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## A dairy-based protein-rich breakfast enhances satiety and cognitive concentration before lunch in young females with overweight to obesity: A randomized controlled cross-over study

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### ABSTRACT

#### Purpose

To investigate if consumption of a high-protein low-carbohydrate breakfast (PRO) leads to a lower subsequent ad libitum energy intake at lunch and the rest of the day compared with ingestion of an isocaloric low-protein high-carbohydrate breakfast (CHO) or no breakfast (CON).

#### Methods

The study was designed as a randomized controlled 3 period crossover study. Thirty young (18–30 years) females with overweight to obesity (BMI >25 kg/m<sup>2</sup>) in random order completed 3 separate experimental days, where they consumed either a PRO, CHO, or CON breakfast test meal followed by an ad libitum lunch meal 3 h after breakfast. Participants were allocated to a sequence group by their inclusion number. PRO and CHO were matched in dietary fiber and fat content. Energy intake at lunch was calculated and dietary records were obtained for the rest of the day to calculate the total daily energy intake and macronutrient intake. Ratings of appetite sensations between meals and palatability of the test meals were assessed using visual analog scale (VAS) sheets in intervals ranging from 10 to 30 min. In addition, blood samples were obtained at multiple time points separated by 10–60 min intervals between breakfast and lunch and were analyzed for appetite-regulating gut hormones, insulin, and glucose. Finally, performance in a cognitive concentration test was tested 150 min after breakfast.

### Results

Compared with CHO and CON, the area under the curves (AUC) for satiety, fullness, and satisfaction in the 3 h after breakfast were significantly higher after PRO, whereas the AUC for hunger, desire to eat, and prospective eating were significantly lower after PRO. The appetite-regulating gut hormones cholecystokinin, glucagon-like peptide-1, and ghrelin in the hours after breakfast, energy intake during the ad libitum lunch meal, and the total daily energy intake did not differ significantly between PRO, CHO, and CON. However, the cognitive concentration test score was 3.5 percentage points higher for PRO, but not CHO, versus CON.

### Conclusion

A dairy-based high-protein low-carbohydrate breakfast increased satiety sensation in the hours after breakfast but did not reduce total daily energy intake compared with an isocaloric low-protein high-carbohydrate breakfast or breakfast omitting. However, performance in a cognitive concentration test before lunch was enhanced after the high-protein low-carbohydrate breakfast, but not the low-protein high-carbohydrate breakfast, compared with omitting breakfast.

Keywords: dietary protein, appetite, ghrelin, cholecystokinin, glucagon-like peptide-1

**Interpretive summary** In light of the global obesity pandemic, developing strategies aimed at improving weight management becomes crucial in the battle against weight gain. In this randomized controlled cross-over study, we examined the impact of three different breakfast options on satiety and subsequent energy intake in young females with overweight to obesity. A dairy-based, high-protein, low-carbohydrate, breakfast enhanced feelings of satiety and reduced hunger sensations in the subsequent hours. However, the daily energy intake was comparable to that observed after consuming an isocaloric low-protein high-carbohydrate breakfast or omitting breakfast. Our findings highlight

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a need for research clarifying the long-term health implications of different breakfast habits.

## INTRODUCTION

The prevalence of obesity worldwide has nearly tripled since the mid-1970s and now more than 11% of adult males and 15% of females are categorized as obese (body mass index (BMI) > 30) (NCD-RisC, 2016), which is a tremendous threat to public health. Overweight and obesity enhance the risk of developing diseases such as type II diabetes mellitus and cardiovascular disease, which alone leads to over 17.5 million deaths each year worldwide (Mendis et al., 2015). Therefore, additional strategies are needed to halt the increasing prevalence of overweight and obesity worldwide. One approach is changing eating behavioral habits, to help regulate energy intake and prevent weight gain.

