL
Control
Before Preload
After Preload

c

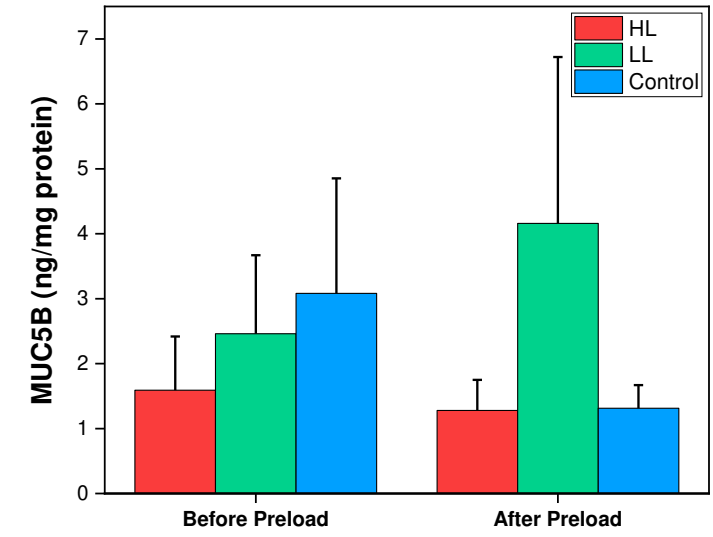

MUC5B (ng/mg protein)
7
6
5
4
3
2
1
0
HL
LL
Control
Before Preload
After Preload

Fig. 7. Total protein (μg/mL) (n=17) (a), α-amylase (U/mg protein) (n=17) (b), and MUC5B (ng/mg protein) (n=9) (c) in saliva for HL (high lubricating), LL (low lubricating) and Control conditions before preload and after preload. Values are means and error bars represent standard error of means (SEMs).

Effects of oral lubricity on satiety, satiation and salivary biomarkers in model foods: A pilot study

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Supplementary Table S1. Palatability of the preloads (HL, LL and Control) measured on a 100-mm VAS scale (n=17).

	HL	LL	Control	p-value[1]
Texture	37±25	43±32	56±27	n.s.
Sweetness	25±22	15±17[a]	41±23[b]	$p < 0.005$
Flavour	26±22	25±24	41±28	n.s.

[1]The lower-case letters (subscripts) denote a significant difference between preloads ($p < 0.05$). Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table S2. Area under the curve (AUC) for appetite ratings (mm) over time for subjects eating HL (high lubricating), LL (low lubricating) or Control preloads, n=17 (mean + SEM).

	HL	LL	Control	p-value[1]
Hunger	8944 ± 874	8548 ± 893	9253 ± 756	n.s.
Fullness	11369 ± 921	10423 ± 881	10160 ± 828	n.s.
Desire to eat	9418 ± 1055	8562 ± 1007	9505 ± 794	n.s.
PFC	10029 ± 961	9069 ± 839	10164 ± 853	n.s.
Thirst	10556 ± 1179	10441 ± 1244	11037 ± 1135	n.s.

[1] Letter n.s. denotes a non-significant difference between the preloads.

Supplementary Table S3. Coefficient of friction[1] of saliva in all three conditions (HL, LL and Control) before preload (a) and after preload (b) at two boundary lubricating regime (0.001 ms^{-1}; 0.005 ms^{-1}) and two mixed lubricating regime (0.05 ms^{-1}; 0.1 ms^{-1}), n=4 (mean + SD).

a	**Coefficient of friction of saliva before preload**							
	Boundary lubricating regime				**Mixed lubricating regime**			
	0.001 m s^{-1}		0.005 m s^{-1}		0.05 m s^{-1}		0.1 m s^{-1}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HL	0.002	0.000	0.003	0.001	0.003	0.001	0.002	0.001
LL	0.005	0.002	0.007	0.002	0.005	0.001	0.005	0.000
Control	0.005	0.002	0.007	0.003	0.006	0.003	0.007	0.001
b	**Coefficient of friction of saliva after preload**							
	Boundary lubricating regime				**Mixed lubricating regime**			
	0.001 ms^{-1}		0.005 m s^{-1}		0.05 m s^{-1}		0.1 ms^{-1}	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
HL	0.004	0.002	0.005	0.003	0.006	0.001	0.004	0.001
LL	0.007	0.002	0.008	0.002	0.009	0.001	0.006	0.002
Control	0.006	0.002	0.007	0.003	0.009	0.004	0.006	0.003

[1]No significant differences were found for friction coefficient for saliva at any entrainment speed between conditions neither before preload or after preload (HL, LL, and Control).

Supplementary Table S4. Pearson's correlations between appetite ratings, energy intake, tribology of saliva and salivary biomarkers, after preload (n=4, MUC5B was not included due to insufficient data). Green colour indicates positive and orange colour a negative correlation with $p < 0.05$ in light colours and $p < 0.01$ in the darker shades.

		Appetite ratings					Energy and water intake				Tribology of saliva							Salivary biomarkers	
		Hunger	Fullnes	Desire to eat	Pros. Consum.	Thirst	Main	Desert	Combined	Water	ES300	ES200	ES100	ES50	ES10	ES5	ES1	Protein	Amylase
Appetite ratings	Hunger	1																	
	Fullnes	-0.922	1																
	Desire to eat	.967*	-.988*	1															
	Pros. Consum.	.987*	-0.949	.986*	1														
	Thirst	0.131	-0.494	0.379	0.257	1													
Energy intake and water	Main	-0.755	0.727	-0.717	-0.673	-0.021	1												
	Dessert	0.607	-0.633	0.673	0.714	0.447	0.030	1											
	Comined	0.291	-0.325	0.365	0.419	0.405	0.384	0.934	1										
	Water	0.674	-0.908	0.836	0.738	0.792	-0.585	0.530	0.281	1									
Tribology of saliva	ES300	0.565	-0.800	0.706	0.581	0.670	-0.747	0.168	-0.112	0.922	1								
	ES200	0.812	-.966*	0.918	0.839	0.609	-0.761	0.477	0.169	.966*	0.927	1							
	ES100	0.881	-.991**	.962*	0.903	0.534	-0.771	0.540	0.223	0.937	0.873	.991**	1						
	ES50	0.894	-.995**	.971*	0.918	0.524	-0.760	0.568	0.253	0.931	0.854	.986*	.999**	1					
	ES10	.955*	-.995**	.998**	.975*	0.415	-0.735	0.647	0.335	0.863	0.747	0.940	.976*	.984*	1				
	ES5	0.474	-0.778	0.678	0.557	0.908	-0.433	0.450	0.261	.970*	0.904	0.880	0.824	0.813	0.714	1			
	ES1	0.574	-0.835	0.741	0.620	0.782	-0.639	0.329	0.076	.975*	.980*	0.944	0.893	0.878	0.779	.968*	1		
Salivary biomarkers	Protein	0.709	-0.758	0.785	0.808	0.507	-0.132	.985*	0.862	0.660	0.330	0.623	0.679	0.703	0.766	0.568	0.476	1	
	Amylase	-0.202	-0.193	0.047	-0.106	0.903	0.042	0.025	0.038	0.586	0.609	0.393	0.278	0.255	0.097	0.766	0.667	0.086	1