



Effect of *in vitro* food oral coating and lubricity on satiety: A randomized controlled trial using milk protein beverages

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ABSTRACT

We investigated the effects of complex textural attributes of food *i.e.* lubricity and oral coating, on appetite ratings, food intake, salivary and gut peptides for the first time. Milk protein-rich beverages (whey and casein) were instrumentally analyzed (tribology, viscosity and adsorption, latter representing oral coating) using *in vitro* measurements. Then these protein beverage preloads differing in their coating properties (low coating, medium coating and high coating) were assessed in two cross-over satiety trials (Study 1, n=37; Study 2, n=15; Total n=52). Fullness ratings increased in the high coating beverage condition ($p < .05$) only after 20 min with limited effects on other time points, suggesting a sporadic effect of oral coating on appetite ratings (n=37). There was a correlation between concentration of protein in saliva and appetite ratings; the higher the concentration of protein in saliva the lower the desire to eat ($r = -0.963$; $p < 0.05$) and prospective food consumption ratings ($r = -0.980$; $p < 0.05$). Human saliva was more lubricating after ingesting preload with high coating properties, thus explaining the results on appetite ratings. There was no effect of oral coating on energy intake and gut peptides (n=15), suggesting that complex textural attributes having influence on oral processing might not have any effect on the later parts of the satiety cascade. Oral coating/ lubricity appears to have a subtle and sporadic effect on appetite suppression, which needs further investigation with changing macronutrients/energy load and degree of coating/ lubricity.

1. Introduction

With the world facing a dramatic increase in obesity over the last decades [1-3], from the multitude of the strategies that seems to address it, food texture is often advocated to be capable of making a meaningful contribution to satiety and consequently weight management [4-6]. In particular, food texture has been shown to have a significant but short-term effect on the control of satiety [4,5] and daily caloric intake [7]. Although the current food design paradigm focuses on viscosity manipulation, portion size, form, and chewiness [8-15]; important constructs in the food textural manipulation such as the lubricity and particularly the mouth-coating properties of food have been rarely studied for their impact on satiety and satiation.

Oral lubrication refers to reduction in friction between two

interactive oral surfaces in relative motions such as tongue-palate [16-19] and in the context of this study – the more lubricating the food is, the friction between tongue and palate would be low. On the other hand, mouth-coating is defined as the residual food that remains attached to the oral surfaces after food is ingested [20]. Often food with higher degree of lubricity are associated with higher *mouth coating*, *i.e.* food spends more time in the oral cavity and gives a pasty perception [21]. These two constructs complement each other to some extent [22].

Recently, food varying in lubricating properties has been shown to have an effect on subjective appetite sensations [5] and snack intake [23]. The mechanism by which lubrication influences food intake is often hypothesized to be associated with mouth coating thereby extending the oro-sensory exposure time leading eventually to a significant reduction in food intake and better appetite control [5,23,24].

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In other words, soft/high lubricating gels were postulated to coat oral surfaces better when compared to gels with hard/low lubricating properties, resulting in reduced snack intake in a previous proof-of-concept snack trial [23]. In the case of lubricity, an association has been found in the literature between fullness and intrinsic oral lubricating properties of saliva as a result of ingesting the non-calorific hydrogels varying in their lubricating properties: the more lubricating the saliva, the higher were the ratings of fullness [5]. Human saliva is an inherently potent nature-engineered lubricant [25,26]. The interaction between food and saliva may influence the formation of a lubricious coating in the mouth, lengthening the oro-sensory exposure time, and in turn may trigger release of gut peptides, suppress appetite and reduce subsequent food intake. However, to date, oral coating has never been quantified in this context and remains to be studied in relation to satiety. Although, instrumental tribological analysis provides quantification of oral lubricity [27], it does not give quantification of real-time oral coating. A new technique *i.e.* quartz crystal-microbalance with dissipation monitoring (QCM-D) can address this gap. QCM-D can be used to emulate the actual coating behavior of the food products by using oral-mimicking surfaces, which have been employed in the current study for the first time and used as a manipulation tool to understand the effect of oral coating and lubrication on satiety [28,29].

Noteworthy that only non-calorific foods such as hydrogels have been used to test the efficacy of lubricity on satiety in literature [5]. Authors demonstrated that hunger and desire to eat ratings decreased whilst fullness ratings increased immediately after preload and remained decreased for 20 min, after preload in the high lubricating condition compared to control ($p < .05$). Nevertheless, such significant effect did not exist after controlling the values for baseline corrections. Therefore, it is of considerable interest to understand the combinatorial effect of food calories and textural manipulation *i.e.* mouth coating in a more realistic food material, and to test its effects on satiety. Our systematic review and meta-analysis have shown that influence of food texture on gut peptides has rarely been studied [4]. Only two studies were noted evidencing that low viscous, liquid food led to a decrease in ghrelin and increase in GLP-1 and PYY levels [30,31]. Hence, in addition to appetite ratings, objective food intake measurements, saliva characterization, various gut peptides, such as ghrelin [32], glucagon-like peptide (GLP-1) and peptide YY (PYY) that are considered to be involved in the regulation of appetite and satiety signalling [33] were measured. Note, satiety here was measured as a consequence of a prior ingestion (preload ingestion).

Dietary proteins such as whey and casein have been reported to have a greater satiating effect as compared to other macronutrients [34,35]. It is known that casein is a 'slow' protein, whilst whey protein is considered as a 'fast' protein mainly on the basis of gastric emptying [36,37]. Consequently, intake of whey protein results in a fast, but transient increase in plasma amino acids that peak in 40 min to 2 hours after its ingestion and returns to baseline values after 3 to 4 hours. In contrast, consumption of casein results in plasma amino acid concentrations to rise slowly, but lasts for at least 7 hours after its consumption [38,39]. Unlike previous literature, this study focused on investigating the immediate and short term (up to 1 hour) effects, therefore examining the first stages of satiety from an oral processing perspective. This was to eliminate, any effect of the protein type particularly in the later stages of satiety generation such as those related to gastric emptying and amino acid release. In addition, although these dairy proteins have been studied for their role on satiety, their lubricity and oral coating properties affecting satiety remain elusive in the literature. The reason for the use of two types of proteins was to achieve different levels of lubricity and mouth coating. To maintain high protein contents in the preloads, protein isolates were used.

Therefore, in the current study, we questioned whether oral coating has an effect on early stages of satiety (from the first bite to post-ingestive stage), from an oral processing perspective. Whey and casein protein beverages differing in their coating properties, achieved *via*

suitable processing were investigated for their satiating effect in two concurrent studies. Study 1 evaluated the effect of three levels of mouth coating: high coating (HC), medium coating (MC) and low coating (LC), together with a control (water), on appetite ratings, food intake, salivary biomarkers and oral lubricity of saliva post ingestion. Study 2 evaluated the effect of two levels of coating: LC and MC using only whey protein focusing on gut peptides with higher quantities of preload. We hypothesized that higher mouth coating will result in higher satiety. However, the influence of lubricity in such mouth coating cannot be fully ignored and is thus measured and discussed simultaneously.

2. Methods

2.1. Participants

Participants in both studies were healthy, 18–55 years old women and men with a BMI of 18.5 – 27.9 kg/m². The subjects were recruited from students and staff of the University of Leeds, UK. Participants were excluded if they were: smokers, had any oral infections/ diseases/ problems in chewing and swallowing, had chronic or acute health conditions that may affect the ability to sense, eat, digest or absorb food. Subjects using prescribed or non-prescribed medication that may interfere with the ability to sense, eat, digest or absorb food were excluded. Pregnant or lactating subjects, or subjects having a food allergy or intolerance were excluded. Also, subjects, who were on a special diet or were taking protein/ fibre supplements, or who could not tolerate protein beverages or had dairy allergies, had a BMI <18.5 kg/m² or >28 kg/m², or having blood-borne diseases were excluded. The studies were approved by University of Leeds MaPS and Engineering joint Faculty Research Ethics Committee (MEEC 16–046, November 2020).

A total of 37 participants (13 males and 24 females) completed study 1 (Table 1a). The age of the participants ranged from 18 to 47 years and the BMI ranged from 19.3 to 27.8 kg/m². A total of 15 participants (10 males and 5 females) completed study 2 (Table 1b). The age of the participants ranged from 18 to 33 years and the BMI ranged from 19.65 to 28.3 kg/m².

Sample size was calculated with G*Power version 3.1.9.3 (Heinrich-Heine-Universität Düsseldorf). The power analysis was a priori one, and was done to determine the number of participants needed for a small effect size ($f = 0.25$) across all four outcomes (as the manipulation of this study involved novel parameter *i.e.* coating properties of the beverages, there was not enough information in the literature in terms of the expected size effect). As such, according to G*Power calculation, 24 participants are required to identify a small effect size ($f = 0.25$, $\alpha = 0.05$ and $1-\beta = 0.80$), where (α) is the significance level and $1-\beta$ is the power, across the 4 groups (high coating, medium coating, low coating and control) with 4 outcome (appetite ratings, food intake, salivary biomarkers and lubricity of saliva), with outcomes varying from 3 to 5 measurements. We targeted to recruit 40 participants to account for any dropouts. The second study was a pilot one due to restricted time and

Table 1
Participants' characteristics^a.

Characteristics	Values
<i>a) Study 1</i>	
Male/Female	13/24
Age (years)	26.51 ± 6.18
Weight (kg)	67.3 ± 10.34
Height (m)	1.69 ± 0.08
BMI (kg/m ²)	23.47 ± 2.26
<i>b) Study 2</i>	
Male/Female	5/10
Age (years)	26 ± 3.7
Weight (kg)	69.9 ± 11.1
Height (m)	1.7 ± 0.09
BMI (kg/m ²)	23.9 ± 3.7

^a Values are means ± SDs.

resources of the project, therefore we targeted for 15 participants.

2.2. Design

Both of the studies were acute, randomized, counterbalanced, cross-over, within-subject and single-blinded, registered at ClinicalTrials.gov as NCT04868461. Participants in both studies were not told the exact aim of the study, instead they were informed that the aim of the study was to investigate the acceptance, pleasantness and taste perception of protein beverages. At the end of the studies, participants were verbally debriefed and the precise purpose of the studies was explained in more details. The studies took place at the University of Leeds, UK, School of Food Science and Nutrition Human trial unit: April – October 2021 for study 1, and February – April 2022 for study 2. Subjects gave their written informed consent before taking part in either of the studies and received £30 for the first study and £100 for the second study as a compensation for their time.