Breakfast omission is the most frequent meal-skipping behavior in the Western part of the world (Pendergast et al., 2016). In light of this, a recent meta-analysis based on 36 cross-sectional studies and 9 cohort studies consistently showed that skipping breakfast is associated with overweight/obesity, and skipping breakfast increases the risk of weight gain (Ma et al., 2020). However, a systematic review and meta-analysis of results from 7 randomized controlled intervention studies ( $n = 425$  participants) with an average duration of 8.6 weeks reported a modest reduction in body mass ( $-0.54$  kg [95% CI:  $-1.05$  to  $-0.03$ ], but no change in % body fat (5 studies) in breakfast skippers compared with participants allocated to consume breakfast during the intervention period (Bonnet et al., 2020). Thus, there are discrepancies between findings that must be further elucidated.

Ingestion of specific macro- and micronutrients such as dietary protein (Leidy et al., 2015), dietary fiber (Pol et al., 2013), and dietary calcium (Kjølbæk et al., 2017) have been proposed to prevent weight gain or induce weight loss. Findings suggest that breakfast with high protein content has the potential to increase postprandial satiety and decrease energy intake (EI) of subsequent meals (Leidy et al., 2015; Moon & Koh, 2020). In line with this, greater increases in anorexigenic gut hormones (glucagon-like peptide-1 (GLP-1), peptide YY (PYY), cholecystokinin (CCK), and leptin) have been reported after high-protein meals compared with isocaloric control meals, as well as a decreased level of the orexigenic hormone ghrelin (Hwalla & Jaafar, 2020; Moon & Koh, 2020). In accordance, dose-dependently increases in GLP-1 and PYY 3–36 in a mixed population of normal weight and overweight participants have been observed after breakfast meals with diverging protein content (Belza et al., 2013). Nevertheless, oth-

ers have reported no positive effect of enhanced protein content in meals on postprandial satiety, EI (Belza et al., 2013; Boelsma et al., 2010; Veldhorst et al., 2009), or the response in PYY (Leidy & Racki, 2010), GLP-1 (Veldhorst et al., 2009), or ghrelin (Belza et al., 2013; Leidy & Racki, 2010; Veldhorst et al., 2009). Thus, the evidence on the effects of high-protein breakfasts on acute changes in satiety and appetite-regulating hormones, as well as the effects on subsequent ad libitum EI is inconsistent. The diverging results in the literature may be related to the protein content of the breakfast meal since previous findings suggest a within-meal protein threshold of 30 g of protein to reach a superior effect of protein on satiety (Leidy et al., 2015; Paddon-Jones & Leidy, 2014).

While skipping breakfast can potentially have a detrimental impact on weight regulation, it may also lead to a decline in cognitive function (Komiya et al., 2016). A meta-analysis of 38 studies revealed a strong correlation between consuming breakfast and improved memory. However, due to the significant heterogeneity in study designs and methods, a definitive conclusion regarding the specific composition of breakfast could not be drawn (Galioto & Spitznagel, 2016).

Based on previous findings described above, we aimed to test the hypothesis that a high-protein low-carbohydrate breakfast (PRO) (~30 g protein) compared with an isocaloric low-protein high-carbohydrate breakfast (CHO) (~5 g protein) or no breakfast would lead to greater satiety and thereby a lower subsequent ad libitum EI at lunch and total daily energy intake (TEI) in young females with overweight to obesity. Furthermore, we aimed to compare cognitive performance through a concentration test conducted 2 1/2 h after the consumption of different breakfast test meals. We hypothesized that breakfast skipping would have an adverse impact on the ability to concentrate. Secondary outcome parameters were changes in plasma glucose, insulin, and satiety-regulating gut hormones, as well as satiety and appetite sensations in the subsequent hours after the breakfast test meals.

## MATERIALS AND METHODS

### Participants

Young (18–30 years), females with overweight to obesity (body mass index (BMI) > 25 kg/m<sup>2</sup>) were recruited through social media and posters in the local city of Aarhus, Denmark. Eligibility was assessed by an online questionnaire (Supplementary files, Table 1) and exclusion criteria were pregnancy, food allergies, needle phobia, mental diseases, chronic and metabolic diseases, use of medication that affects appetite, physical train-

ing > 5h/week, > 5 kg weight change in the previous 6 mo, irregular menstrual cycle, not liking lasagna, or participation in other research studies including diet intervention and/or blood sampling. Eligible females went through a telephone screening, and if accepted a meeting was scheduled for further information and measurements of height, weight, body composition, and habituation to the cognitive test. All participants provided written consent before any data was collected. In total 58 females were included in the randomization and 30 completed the experimental period. A consort flow diagram of the number of participants from the start of inclusion to the final analyzes is shown in Figure 1. Subject characteristics are shown in Table 1. The study was conducted in accordance with the Declaration of Helsinki, approved by the Central Denmark Region Committees on Health Research Ethics (Journal no. 1-10-72-220-19), and was registered at Clinicaltrials.gov (ID: NCT04652713).

### Study design

The study was conducted as a randomized, controlled, crossover trial composed of 3 experimental days with at least one day washout between trials (mean:  $12.1 \pm 9.7$  d; range: 2–36 d). In notion, variations in fluctuations of sex hormones were calculated for, as the participants were tested in the follicular phase of their menstrual cycle, where they are least affected, or when on birth control since estrogen may inhibit food intake, and progesterone and testosterone may stimulate appetite (Hirschberg, 2012). One of 3 different breakfast test meals was served in random order. There were 6 sequence groups ( $3 \times 2 \times 1$ ) and participants were allocated to a sequence group by their inclusion number which was matched to a predefined sequence group. The number of participants allocated to each sequence group ( $n = 3, 4, 4, 4, 7,$  and  $8$ ) differed due to dropouts, which disturbed the predefined matching of inclusion number and sequence group. The breakfast meals consisted of either a high-protein low-carbohydrate breakfast (PRO), an isocaloric low-protein high-carbohydrate breakfast (CHO), or a control day omitting breakfast (CON). Samplings and tests were conducted at the Section of Sport Science, Department of Public Health at Aarhus University, Denmark in the period between November 2020 and August 2022.

### Experimental days

On experimental days (Figure 2), the participants arrived at the laboratory at approximately 8:30 a.m. after overnight fasting since 8 p.m. the previous day. Also, the participants were instructed to withstand

**Table 1.** Anthropometric and eating behavior characteristics of the participants

Characteristic	Mean $\pm$ SD <sup>4</sup>
Anthropometrics	
Age (years)	24.2 $\pm$ 2.2
Height (cm)	168.4 $\pm$ 7.5
Weight (kg)	85.2 $\pm$ 11.8
BMI (kg/m <sup>2</sup> ) <sup>1</sup>	30.0 $\pm$ 3.5
Body fat (%)	44.2 $\pm$ 6.2
FFM (kg) <sup>2</sup>	45.6 $\pm$ 5.5
Eating behavior	
Restrained eating <sup>3</sup>	2.57 $\pm$ 0.71
Emotional eating <sup>3</sup>	3.00 $\pm$ 0.93
External eating <sup>3</sup>	3.42 $\pm$ 0.56

<sup>1</sup>BMI, Body-mass index.

<sup>2</sup>FFM, Fat-free mass.