2.3. Session procedure

Before taking part in the studies, subjects were first screened for eligibility using an online health screening questionnaire.

Study 1. A schematic overview of the study protocol is presented in Fig. 1a. A total of 66 subjects were screened, of which 37 were included in the study and further analysis (26 did not meet the inclusion criteria,

3 withdrew from the study, see Supplementary Figure S1 CONSORT Flow Diagram – Study 1). Each participant was asked to come to the laboratory on four different occasions with a 7 day washout period in between each session. Participants were instructed to fast for 11 h (10.00 pm onwards) and to refrain from drinking (except water) for 24 h before each session. Alcohol consumption was prohibited. Each session lasted for 1.5 h. Participants were asked to come to the laboratory at 8.40 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). Participants then provided baseline appetite ratings on a 100-mm visual analogue scale (VAS), and a first sample of whole mouth saliva was collected. After that, at 9.00 am, they were given a fixed amount of preload (200 mL) – protein beverages differing in their mouth coating and lubrication properties or water (control). Participants were instructed to drink the whole amount of each preload. Immediately after the preload, participants rated their appetite, and the second sample of saliva was collected. Appetite was rated at every 10 min intervals for a duration of 30 min. Before the *ad libitum* breakfast (30 min after preload), the last sample of saliva was collected, and after consuming the breakfast participants completed the last appetite ratings. In total, appetite was rated at 6 time points: -10 min, 0 min, 10 min, 20 min, 30 min and 50 min. Saliva was collected at 3 time points: before preload,

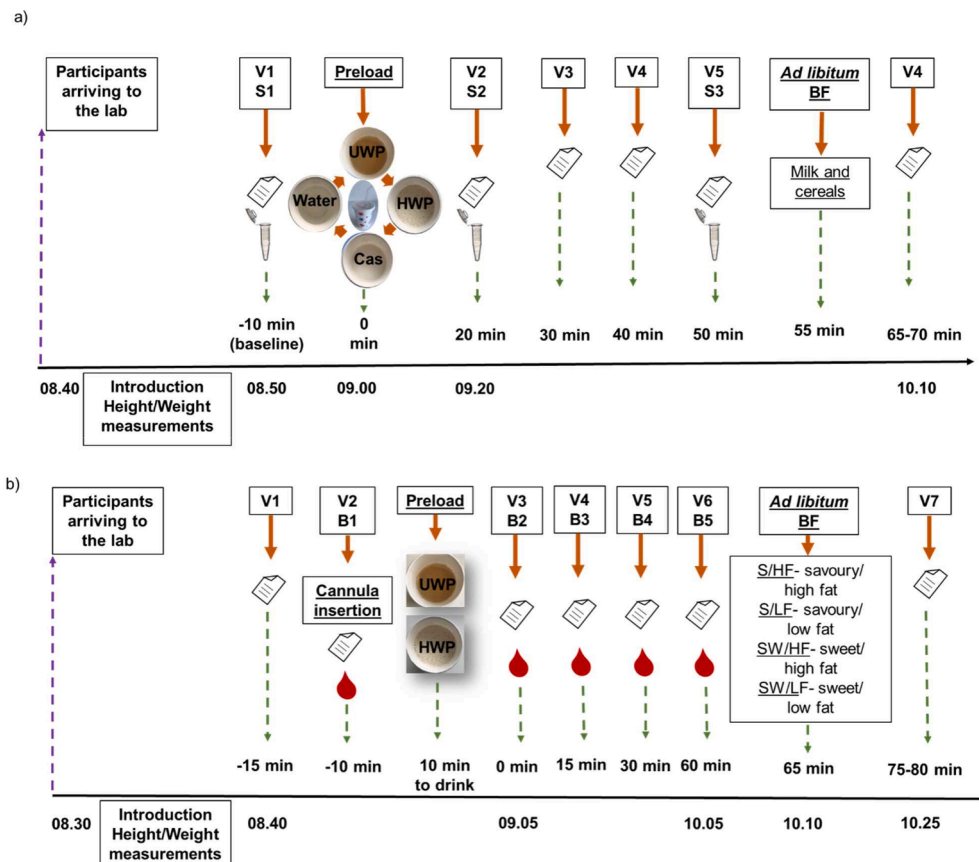


Fig. 1. Overview of the study protocols. **Study 1** - VAS (visual analogue scales) are represented by letter V, 6 in total (V1-V6). Collection of saliva is represented by letter S, 3 in total, for each visit (S1-S3). Preloads were banana-flavoured sweetened protein beverages containing 15 g protein per 100 g water (read the pictures of the preload/ beverages in clockwise direction) – UWP (unheated whey protein beverage), HWP (heated whey protein beverage), Cas (casein beverage) and control (water) served in a cup with the lid on and a straw (see the picture in the middle of the beverages). BF represents breakfast – *Ad libitum* BF. Each visit lasted for around 1.5 h; **Study 2** - VAS (visual analogue scales) are represented by letter V, 7 in total (V1-V7). Collection of blood is represented by letter B, 5 in total, for each visit (B1-B5). Preload were banana-flavoured sweetened protein beverages containing 15 g protein per 100 g water – UWP (unheated whey protein beverage) and HWP (heated whey protein beverage) served in a cup with the lid on and a straw (see the picture in the middle of the beverages in study 1). BF represents breakfast – *Ad libitum* BF. Each visit lasted for around 1 h 45 min.

immediately after preload and 30 min after preload. Also, oro-sensory exposure time for the preload and salivary flow rate at each time point of collection was measured. The *ad libitum* breakfast consisted of cereals (Wholegrain Malties and Wholegrain Brown Flakes, produced by Sainsbury's Supermarkets Ltd, London, UK), milk (Semi Skimmed Milk, produced in UK) along with water and tea or coffee (participants' choice). Participants were provided with a full container (1125.3 kcal/350 g) of Wholegrain Malties (cereals), a full container (990.7 kcal/330 g) of Wholegrain Brown Flakes (cereals) and a full jug (486 kcal/1000 g) of milk. They were asked to eat to a comfortable level of fullness and were told that more food could be provided if they wanted more. To make sure participants are provided breakfast they are familiar with and to diminish the effect of anything novel, milk and cereals were provided as the most common food eaten across the UK during breakfast time.

Study 2. A schematic overview of the study protocol is presented in Fig. 1b. A total of 45 subjects were screened, of which 15 were included in the study and further analysis (26 did not meet the inclusion criteria, 4 withdrew from the study, see Supplementary Figure S2 CONSORT Flow Diagram – Study 2). Each participant was asked to come to the laboratory on two different occasions with 7 days washout between sessions. Participants were instructed to fast for 11 h (10.00 pm onwards) and to refrain from drinking except water for 24 h before each session. Alcohol consumption was prohibited. Each session lasted 1 h 45 min. Participants were asked to come to laboratory at 8.40 am.

Similar to study 1, in the first session, weight and height were measured. Participants then provided baseline appetite ratings (-15 min) on a 100-mm visual analogue scale (VAS), after which a cannula was inserted in their forearm. Five minutes later, another VAS was provided by the participants and this was used to check for any effect of cannula insertion on the appetite responses. Immediately following this, a fasting (-10 min) blood sample (pre-preload) was collected and a fixed amount of preload – 400 mL (whey protein beverages of varying coating properties) was given to participants to drink it all. Participants were instructed to drink the beverage within 10 min and a stopwatch was placed in front of them with a 10 min count down time. After finishing the preload, the third VAS was given to participants and second blood sample (post-preload) was collected (0 min). After this, VAS and blood were collected every 15 min for a duration of 30 min. The next VAS and blood was collected after a further 30 min had elapsed (at 60 min). The last VAS was collected after the *ad libitum* breakfast. In total, appetite was rated at 7 time points: -15 min, -10 min (after cannula insertion), 0 min, 15 min, 30 min, 60 min and after *ad libitum* breakfast. Blood was collected at 5 time points: -10 min (pre-preload/fasting), 0 min (post-preload), 15 min, 30 min and 60 min. The *ad libitum* breakfast consisted of: 1. savoury/high fat food (S/HF) – plain bagel (New York Bakery Co., produced by Waitrose and Partners Meanwood, Leeds, UK) with cream cheese (Philadelphia Original Soft Cheese, produced by Waitrose and Partners Meanwood, Leeds, UK); 2. savoury/low fat food (S/LF) – crackers (Jacob's Crackers, produced by Waitrose and Partners Meanwood, Leeds, UK) with cottage cheese (Morrisons Low Fat Cottage Cheese, produced by Wm Morrisons Supermarkets PLC, Bradford, UK); 3. sweet/high fat food (SW/HF) – chocolate and butter pastries (Morrisons Chocolate and Butter Brioche Rolls, produced by Wm Morrisons Supermarkets PLC, Bradford, UK); 4. sweet/low fat food (SW/LF) – apples and pineapples (Morrisons Pink Lady Apples and Pineapples, produced by Wm Morrisons Supermarkets PLC, Bradford, UK). The breakfast was served along with water, milk and tea or coffee (at participants' choice). Participants were provided with 282 kcal/141 g of bagel and cream cheese (S/HF), 194 kcal/112 g of crackers and cottage cheese (S/LF), 621 kcal/180 g chocolate and butter brioche (SW/HF), 151 kcal/350 g of fruits (SW/LF), and 243 kcal/500 g of milk. In total, participants were provided 1491 kcal for breakfast. They were asked to eat to a comfortable level of fullness and were told that more food could be provided if they wanted.

2.4. Preload preparation and instrumental measurements

Study 1. Four preloads were tested in this study: unheated whey protein solution (referred to as UWP), heated whey protein solution (referred to as HWP- heating was used to achieve different levels of mouth coating), casein solution (Cas), and water which acted as a control. Whey protein isolate and casein were purchased from MYPROTEIN (Manchester, UK). The powders were bought unflavoured, and were subsequently flavoured using banana essence in our laboratory. The flavour was purchased from Special Ingredients (Special Ingredients Ltd, Chesterfield, UK). The beverages were sweetened by adding small amount of stevia granulated non-nutritive sweetener purchased from a local supermarket (Leeds, UK). On average, a minimum of 10 g of whey/casein protein per 100 g of water is required to detect an effect on satiety [40]. Consequently, each protein beverage in our study contained 30 g of protein powder to a total of 200 mL solution, *i.e.* 15 g per 100 g water (see Table 2 Study 1 for beverages recipe). The control was 200 mL water which contained the sweetener and banana flavour in an appropriate proportion to match the taste and flavour of the protein beverages based on a small pilot trial. The whey and casein protein powders were dissolved in distilled water and were left to stir on a magnetic stirring plate for 2 h until a complete hydration was obtained. For the heated whey protein beverage, the protein solution was heated at 80 °C for 8.5 min in a water bath at 80 rpm (OLS26, Aqua Pro, Grant Instruments, Royston, UK). Before serving it to the participants, the HWP beverage was blended for 30 sec with a hand blender (Braun, Germany) and served at room temperature similar to the other beverages or water.

The protein beverages and the control (water) were poured into opaque cups. Each cup had a lid on and the participant drank the preload through a straw. Each participant received a total amount of 200 mL of each protein beverage or control (water) on different testing days. The preloads were prepared a day prior to each test day and kept in the fridge overnight at 4 °C and served to the participants at room temperature. All the preloads, except water contained around 105 kcal (see Table 3 Study 1 for nutritional composition). Water was selected as a control due to its protein free content and lack of coating/lubricating properties.

Study 2. Two preloads were tested in this study: whey protein solution (unheated, UWP) and whey protein solution (heated, HWP). In order to exclude any effect of protein type and focus on texture solely, the whey protein beverages have been chosen. The ingredients were identical to those used in the study 1, with the same preparation method. However, the amount (kcal) of the beverages in this study was doubled to account for blood collection. The gut peptides need higher calories load to see an increase/decrease [41]. Each protein beverage contained 60 g of protein powder to a total of 400 mL solution, *i.e.* 15 g per 100 g water (see Table 2 Study 2 for beverages recipe and Table 3 Study 2 for

Table 2
Recipe of preloads – Study 1 and Study 2.

	UWP ^a	HWP ^b	Cas ^c	Control (Water)
Study 1				
Protein (g)	30	30	30	–
Water (g)	169	169	169	197.9
Flavour –banana (mL) ^d	0.5	0.5	0.5	2
Stevia sweetener (g)	0.5	0.5	0.5	0.1
Total (g)	200	200	200	200
Study 2				
Protein (g)	60	60	–	–
Water (g)	338	338	–	–
Flavour –banana (mL) ^d	1	1	–	–
Stevia sweetener (g)	1	1	–	–
Total (g)	400	400	–	–

^a UWP (unheated whey protein)

^b HWP (heated whey protein)

^c Cas (Casein)

^d Firstly, 0.5 g of banana flavour was diluted in 50 g of water, and then 2 mL of the diluted solution was added to the control (water).

Table 3
Nutritional information of the preloads – Study 1 and Study 2.

Food item	Weight (g)	Energy (kcal)	Protein (g)	Carbohydrate (g)	Sugar (g)	Fat (g)
Study 1						
UWP ^a / HWP ^b	30	119.7	27	0.75	0.75	0.009
Cas ^c	30	105	24.6	1.41	1.38	0.21
Control (Water)	200	–	–	–	–	–
Study 2						
UWP ^a / HWP ^b	60	239.4	57	1.5	1.5	0.18

^a UWP (unheated whey protein)

^b HWP (heated whey protein)

macronutrient composition of the beverages). There was no significant difference in palatability in terms of texture, sweetness and flavour, likewise on liking and wanting ($p > .05$) between the conditions in both studies (see Table 4a for study 1 and Table 4b for study 2).

Viscosity, lubricity and mouth coating of the preloads were measured using rheometer, tribometer and QCM-D, respectively. The apparent viscosity of the beverages was measured with a rheometer (Kinexus Ultra+, Malvern Instruments Ltd, Worcestershire, UK) using a plate-plate geometry (diameter 60 mm) with a gap size of 0.5 mm at shear rates ranging from 0.01 to 1000 s^{-1} at 37 °C. The viscosity data at highest shear rates (1000 s^{-1}) was used to scale the tribology data [42, 43]. This was done to remove the confounding factor of viscosity from lubricity. For lubricity of the preloads or the saliva, 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 oral surfaces for the oral tribology measurements (surface roughness of the PDMS tribopairs, $R_a < 50$ nm). For the mouth coating analyses, PDMS-coated QCM-D (Quartz crystal microbalance with dissipation) sensors were designed to emulate oral surfaces [28,29,44-46]. For the preparation of PDMS-coated QCM-D sensors, briefly, 100 μ L of 0.5 wt% PDMS solution was placed on the substrate and was spin-coated at 5000 rpm speed for 60 s. QCM-D can simultaneously measure the shifts in frequency and dissipation at different overtones occurring during adsorption and provide wealthy information on the mass of the adsorbing film corresponding to coating. All the protein solutions (Cas, UWP and HWP) were supplied into QCM-D chamber containing the PDMS sensors by a peristaltic pump with a flow rate of 100 μ L/min at 25 °C. The first step was to inject water until a stable baseline was observed.

Table 4
Palatability of the preloads (Control, HC, MC and LC) measured on a 100-mm VAS scale (n=37) (means \pm SDs) for Study 1 (a); Palatability of the preloads (MC and LC) measured on a 100-mm VAS scale (n=15) (means \pm SDs) for Study 2.

a)	Control	HC	MC	LC	p-value ¹
Texture	47.5 \pm 26.7	51.1 \pm 25	54.4 \pm 26.7	56.3 \pm 25.7	n.s.
Flavour	34.2 \pm 25.9	28.8 \pm 23.6	33.2 \pm 23.8	40.4 \pm 28	n.s.
Sweetness	16.1 \pm 21.8	18.2 \pm 17	20.6 \pm 20.8	22.9 \pm 18.6	n.s.
Liking	35.5 \pm 23.2	36 \pm 23.5	36.1 \pm 28.1	42.5 \pm 27.9	n.s.
Wanting	35.6 \pm 26.8	35.7 \pm 24.8	34.9 \pm 27.7	39.7 \pm 28.6	n.s.
b)	MC	LC	p-value ¹		
Texture	49.4 \pm 27.9	58.2 \pm 20.6	n.s.		
Flavour	47.1 \pm 23.6	39 \pm 25.2	n.s.		
Sweetness	25 \pm 22.7	21.9 \pm 20.4	n.s.		
Liking	44.4 \pm 24.9	44 \pm 25.1	n.s.		
Wanting	41.7 \pm 24.7	38.7 \pm 20.6	n.s.		

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

Subsequently, for the adsorption of protein (0.1 mg/mL) solutions on PDMS surfaces, solutions were injected into the system for two hours, allowing the system to equilibrate, followed by rinsing in water for 30 min. The data were fitted using the Voigt model for viscoelastic solids (namely, ‘‘Smartfit Model!’’) by Dfind software (Q-Sense, Sweden) to obtain the mass of the hydrated protein layers, in other words oral coating. For improved visualization only the 5th overtone has been used in graphs (see frequency shifts in Figure S3a, Supplementary Information and dissipation shifts in Figure S3b, Supplementary Information) plots. A minimum of three replicates were measured for each beverage sample for all three instrumental analysis – viscosity, lubricity and coating and a detailed method and protocol for all three measurements are described in our previous studies [5,27-29].

2.5. Appetite ratings

Study 1. Participants rated their appetite at 6 time points using a 100-mm VAS scale, which has been shown to be valid and reliable scale used for appetite research [47,48]; the scale anchor points ranged from ‘not at all’ to ‘extremely’. Time points were: -10 min, 0 min, 10 min, 20 min, 30 min and 50 min on each testing day. Rating scales included hunger, fullness, desire to eat, prospective food consumption (how much food they think they could consume) and thirst. Ratings were also performed for mood - content, mental alertness, and nausea, knowing that there is a correlation between mood and food intake [49-51]. In addition, participants rated wanting and liking immediately after drinking the preloads only, as well as palatability and acceptability of the preloads (including control) in terms of texture, flavor, and sweetness using the same 100-mm VAS scale, where anchor points ranged from ‘not at all’ to ‘extremely’. The time point of -10 min will be referred to ‘before preload’ and 0 min to ‘after preload’ throughout this article.

Study 2. Participants rated their appetite at 7 time points using the same 100-mm VAS as in study 1. The time points were: -15 min, -10 min (after cannula insertion), 0 min, 15 min, 30 min, 60 min and after *ad libitum* breakfast. Like in study 1, participants rated wanting and liking, as well as palatability and acceptability of the preloads in terms of texture, flavor, and sweetness immediately after drinking the preloads only, using the same 100-mm VAS scale, where anchor points ranged from ‘not at all’ to ‘extremely’. Blood was collected at 5 time points: -10 min (pre-preload/fasting), 0 min (post-preload), 15 min, 30 min and 60 min. The time point of -10 min will be referred to ‘before preload’ and 0 min to ‘after preload’ throughout this article.

2.6. Energy intake

For both studies, *ad libitum* foods and beverages were weighed (to the nearest 0.1 g) prior to being served to the participants and were re-weighed after the participant had finished eating to determine the amount of food and beverage actually consumed by each participant. For completeness in reporting, the food intake was initially calculated in grams and the weights of carbohydrate, protein and fat were converted to energy using appropriate factors (3.75, 4 and 9).

2.7. Oro-sensory exposure time and salivary flow rate- study 1

Oro-sensory exposure time of the preloads was measured using a countdown timer (Fisher Scientific Ltd, UK). On average, the time for these beverages to be drunk can vary between 5 to 15 min [40,52]. Participants were instructed to press 'Start' on the timer when they began to drink the preload (at their first sip) and press 'Stop' when they finished drinking; they were instructed the procedure would not last more than 15 min. Note, this is not a direct measurement of oro-sensory exposure time, it is a measure of a duration for the preload to be consumed, which gives an indirect approximation of the oro-sensory exposure time.

Salivary flow rate was measured every time saliva was collected (at three time points on each visit) before and after preload and 30 min after preload. The same countdown timer (Fisher Scientific Ltd, UK) was used starting from 5 min. Again, participants were instructed to 'Start' the timer when they first started spitting into the tube and 'Stop' when they finished (at \approx 2 mL of saliva); they were told the procedure should not take more than 5 min.