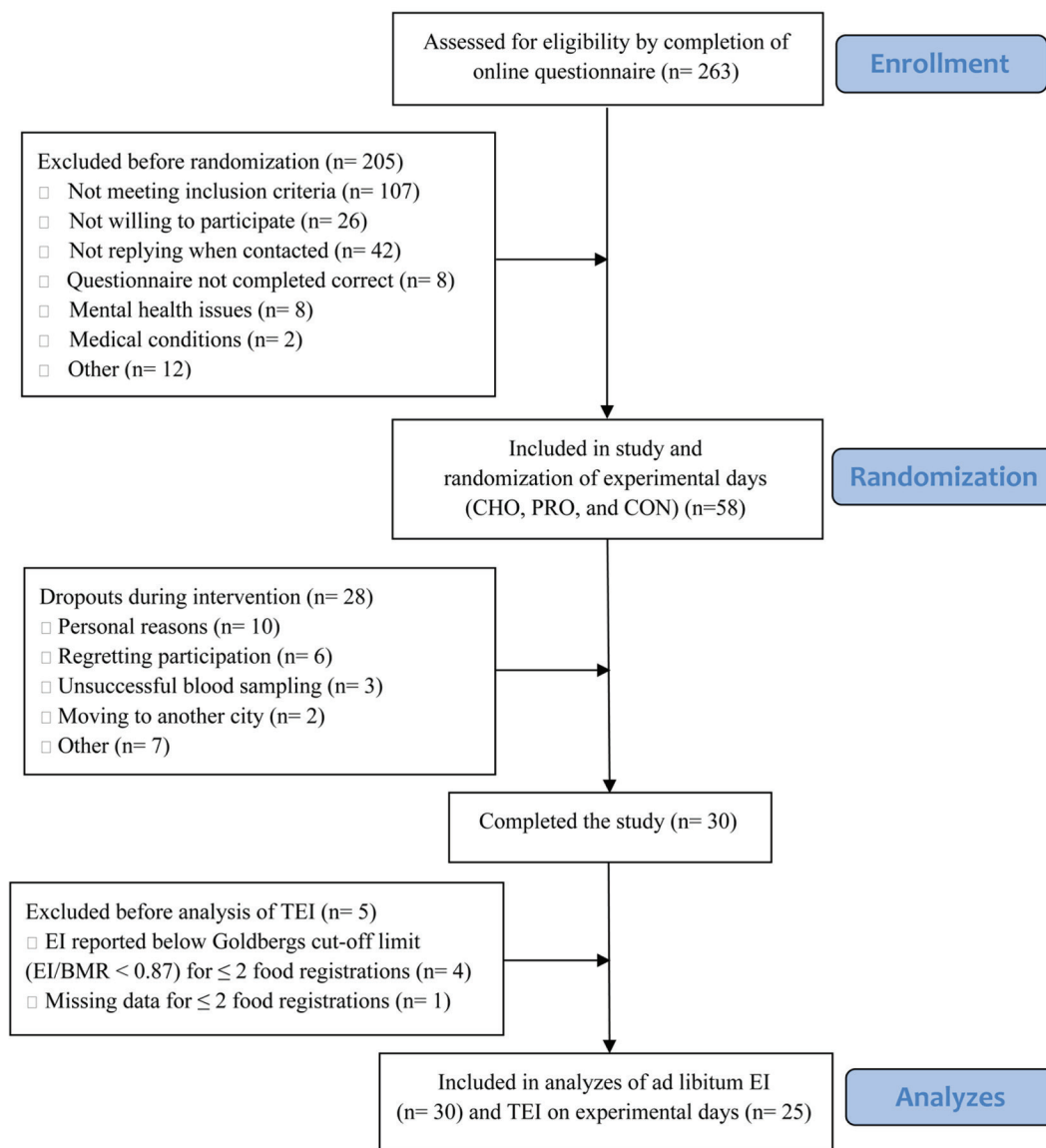
<sup>3</sup>Mean score in the subcategory of the modified Dutch eating behavior questionnaire (DEBQ); a score of 1 = never, 2 = seldom, 3 = occasionally, 4 = often, and 5 = always.

<sup>4</sup>SD, Standard deviation.  $n = 30$ .

alcohol and strenuous physical exercise the day before the experimental day. The participants were instructed to consume a glass of water before arriving at the laboratory. Upon arrival, baseline blood samples were taken (pre-breakfast), and a visual analog scale (VAS) sheet assessing appetite and satiety sensations was completed. Afterward, the breakfast meal (randomized by inclusion order) was served along with a glass of water (150 mL) and a VAS sheet assessing the palatability of the breakfast meal (not provided at CON). The participants were instructed to consume all foods and beverages within 15 min. Seven VAS sheets assessing appetite and satiety were completed 10, 30, 60, 90, 120, 150, and 170 min post-breakfast (+10, +30, +60, +90, +120, +150, and +170 min) and cognitive function was assessed by a paced auditory serial addition task (PASAT) (Tombaugh, 2006) performed at +150 min. An ad libitum lunch meal was served 180 min post-breakfast (+180) and the palatability was assessed by a VAS sheet. Immediately after lunch (post-lunch), satiety and appetite sensations were assessed by a VAS sheet. Sampling of venous blood and measurements of blood glucose was done pre-breakfast, at +10, +30, +60, and +170 min as well as at +120 min for blood glucose. During the experimental day, participants were allowed to do sedentary activities between time points such as reading, writing, or using a computer.