2.8. Lubrication properties and viscosity of human saliva – study 1

As illustrated in Fig. 1a, saliva was collected at three time points. Participants were asked to spit 2 mL of saliva into a pre-cooled tube. The collected saliva from each participant at three different time points was pre-processed according to previously reported method [5,53]. Briefly, the samples were centrifuged for 5 min at 4000 g and the precipitate containing cell debris was discarded. Approximately, 2 mL of the supernatant was made up to 4 mL volume using pre-chilled 20 mM phosphate buffer (pH 7) (i.e. 16 vol% unstimulated whole human saliva) [53] and was stored at -80 °C until analysis of total protein, α -amylase and MUC5B, respectively. Tribology and rheology were performed to determine the lubrication and viscosity properties of pooled saliva before, after preload and 30 min after preload (immediately before the *ad libitum* breakfast). The friction coefficient results are presented as a function of product of entrainment speed, scaled to viscosity. Friction forces in the presence of saliva collected at different time points, and after consuming preloads or controls, were compared at boundary (BL, 0.0001 Pa m) and mixed (ML, speed of 0.005 Pa m, 0.01 Pa m) lubrication regimes [54].

2.9. Biochemical assays of salivary biomarkers - study 1

Saliva from individual were collected and analysed for oral biomarkers such as protein, mucins (MUC5B), salivary amylase as well as salivary lubricity to unravel oral mechanism behind satiety (if any) effects observed. Supernatants (i.e. unstimulated whole human saliva, diluted 1:1 v/v with water) collected in 250 μ L 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). 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 μ g/mL for protein.

2.10. Biochemical assays of gut peptides - study 2

Blood samples were collected to analyse for gut peptides as biomarkers of later stages of satiety. Blood samples were collected using cannulation by two trained personnels. A total of 25 mL (5 mL on each time point – 5 time points per session) of blood was collected on each visit (50 mL for whole study) (Fig. 1b). Out of the 5 mL blood, 3 mL were

placed in pre-cooled tubes for gut peptide analysis and 2 mL in pre-cooled tubes for glucose analysis. Immediately after collection, blood was centrifuged at 1500 g for 10 min at 4 °C. Afterwards, 250 μ L of plasma (for each appetite biomarker/ gut peptide and glucose) was placed in a 2 mL Eppendorf tube and was stored at -80 °C until biochemical analysis.

The plasma samples were analysed by BIOIATRIKI Central Lab (Athens, Greece). The analysed gut peptides were total ghrelin, GLP-1 (Glucagon-like peptide-1) and PYY (Peptide tYY). Total ghrelin was analysed using RayBio® Human Ghrelin ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No.ELH-GHRL-1). GLP-1 was analysed using RayBio® Human GLP-1 ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No. ELH-GLP137-1), and PYY was analysed using RayBio® Human PYY ELISA kit (RayBiotech, Norcross GA, USA) (Cat. No.ELH-PYY-1). The plasma level of glucose was determined by using Hexokinase test (enzymatic ultra-violet) (ROCHE, Basel, Switzerland) using a HITACHI cobas 800c system/701 analyser.

The protocol was the same for all gut peptides and glucose analysis. The assays employed an antibody specific for human GHRL/Ghrelin, GLP-1 and PYY coated on a 96-well plate. Standards and samples were pipetted into the wells and GHRL/Ghrelin, GLP-1 and PYY present in a sample were bound to the wells by the immobilized antibody. The wells were washed and biotinylated anti-human GHRL/Ghrelin, GLP-1 and PYY antibody was added. After washing away unbound biotinylated antibody, HRP-conjugated streptavidin was pipetted to the wells. The wells were again washed, a TMB substrate solution was added to the wells and color develops in proportion to the amount of GHRL/Ghrelin, GLP-1 and PYY bound. The stop solution changed the color from blue to yellow, and the intensity of the color was measured at 450 nm.

2.11. 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 each time point. Overall appetite ratings, food intake, salivary and gut peptide, and lubricating capacity of human saliva were measured after ingesting the preloads. The differences in palatability nausea, mental alertness and content mood after ingesting the preloads were also assessed by repeated measures ANOVA. In Study 1, a 4 \times 5 level factorial repeated measures ANOVA was used to examine the main effect on appetite ratings of the intervention condition (Control, HC, MC, LC), time (post-preload, 10 min, 20 min, 30 min after preload and after *ad libitum* breakfast) and condition*time interaction. In study 2, to check if cannula insertion affected the appetite ratings, there were 2 baseline time points – one before cannula insertion and one after. After comparing the means between these 2 time points, using paired *t*-test, the first one was selected for further analysis since there was no significant difference between them. Therefore, in study 2, a 2 \times 5 level factorial repeated measures ANOVA was used to examine the main effect of the intervention condition (MC, LC), time (post-preload, 15 min, 30 min, 60 min after preload and after *ad libitum* breakfast) and condition*time interaction on appetite ratings. Analysis of appetite ratings and gut peptides were also compared after controlling for baseline ratings using the analysis of difference from baseline. As the textural manipulation of food (protein beverages) in this study was quite subtle and oral coating is used as a construct for the first time, there is uncertainty about the immediate post-preload 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 and discussed. Where the assumption of sphericity had been violated, indicated by Mauchly's test, Greenhouse-Geisser corrected tests are reported. Significant differences were calculated by Bonferroni corrected post-hoc *t*-tests and was set at $\alpha < 0.05$ level. Area under the curve (AUC) analysis of appetite data has

also been performed on both study 1 and study 2. Data were plotted using the software Origin® (OriginPro 2018; OriginLab Corporation, Northampton MA, USA).

3. Results

3.1. Preload characteristics

For lubricity (expressed as friction coefficient at product of entrainment speed and high shear viscosity), the most lubricating beverage was heated whey protein (HWP) followed by unheated whey protein (UWP) and the least lubricating was casein (Cas) independent of the regimes (Fig. 2a). It is worth noting that BL refers to the regime where the speed is low and the lubricity is attributed to the surface properties of the proteins rather than viscosity, whilst in the ML there is a continuous film of protein most likely formed between the oral palate and the tongue [19]. Of more importance, it is clear that HWP could form a lubricating film independent of the speeds, which might be attributed its surface properties.

As shown in Fig. 2b, the adsorbed mass is higher for Cas, followed by heated whey protein HWP and unheated whey protein UWP. Noteworthy that QCM-D provides a quantitative measure of adsorbed mass

on a solid as well as mucin-coated surface and often used as proxy for measuring oral coating for oral and pharmaceutical applications to measure mucoadhesion [26,55-57]. However, to our knowledge, relationship between QCM-D-derived adsorbed mass and intensity of sensorial oral coating derived from a trained sensory panel remains to be reported. Nevertheless, considering oral mimicking surfaces are used in this study to measure adsorbed mass, we will refer this “adsorbed mass” data as a measure of coating in mouth and use the term “oral or mouth coating” henceforth. In other words, Cas has the highest mouth coating behaviour (Fig. 2b) among the experimental samples followed by HWP with medium mouth coating behaviour and UWP has a low coating behaviour. Summarising the textural measurements, the beverages presented the following properties: Cas (casein) –low lubricating/high coating, UWP (unheated whey protein) –medium lubricating/low coating and HWP (heated whey protein) –high lubricating/medium coating. Taking into account the most novel aspect of textural attributes *i.e.* coating perspectives, preloads will be henceforth called as HC – high coating, MC – medium coating and LC – low coating.

3.2. Appetite ratings

Figs. 3 and 4 show the appetite ratings over time in study 1 and study

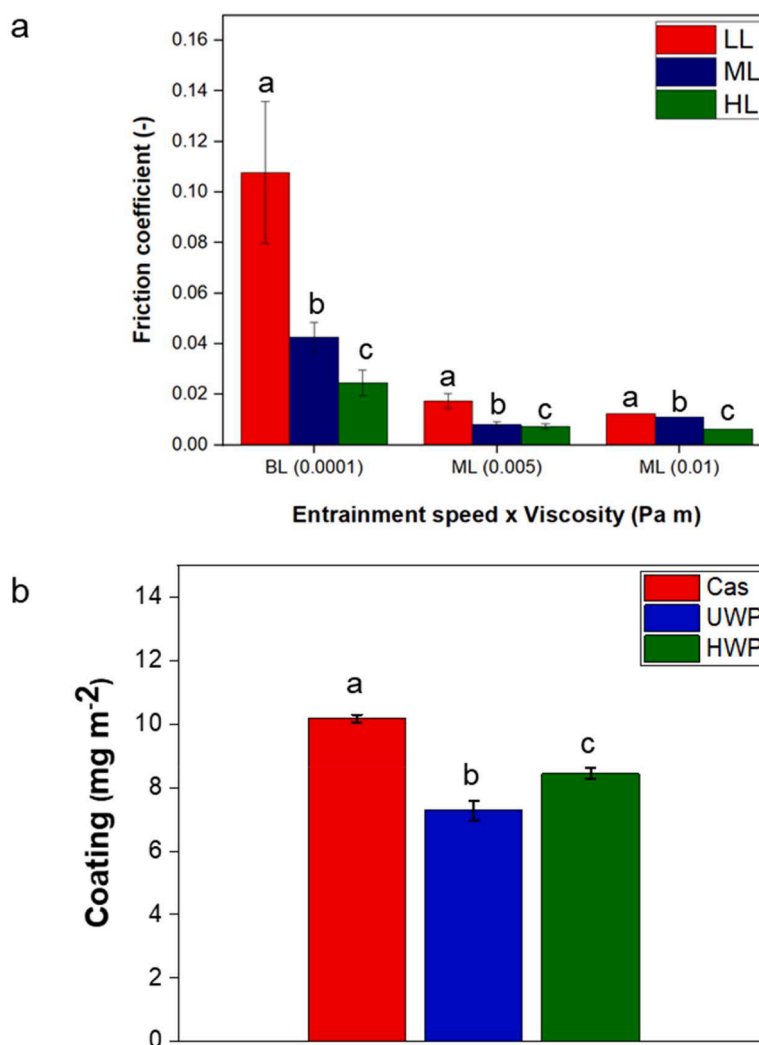


Fig. 2. Mean friction coefficient as a function of entrainment speed scaled to viscosity (c) at boundary (0.0001 Pa m) and mixed (0.005; 0.01 Pa m) lubrication regimes and (b) coating expressed through adsorbed mass per unit area of the beverages included in the study (Cas – Casein, unheated whey protein – UWP and heated whey protein – HWP). Values are means and error bars represent standard error of means (SEMs). Different letters denote a significant difference between beverages ($p < .05$). BL = boundary lubrication regime, ML = mixed lubrication regime. A lower friction coefficient represents higher lubrication performance of the beverages. All measurements were carried out at 37 °C.

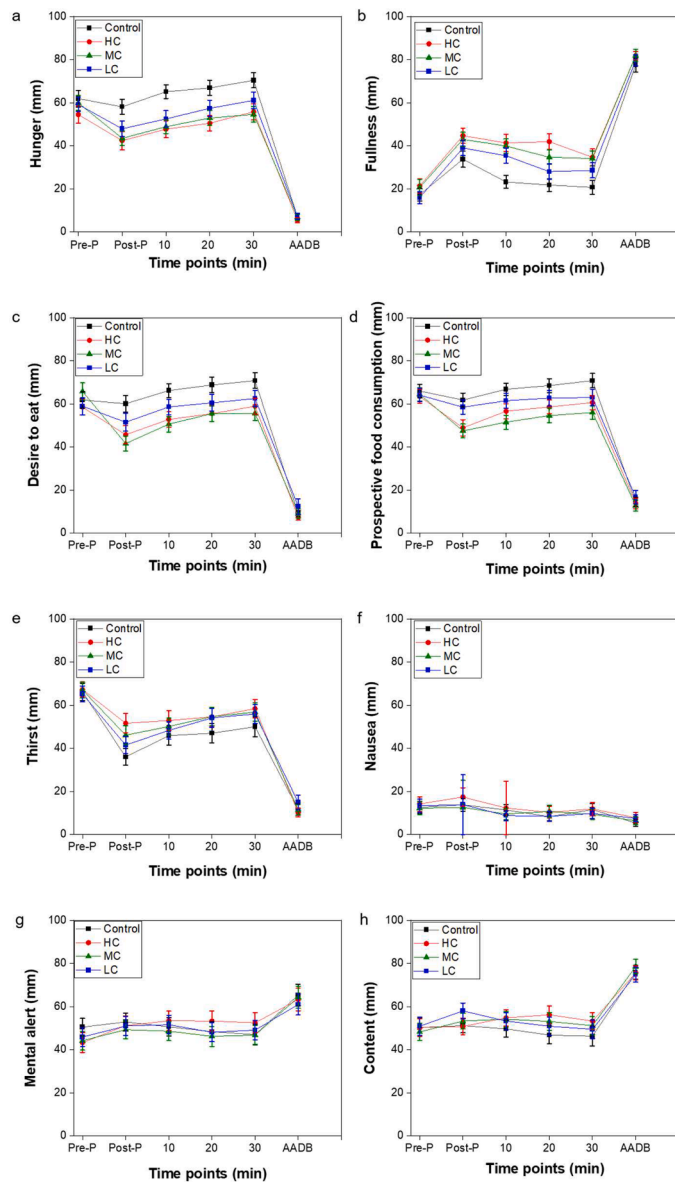


Fig. 3. Study 1. Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) mental alert, and (h) content over time: pre-preload (Pre-P), post-preload (Post-P), 10 min, 20 min, 30 min and after *ad libitum* breakfast (AADB) in Control, HC (high coating), MC (medium coating) and LC (low coating) conditions. Values are means and SEMs (n=37).

2 respectively. Both figures indicate a decrease in hunger (Figs. 3a and 4a), desire to eat (Figs. 3c and 4c), prospective food consumption (Figs. 3d and 4d) and thirst (Figs. 3e and 4e) immediately post-preload and with a slight increase 10 min post-preload and reaching the baseline ratings at 30 min post-preload in study 1 and at 60 min post-preload in study 2. Opposite trend can be observed for fullness (Figs. 3b and 4b) for both studies where fullness increased immediately post-preload and with a slightly decrease 10 min post-preload and reaching the baseline ratings at 30 min post-preload in study 1 and at 60 min post-preload in study 2. We also assessed for the feelings of nausea, as well as the mood of participants (mental alertness and content) after ingesting the preloads, in both studies (see means and SDs for nausea, content and mental alertness in Supplementary Table S1 – study 1 and Supplementary Table S2 – study 2). A plateau-like pattern for nausea (Figs. 3f and 4f), content (Figs. 3h and 4g) and mental alert (Figs. 3g and 4h) was observed, with a slight increase in content and mental alertness after the

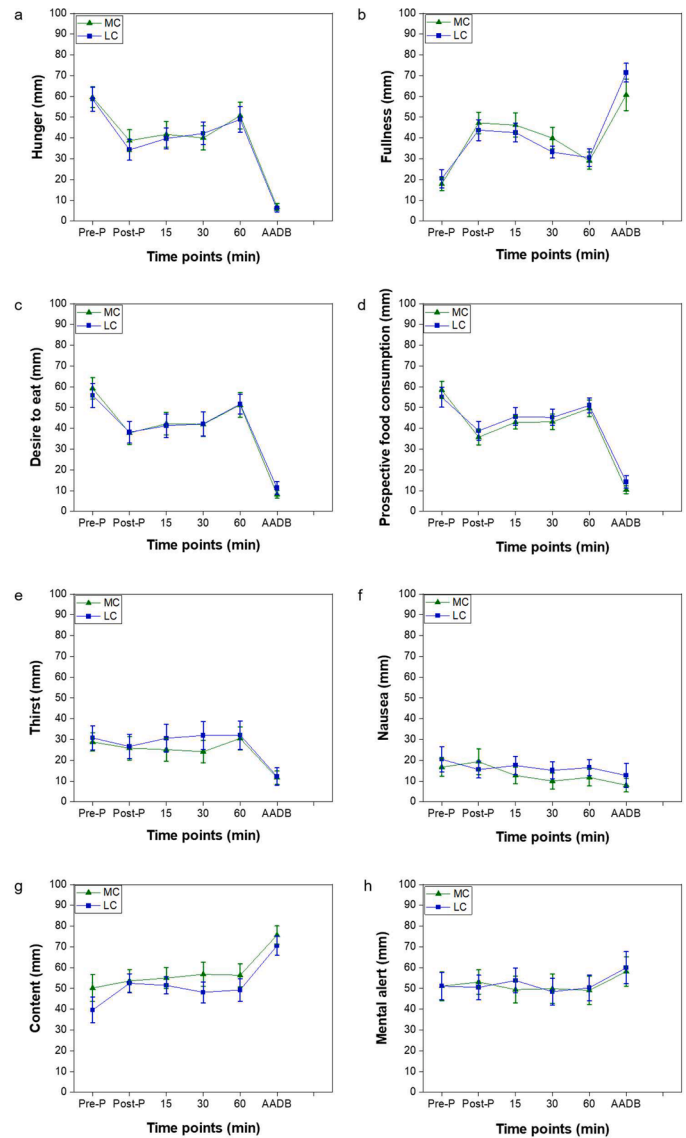


Fig. 4. Study 2. Ratings (mm) for (a) hunger, (b) fullness, (c) desire to eat, (d) prospective food consumption (PFC) (e) thirst, (f) nausea, (g) mental alert, and (h) content over time: pre-preload (Pre-P), post-preload (Post-P), 15 min, 30 min, 60 min and after *ad libitum* breakfast (AADB) in MC (medium coating) and LC (low coating) conditions. Values are means and SEMs (n=15).

study finished – after *ad libitum* breakfast (AADB – for both the studies). However, for content there was a significant difference between conditions at three time points: pre-preload, 30 min and 60 min post-preload in study 2. Participants were more content in MC compared to LC conditions ($p < .05$) in study 2 (see Supplementary Table S2).

For study 1, there was a significant effect of condition (hunger: $F(3, 108) = 12.61$; fullness: $F(3, 108) = 14.17$; desire to eat: $F(3, 108) = 7.32$; prospective food consumption: $F(3, 108) = 10.78$; and thirst: $F(3, 108) = 2.69$), all at $p < .001$); a significant effect of time (hunger: $F(5, 180) = 125.71$; fullness: $F(5, 180) = 130.84$; desire to eat: $F(5, 180) = 119.77$; prospective food consumption: $F(5, 180) = 130.70$ and thirst: $F(5, 180) = 113.80$), all at $p < .001$); and a significant effect of condition*time interaction (hunger: $F(15, 540) = 3.90$; fullness: $F(15, 540) = 2.70$; desire to eat: $F(15, 540) = 4.99$; prospective food consumption: $F(15, 540) = 4.13$; and thirst: $F(15, 540) = 2.43$), all at $p < .001$). Post-hoc pairwise comparison tests revealed that there was a significant difference between all three protein beverages - HC, MC and LC and Control (water): hunger, desire to eat, prospective food consumption and thirst

significantly decreased immediately post-preload maintaining its effect until 30 min post-preload after ingesting HC, MC and LC preloads compared to Control ($p < .05$). Fullness significantly increased in HC, MC and LC compared to Control immediately post-preload maintaining its effect until 30 min post-preload ($p < .05$). A sporadic effect of the condition between three protein beverages on some of the appetite sensations were seen. For instance, the participants felt significantly fuller in HC compared to LC, 20 min post-preload ($p < .05$), and felt they could eat significantly less (prospective food consumption) in MC and HC compared to LC immediately post-preload ($p < .05$), and significantly less in MC compared to LC 10 min post-preload ($p < .05$). Appetite ratings means and SDs are given in Supplementary Table S3a, study 1.

After controlling for baseline ratings, main effects of condition, time and condition*time interaction across all appetite ratings were confirmed, with the exception of thirst (no effect of condition anymore). Readers may refer to Supplementary Table S3b for more details on the main effects after controlling for baseline ratings, study 1.

In study 2, there was an effect of time only (hunger - $F(5, 70) = 35.165$; fullness - $F(5, 70) = 26.824$; desire to eat - $F(5, 70) = 38.521$; prospective food consumption - $F(5, 70) = 41.333$ and thirst - $F(5, 70) = 6.700$ all at $p < .05$). There was no effect of condition (hunger - $F(1, 14) = 0.591$; fullness - $F(1, 14) = 0.003$; desire to eat - $F(1, 14) = 0.001$; prospective food consumption - $F(1, 14) = 0.301$ and thirst - $F(1, 14) = 0.693$ all at $p > .05$) or condition*time interaction (hunger - $F(5, 70) = 0.659$; fullness - $F(5, 70) = 1.627$; desire to eat - $F(5, 70) = 0.408$; prospective food consumption - $F(5, 70) = 1.041$ and thirst - $F(5, 70) = 0.436$, all at $p > .05$). A post-hoc pairwise comparison test revealed that hunger, desire to eat, prospective food consumption and thirst significantly decreased immediately post-preload and was maintained up to 60 min post-preload ($p < .05$). The opposite was observed for fullness where it significantly increased immediately post-preload and was maintained up to 60 min post-preload ($p < .05$). However, all the appetite sensations had the same levels irrespective of the condition *i.e.* participants reported the same levels of appetite ratings in both MC and LC conditions ($p > .05$). Appetite ratings means and SDs are given in Supplementary Table S4a, study 2.