The participants registered their food intake using a commercially available diet registration software (MadLog, MadLog ApS, Denmark) the day before the experimental day, and for the remainder of the experimental day, when the participant had left the laboratory. From these data, TEI as well as the daily dietary intake of protein, carbohydrates, fat, fiber, and calcium was estimated. The potential underestimation

## CONSORT Flow Diagram



**Figure 1.** CONSORT flow diagram. CHO, low-protein high-carbohydrate breakfast test meal. PRO, high-protein low-carbohydrate breakfast test meal. CON, control omitting breakfast. EI, energy intake. TEI, total energy intake. BMR, basal metabolic rate.

of single-day registrations was assessed using Goldberg's cut-off value ( $TEI/BMR \leq 0.87$ ) for adults with low physical activity levels (Black, 2000). Furthermore, the participants were instructed to replicate food intake and physical activity levels the day before the first experimental day on the days before experimental d 2 and 3. On the final day of testing, participants filled out a modified Dutch eating behavior questionnaire (DEBQ) (van Strien et al., 1986) after lunch.

### Test meals

The CON meal consisted of a glass of water (150 mL, 0 kJ). The breakfast meals differed in protein content (PRO: 32.4 g; CHO: 5.2 g) and carbohydrate content (PRO: 29.4 g; CHO: 64.3 g), however, not in energy content ( $1,260 \pm 69$  kJ), fiber content ( $3.5 \pm 0.57$  g), fat content ( $2.4 \pm 0.28$ g), and weight ( $485 \pm 5$  g) (Table 2). Test meals were prepared and served by research personnel. The participants, but not the researchers, were blinded to the randomization order of the breakfast meals. PRO consisted of 300 g of high-protein,

**Table 2.** The nutritional content of the breakfast test meals

	Energy [kJ] <sup>1</sup>	Weight [g] <sup>2</sup>	Carbohydrate [g] <sup>2</sup>	Protein [g] <sup>2</sup>	Fat [g] <sup>2</sup>	Fiber [g] <sup>2</sup>
High protein breakfast (PRO)	1211	480	29.4	32.4	2.6	3.9
Cheasy® 0.2% vanilla skyr	747	300	12.3	28.2	0.6	0.9
Oats	464	30	17.1	4.2	2.0	3.0
Water	0.0	150	0.0	0.0	0.0	0.0
High carbohydrate breakfast (CHO)	1309	490	64.3	5.2	2.2	4.1
Whole grain toast	623	60	25.8	4.7	2.0	3.4
Raspberry jam	248	30	13.5	0.2	0.2	0.7
Apple juice	438	250	25.0	0.3	0.0	0.0
Water	0.0	150	0.0	0.0	0.0	0.0
Control “breakfast” (CON)	0.0	150	0.0	0.0	0.0	0.0
Water	0.0	150	0.0	0.0	0.0	0.0

<sup>1</sup>kJ, kilojoule.<sup>2</sup>g, grams.

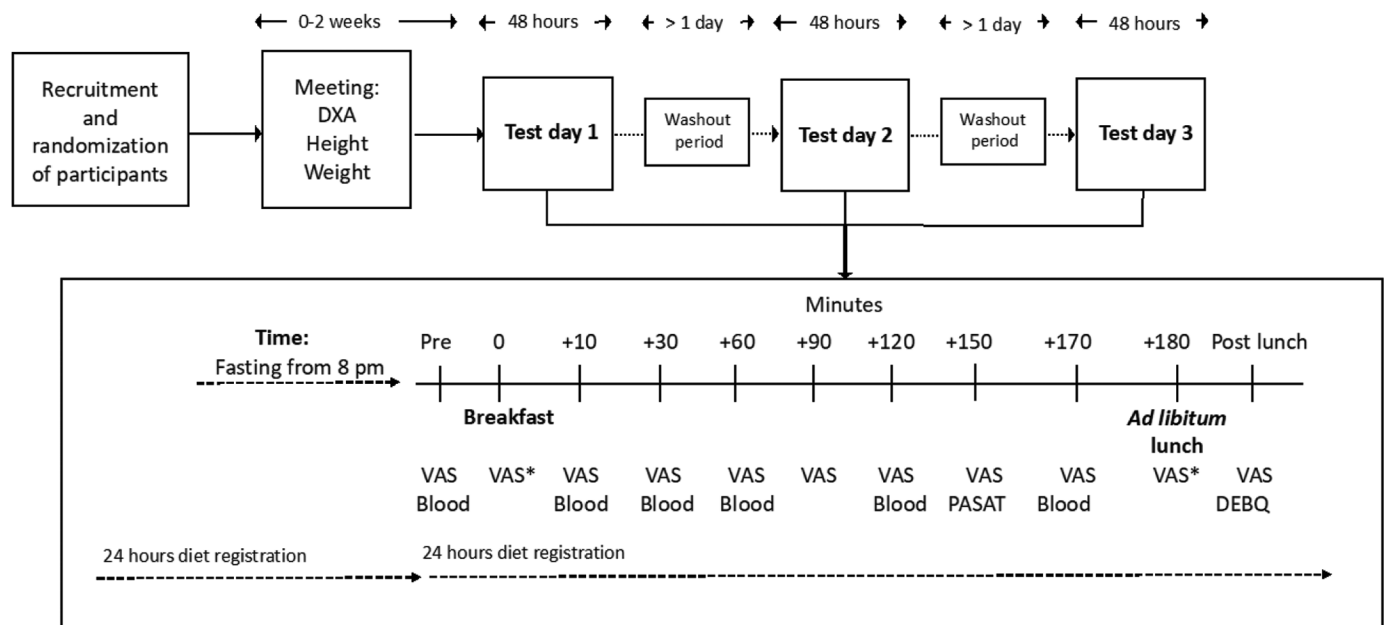
drained yogurt “skyr” (Arla Foods Amba, Denmark), with a protein content of 9.4 g/100 g, and 30 g of oats (REMA 1000 Denmark A/S). CHO consisted of 60 g of whole grain bread (REMA 1000 Denmark A/S) with 30 g of raspberry jam (REMA 1000 DANMARK A/S) and 250 g of apple juice (REMA 1000 Denmark A/S). A glass of water (150 mL) was served with all meals. See Supplementary files, Figure 1 for an illustration of meals.

The ad libitum lunch meal consisted of 1,200 g of heated lasagna (Lasagna Bolognese, REMA 1000 DANMARK A/S, 542 kJ/100 g; macronutrient content g/100 g: protein 6.7, fat 4.4 and carbohydrate 15)

served with 150 mL of water. Participants were taken to a quiet, undisturbed place and were instructed to eat until comfortably satiated. After the lunch meal, research personnel weighed the leftovers to estimate the ad libitum EI at lunch.

### Blood sampling

Fingertip blood samples were analyzed for blood glucose concentration immediately after collection using a HemoCue Meter (HemoCue glucose 201 RT, Denmark). Venous blood samples (n = 450) were collected from the *median cubital* vein in the crevice of the elbow



**Figure 2.** Overview of the study period and timeline of the measurements on the experimental days. Visual analog scale (VAS) sheets to assess appetite and satiety sensations, and palatability during meal consumption. Blood sampling to assess plasma levels of glucose and gut hormones and serum levels of insulin. PASAT: Paced Auditory Serial Addition Task. DEBQ: modified Dutch eating behavior questionnaire provided on the last experimental day.

into 2 tubes; a serum tube with a coagulation activator for insulin analysis and a plasma tube containing EDTA (EDTA) and proteinase inhibitors for analyzes of appetite-regulating hormones. The plasma tube was centrifuged immediately after collection, whereas the serum tube was centrifuged after coagulation for ~60 min at room temperature. All blood samples were centrifuged at 4 °C, at 1,300 g for 10 min, and immediately stored at -80 °C until further analyzes. The blood samples from all time points were later analyzed by double determination for serum insulin (The Department of Biochemistry, Aarhus University Hospital, Aarhus, Denmark), total plasma ghrelin (MyBioSource, Inc.; Human Ghrelin (GHRL) ELISA Kit; catalog number: MBS2700428), plasma cholecystokinin (CCK) (MyBioSource, Inc.; Human Cholecystokinin (CCK) ELISA Kit; catalog number: MBS2700293), and plasma glucagon-like-peptide-1 (GLP-1) (RayBiotech Life, Inc.; Human GLP-1 (1-37a) ELISA; catalog number: ELH-GLP137).