After controlling for baseline ratings, the same effect of time was noticed in study 2, with no effect of condition or condition*time interaction (see Supplementary Table S4b for more details on the main effects after controlling for baseline ratings, study 2).

In terms of the area under the curve (AUC), for study 1, (Supplementary Table S5), all appetite ratings displayed significantly higher AUC in control than in the rest of the conditions ($p < .05$). In study 2, in terms of AUC there was no significant difference between conditions for all appetite ratings (Supplementary Table S6).

3.3. Energy intake

For *ad libitum* energy intake at breakfast, there was no statistical difference between the conditions for both studies: Control, HC, MC and LC, $F(3, 108) = 2.139, p > .05$ (Fig. 5a); MC and LC, $F(1, 14) = 0.679, p > .05$ (Fig. 5b). Therefore, the total amount of food participants consumed was almost the same in all conditions in both studies. The same was observed for water; no significant difference between groups in the water intake in both studies. However, there was a significant difference between the type of breakfast participants ate in study 2 (Fig. 5c). Participants opted for SW/LF compared to the rest S/LF, S/HF and SW/HF ($p < .05$) based on the total meal energy intake (Fig. 5c).

3.4. Oro-sensory exposure time and salivary flow rate - Study 1

The oro-sensory exposure time and salivary flow rate has been assessed for each condition. The oro-sensory exposure time was significantly longer in HC and MC compared to Control and LC ($p < .05$) (Fig. 6a). For the salivary flow, there was no significant difference

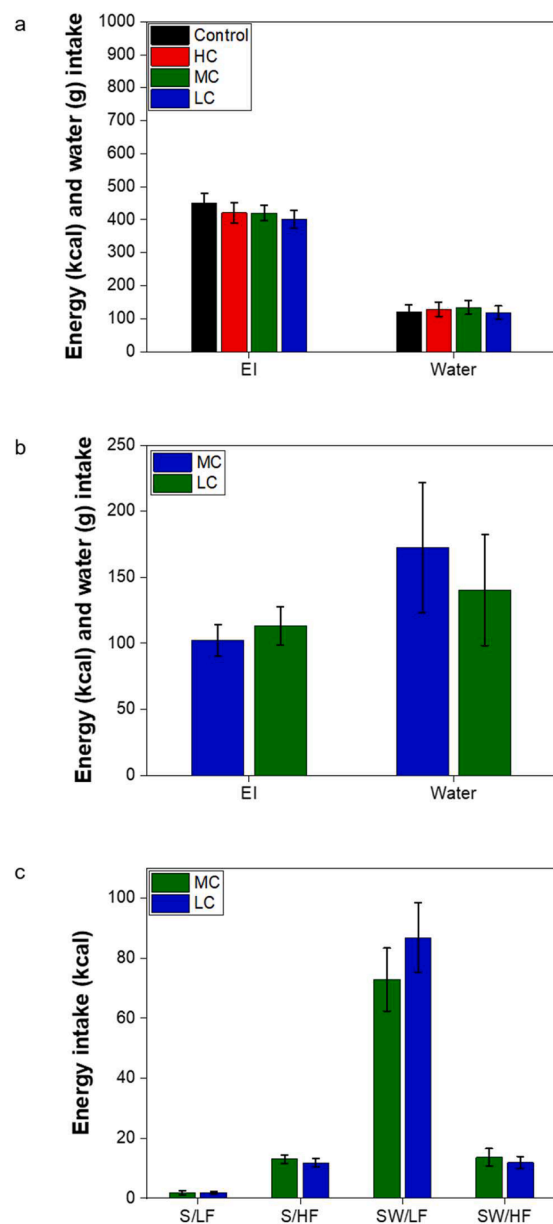


Fig. 5. Energy intake (kcal) and water intake (g) for study 1 (a), study 2 (b) and energy intake depending on breakfast type for study 2 (c). Values are means and SEMs.

between conditions at any time point: pre-preload, post-preload and 30 min post-preload ($p > .05$) (Fig. 6b).

3.5. Lubricating performance and viscosity of saliva

To check if there were differences in lubrication properties of saliva between conditions before, after the intervention and 30 min after intervention, tribological and rheological measurements were performed on the collected pooled saliva. There was no significant difference in the lubrication properties of saliva expressed through friction of coefficient between conditions (Control, HC, MC and LC) before preload (Fig. 7a) which means that the baseline conditions were similar. However, there was a significant difference in the lubrication properties of saliva between conditions immediately after preload (Fig. 7b). Saliva showed to be more lubricious in HC and Control compared to MC and LC ($p < .05$); and in Control compared to HC ($p < .05$) in boundary regime (BL 0.005); more lubricating in Control and HC compared to MC and LC

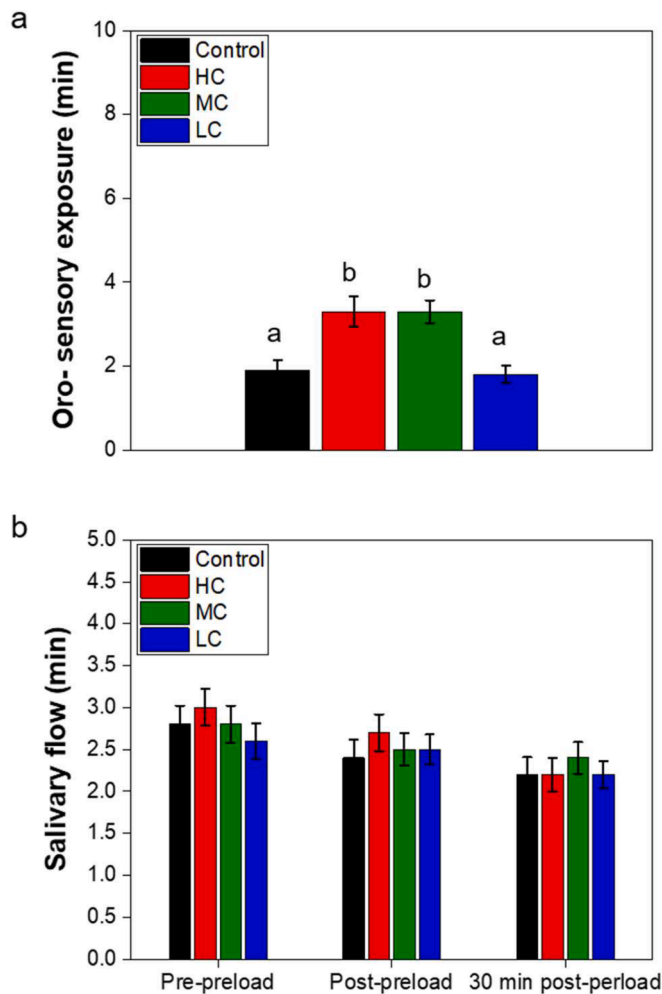


Fig. 6. Oro-sensory-exposure time (min) (a) and salivary flow (min) (b) between conditions Control, HC (high coating), MC (medium coating) and LC (low coating). Values are means and error bars represent standard error of means (SEMs). Different letters indicate significant differences ($p < .05$), ($n=37$).

($p < .05$) in mixed regimes (ML 0.05 and ML 0.1). After 30 min post-preload (Fig. 7c), strikingly saliva was more lubricious in LC compared to Control ($p > .05$) in mixed regime (ML 0.1). Also, viscosity of saliva was measured and there was no significant differences in its level of viscosity (at orally relevant shear rate of 50 s^{-1}) between the conditions across all time points: before, after and 30 min after preload ($p < .05$) (see Fig. 7d).

3.6. Salivary biomarkers

The total concentration of protein (Fig. 8a) and α -amylase (see Fig. 8b) were assessed for each condition at three time points: pre-preload, post-preload and 30 min post-preload. For both protein and α -amylase activity at baseline/pre-preload there was no significant difference between conditions. Post-preload, for the protein activity, there were significant differences between all conditions, with the highest activity in LC followed by MC, HC and Control with the lowest protein activity ($p < .05$). No significant difference was noted 30 min post-preload in protein activity between conditions. For the α -amylase activity, there was a significant difference between HC condition and Control immediately post-preload ($p < .05$), which persisted after 30 min (Fig. 8b). For total protein, there was an effect of time $F(2, 72) = 44.753$, $p = .001$, condition $F(3, 108) = 40.033$, $p = .001$ and condition*time interaction $F(6, 216) = 53.412$, $p = .001$. The same was

noted for α -amylase. There was an effect of time $F(2, 70) = 16.416$, $p = .001$, condition $F(3, 105) = 3.910$, $p = .011$ and condition*time interaction $F(6, 210) = 3.595$, $p = .002$ on salivary α -amylase concentration. Further, mucin (MUC5B) content was determined in saliva samples, however out of 37 saliva samples, MUC5B was only found in 4 samples, which could be due to precipitation of mucin during freeze-thaw cycle of saliva processing. Therefore, these results cannot be treated as robust and have been only included in Supplementary file for the record (see Supplementary Figure S4).

3.7. Gut peptides

There was no difference in the fasting levels between MC and LC conditions for glucose, total ghrelin and PYY (all $p > .05$) as shown in Table 5. However, fasting levels between conditions significantly differed for GLP-1 ($p < .05$), reasons for this are not clear, but may be related to the high variation.

Although we calculated both absolute and controlled for baseline data, we will focus on the controlled for baseline results in this section (results for absolute data can be seen in Supplementary Table S7). Therefore, after controlling for baseline, there was no main effect of condition for glucose $F(1, 14) = 0.165$ (Fig. 9a) and all gut peptides: total ghrelin $F(1, 14) = 0.209$ (Fig. 9b), GLP-1 $F(1, 14) = 1.776$ (Fig. 9c) and PYY $F(1, 14) = 0.204$ (Fig. 9d) (all $p > .05$). There was a main effect of time for glucose, with this getting significantly decreased 30 and 60 min after preload $F(3, 42) = 39.336$, $p = .001$. For the rest of the gut peptides there was no main effect of time: total ghrelin $F(3, 42) = 1.785$, GLP-1 $F(3, 42) = 0.719$, PYY $F(3, 42) = 1.999$ (all $p > .05$). There was a significant effect of condition*time interaction for PYY only $F(3, 42) = 3.674$, $p = .019$. For the rest there was no condition*time interaction effect: glucose $F(3, 42) = 0.349$, total ghrelin $F(3, 42) = 0.383$ and GLP-1 $F(3, 42) = 1.994$ (all $p > .05$) (see Fig. 9a-d for glucose and all gut peptides).

4. Discussion

In the current study, we investigated the effect of mouth-coating and lubricity on appetite control, food intake, salivary biomarkers (Study 1) and gut peptides (Study 2) using texture-manipulated protein beverages as preloads. In order to achieve different textural properties of the preloads, whey and casein protein beverages were subjected to heat treatment method. This appears to be the first occasion in which this experimental approach has been employed. Additionally, we explored the lubricating properties of human saliva after ingesting these preloads. To understand if the time of the preloads in the mouth affected in any way the results we also investigated the oro-sensory exposure time and salivary flow.

With reference to the appetite ratings, in study 1, an effect of protein intake versus Control (water) irrespective of mouth coating (HC, MC, LC) properties in reducing hunger, desire to eat, prospective food consumption and increasing fullness was observed immediately after ingestion, which continued 30 min after. Interestingly, in study 1, a sporadic effect of mouth coating was noticed where fullness increased in HC condition vs LC 20 min after preload, meaning that participants felt fuller after ingesting beverages with high coating properties compared to low coating. However, this effect was absent at 10 and 30 min. Also, a decrease in prospective food consumption ratings (how much participants felt they could eat after the preloads) was noted with participants feeling like that they could eat less after HC and MC immediately as well as after 10 min after preload intake compared to LC, but not at 20 and 30 min after. As such, we could see a clear effect of protein intake vs Control at some time points which is well-reported in literature [58,59] and a much more sporadic effect of HC (high coating) vs LC (low coating) on appetite sensations, which is reported for the first time in literature. This sporadic effect of coating could be explained by several factors. Firstly, the oro-sensory exposure time of the preloads was higher in HC and MC

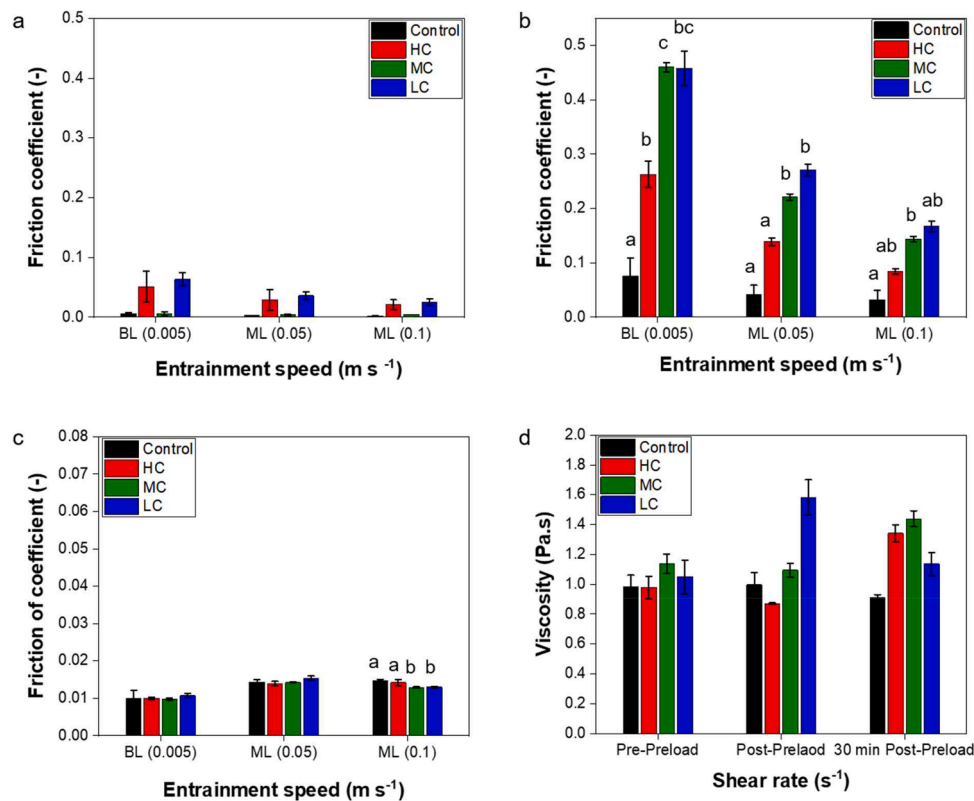


Fig. 7. Friction coefficient of saliva before preload (a), after preload (b) and 30 min after preload (c) at boundary (0.005 m s⁻¹ speed) and mixed (0.05 m s⁻¹; 0.1 m s⁻¹ speed) lubrication regimes and viscosity (d) of saliva as a function of orally-relevant shear rate of 50 s⁻¹, in all four conditions of Control, HC (high coating), MC (medium coating) and LC (low coating), n = 37. 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. Different letters indicate significant differences ($p < .05$), (n=37).

vs LC. Therefore, the beverages had higher residence time in the mouth, and consequently experienced higher feelings of fullness in HC and MC compared to LC. Secondly, saliva was more lubricating after eating HC and MC vs LC preloads immediately after consuming the preloads, which suggests that intrinsic lubrication might have contributed to the initial appetite scores [5,23,24]. Thirdly, there was a negative association between protein activity in saliva and prospective food consumption and desire to eat, such that the higher the protein activity in saliva the lower was the desire to eat and the less feelings of prospective food consumption. However, one should be cautious drawing conclusions from these results as the effect of coating was sporadic and was not consistent across all appetite sensation and at all time points.

With respect to appetite ratings in study 2, there was no difference in the gut peptides between the conditions MC and LC. It is not a surprise as this comes in accordance with the results of study 1 where there also was no difference between MC and LC in hunger or fullness ratings, post preload or 30 min after. Although in the study 1 the level of coating and lubricity in the preloads was clearer (lubricity – low, medium and high; coating – low, medium and high), in order to exclude any effect of the protein type (Casein – high coating/low lubricating, Heated Whey protein – medium coating/high lubricating, Unheated Whey protein – low coating/medium lubricating), the two whey protein beverages -Heated Whey protein – MC (medium coating) and Unheated Whey protein – LC (low coating) were selected for study 2. As such, it appears that a subtle change in texture (lubricity/coating) properties of the preloads does not influence the appetite ratings which corroborates with previous studies [5,23]. This is in contrary to studies where manipulation of the preloads texture is stronger such as liquid vs solid, low viscous vs high viscous or low viscous vs gels [4,30]. Although the lubricity and coating parameters were statistically different between preloads, it was not large enough to influence appetite ratings. To see a physiological

effect, future studies may need to create preloads with at least 10-folds difference in such oral processing parameters to see modulation of appetite ratings, especially when it comes to new concepts such as lubricity and coating.

We also attempted to understand the effect of texture in combination with macronutrients/energy load on appetite. From this perspective, it could be seen that the effect lasted up to 30 min. In our previous study [5] where the texture (lubricity) was expressed through non-caloric preloads (hydrogels), the effect of lubricity on appetite ratings was immediate and very short (10 min) compared to the current study where effect of coating was combined with macronutrients/energy load expressed through protein beverages preloads and the effect lasted up to 30 min. A combinatorial effect of texture and macronutrients/energy has been demonstrated.

Regarding energy intake, there was no effect of oral coating in both studies. In study 1, the energy intake was similar in all conditions – HC, MC, LC and Control. A previous study in the literature reported an immediate effect of food texture (oral lubricity) on snack intake, with this being lower in high lubricating condition compared to low lubricating one [23]. It may suggest that the effect of texture (coating/lubricity) could be immediate regardless of the presence or absence of macronutrients/energy load in the preloads. Noteworthy, the study design involved breakfast, and breakfast is often a habitual meal *i.e.* people may or may not eat breakfast. Hence, merely the breakfast study design might have affected the food intake results to a certain extent.

In study 2 we changed the content of *ad libitum* breakfast because of two main reasons – 1) to exclude any learned experience on energy intake from the previous study (half of the participants were from study 1, and 2) to investigate the effect of oral coating on the preference of chosen food (S/LF, S/HF, SW/LF and SW/HF). Despite this, the energy intake has been similar in both MC and LC conditions. Moreover, it was

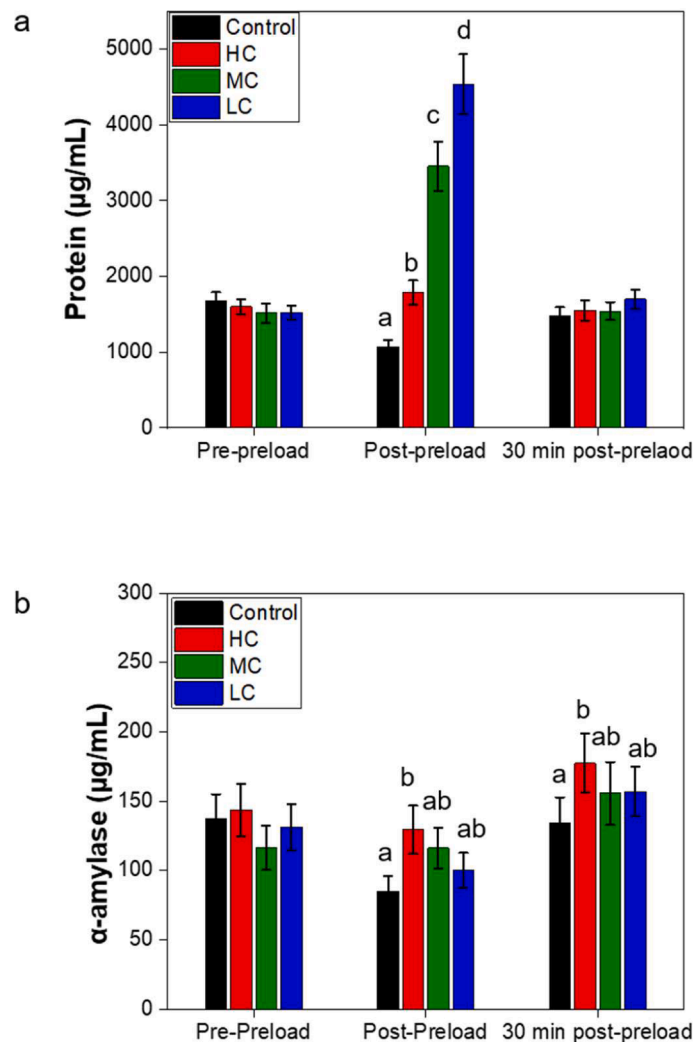


Fig. 8. Total protein ($\mu\text{g/mL}$) ($n = 37$) (a) and α -amylase ($\mu\text{g/mL}$) ($n = 37$) (b) in saliva for Control, HC (high coating), MC (medium coating) and LC (low coating) conditions Pre-preload, Post-preload and 30 min post-preload. Values are means and error bars represent standard error of means (SEMs). Different letters indicate significant differences ($p < .05$), ($n=37$).

Table 5

Absolute fasting levels of glucose, total ghrelin, GLP-1 and PYY before consumption of preloads. Data are mean and SDs, ($n=15$).

Fasting levels	Medium Coating (MC)	Low Coating (LC)	P value
Glucose, mg/dL	92.33 \pm 5.72	89.2 \pm 8.77	0.236
Total ghrelin, pg/mL	884.2 \pm 809	596.73 \pm 483.42	0.158
GLP-1, pg/mL	25.63 \pm 10.91	32.12 \pm 19.88	0.029
PYY, ng/mL	674.87 \pm 196.67	680.27 \pm 310.73	0.958

noted that participants chose SW/LF compared to the rest (S/LF, S/HF and SW/HF) irrespective of the condition (MC and/or LC). This could suggest that participants deliberately opted for more healthy choices irrespective of study conditions (texture manipulation). Therefore, it can imply that the changes in the texture of the preloads (manipulation of coating) in both studies, were too subtle to trigger a physiological (body signals) response in relation to food intake (participants to eat significantly more or less depending on the conditions).

To add further to the understanding of the mechanism behind coating and appetite, we measured glucose and gut peptides such as total ghrelin, GLP-1, PYY. While the trend in glucose levels with a plateau up to 15 min and a sharp decrease after 15 min up to 60 min was in alignment with literature [4,59,60], there was no significant difference in glucose levels between the conditions MC and LC. Our findings

are in agreement with previous works on texture that reports no differences in glucose levels, although the differences in texture (in the previous reported studies) were clearer, such as solid *versus* liquid [61, 62] compared to this study – medium coating *versus* high coating.

The same was noted for total ghrelin, GLP-1 and PYY with no effect of condition/texture and time. This does not come as a surprise as previous studies showed no effect of texture on ghrelin [31,61,62], GLP-1 and PYY [9,30]. Recent studies have suggested that gut peptides such as GLP-1 and/or PYY can be released in proportion to the energy load and macronutrients [30,63,64], indicating that the higher the energy and fat load of the test meal/preload is, the more GLP-1 and PYY is released. Therefore, meals that have only protein with low or/and equal energy load may explain to some extent the lack of differences in the results of the gut peptides in the current study. For instance, an effect of food texture on ghrelin, GLP-1 and PYY has been shown in preload starting with 300 kcal [31,65], while in the current study the preloads were of 239 kcal. Therefore, it may be suggested that the preloads in our study did not have enough kcal load to elicit reduction in ghrelin and release of GLP-1 and PYY. Likewise, it can be suggested that texture alone is not enough to trigger a physiological/ gut hormonal response, which is in the later stages of the satiety cascade. It seems that oral processing has a limited or no effect on gut hormonal response when the manipulation of food texture is subtle, based on one macronutrient only that has a reduced amount of kcal.

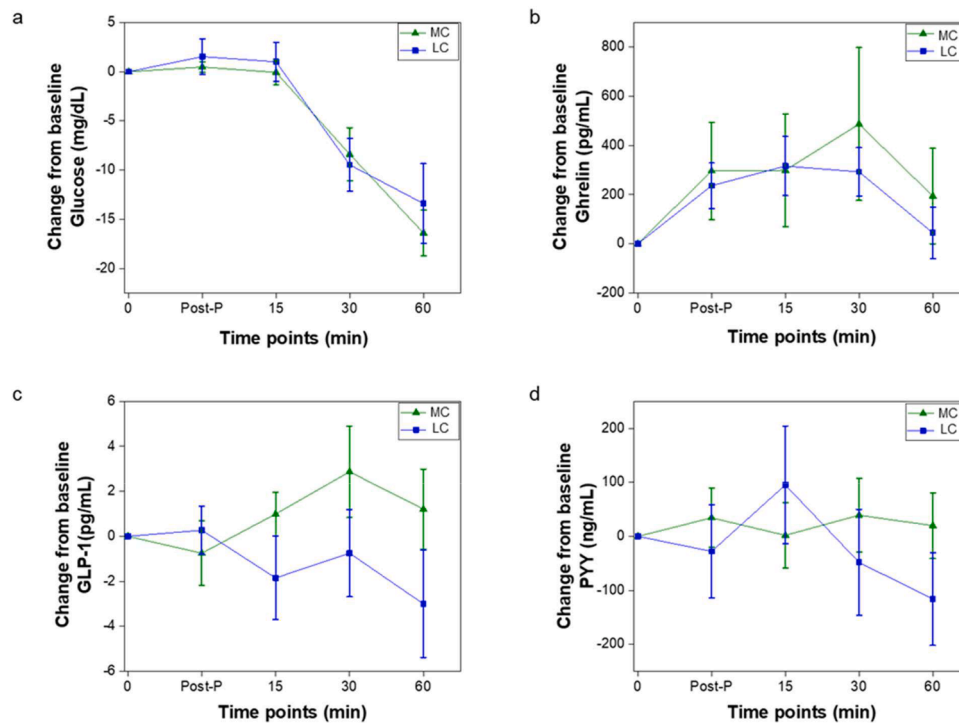


Fig. 9. Postprandial profile of glucose (a), total ghrelin (b), GLP-1 (c) and PYY (d) after ingesting the preloads differing in their coating properties (MC and LC). Data are represented as means and SEM of the means, (n=15).

This brings us to the question, does texture (lubricity and coating) influence satiety and satiation? There was a clear effect of protein beverages vs control and a sporadic effect of coating on some appetite sensations at certain time points which was also observed with a consequent increase in salivary lubricity. However, at this stage it is unclear and premature to give a robust answer to this question. Nevertheless, there is certainly room for more research into the area especially in relation of the interaction between saliva and food with high coating properties.

Although viscosity, lubricity and coating measurements were done outside the mouth using well-established *in vitro* techniques, such parameters have shown correlation with sensory smoothness in previous studies involving various model and real foods [27,66-68]. However, a more representative equipment such as use of a recently developed 3D biomimetic tongue-emulating surfaces [69] alongside real measurement of *in vivo* mouth coating in human will be more definitive to quantify and confirm the real differences in coating of preloads. Also, one should be cautious with difference in energy load across the preloads versus control (water). Although the level of the energy load in study 1 was similar in all three protein preloads, the control one (water) had no energy load. Therefore, this might have affected the results to a certain extent.

The strength of this study is showing the importance of saliva in underpinning the mechanism of oral lubricity/coating in the context of satiety. When saliva interacts with food high in coating properties, strikingly it becomes more lubricating which might help to coat oral surfaces better and for longer time and in turn led to higher ratings in fullness and lower ratings in desire to eat and prospective food consumption in this study. Thus, this study offers a novel textural construct of oral coating along with consequent changes in salivary lubricity in the context of satiety.

However, one should interpret such results with caution as the effect of coating was not consistent across all appetite sensations and at all-time points, a sporadic effect as mentioned above. Future research should investigate whether the effects of coating are observed in a repeated exposure and long-term design. In addition, future research should aim at creating preloads with a higher degree of difference in

coating properties between preloads and examine its effect on satiety. Additionally, a 2×2 (low lubricating, high lubricating, low coating, high coating) design would also add valuable information to the literature. In addition, investigating the effect of oral coating on satiety and satiation in an *ad libitum* intake design would add valuable information to the mechanism proposed above. Noteworthy, two different protein types (casein and whey protein) were used to create three different coating properties and such coating structure resulted from different conformation of the protein from a molecular viewpoint. Although we focused on early stage satiety generation over 50 min post preload to diminish any effects associated with digestion, amino acid release of the proteins and gastric emptying, such difference in structural and corresponding physiological degradation of the different protein types in the early timepoints and its impact on satiety cannot be fully ignored.

5. Conclusions

Herein, new kinds of textural manipulation involving measuring lubricity and mouth coating of milk protein-based beverages demonstrate that combining lubricity/coating with macronutrients/energy load can prolong the effect on appetite ratings only sporadically. For the first time, human saliva has been analysed and linked to the mechanism of oral lubrication/coating in a satiety context. There was no effect of oral coating on energy intake and gut peptides (n=15), suggesting that complex textural attributes having influence on oral processing might not have any effect on the later parts of the satiety cascade. Although, oral lubricity/coating as a textural manipulation strategy is in its infancy stage, further investigation requires increased differences in the degree of coating/ lubricity between preloads to offer a clear and sustained effect on reducing appetite ratings.

CRedit authorship contribution statement

Ecaterina Stribițaia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Catherine Gibbons:** Writing – review & editing,

Supervision, Methodology, Conceptualization. **Graham Finlayson:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Kwan-Mo You:** Investigation, Formal analysis. **Andrea Araiza-Calahorra:** Methodology, Formal analysis. **Maryam S. Hafiz:** Methodology, Investigation, Formal analysis. **Lucy R. Ellis:** Investigation, Formal analysis. **Christine Boesch:** Writing – review & editing, Methodology, Conceptualization. **Joanna H. Sier:** Methodology, Formal analysis. **John Blundell:** Writing – review & editing, Supervision, Methodology, Conceptualization. **Anwasha Sarkar:** Writing – review & editing, Supervision, Project administration, Methodology, Conceptualization.

Declaration of competing interest

There are no known conflicts of interest associated with this publication.

Data availability

Data will be made available on request.

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Supplementary materials

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