### Questionnaires

Sensations of satiety, fullness, hunger, prospective eating, satisfaction, and desire to eat before, between, and after the breakfast meals and ad libitum lunch were assessed by the area under the curve (AUC) derived from VAS sheets. Furthermore, an additional set of VAS sheets was employed during the consumption of both the breakfast and lunch test meals to assess the palatability. VAS sheets were based on a 100 mm scale with the left anchor point being 'not at all' and the right anchor point being 'extremely much'. The order of the VAS questions assessing sensations of satiety and hunger was randomized between time points (Supplementary files, Table 1). On the final experimental day, the participants completed a 16-question DEBQ adapted from Strien et al. (van Strien et al., 1986) after lunch (Supplementary Files, Table 1). The questionnaire, split into 3 sections (restrained, emotional, and external), assessed dominant eating behaviors, and participants were evaluated using the average score (1-5) from each section.

### Paced Auditory Serial Addition Task (PASAT)

The PASAT test (Tombaugh, 2006) assessed information processing speed and attention differences between experimental days and was conducted in a separate and quiet room. The participants were by randomization assigned to either an A or B test sheet. The test consists of 61 single-digit numbers presented at 3-s intervals by an audio file. During the test, the participants had to add each number to the previous one and say the result

out loud. The test personnel recorded the result, and the percentage of correct answers was calculated.

### Body composition

All participants had their body composition determined by a GE Lunar iDXA series scanner (GEHealthcare, Madison, WI, United States) equipped with the enCORE software v16.0 (GEHealthcare, Madison, WI, United States). Scans were performed in the morning after the participants had fasted overnight.

### Sample size calculation

With reference to a previous comparable study (Nielsen et al., 2018), it was calculated that 30 participants were needed to detect a mean difference of 400 kJ in the ad libitum lunch between experimental days when assuming a standard deviation (SD) of 750 kJ, 80% power and a significance level of 0.05. The trial was stopped as the wanted sample size was acquired.

### Statistical analyzes

Statistical analyzes were done with GraphPad Prism 9 (GraphPad Software, California) at a 0.05 significance level. PASAT scores were analyzed using Stata/IC16 (StataCorp, College Station, TX, USA), as the data had to be adjusted for order effect ( $P < 0.001$ ). We found no order effect on ad libitum lunch energy intake ( $P = 0.58$ ) or total daily energy intake on experimental days ( $P = 0.71$ ). Values of biological origin such as appetite- and satiety-regulating gut hormones and plasma levels of glucose and insulin were not tested for order effect due to the acute nature of the study interventions. All participants who completed all 3 experimental days and had valid EI, TEI, and blood sampling data from at least 2 of those were included in the respective analyzes. Five participants were excluded from the analyzes of TEI and intake of macro- and micronutrients due to Goldberg's cut-off limit ( $n = 4$ ) and missing data ( $n = 1$ ). Furthermore, 5 participants did not provide blood samples ( $n = 25$  missing samples). In the analyzes of blood hormone levels, outliers ( $\text{mean} \pm 1.96 \times \text{SD}$ ) ( $n = 3$  samples for ghrelin) and samples with coefficients of variation (CV)  $> 15\%$  were excluded from the final statistical analyzes ( $n = 53, 92,$  and  $8$  samples for ghrelin, CCK, and GLP-1, respectively). QQ plots were used to assess the normal distribution, and insulin data was log-transformed as it was non-normally distributed. Adjusted PASAT scores were analyzed with a mixed-effects model using intervention as fixed effect and subject as random effect, and a post hoc Bonferroni correction was performed. To analyze for

differences in ad libitum lunch EI between the intervention days, we used a one-way repeated measurements (RM) ANOVA. A mixed-effects model was used with intervention (PRO, CHO, CON) in the fixed part of the model and participants in the random part to analyze for differences in TEI and daily dietary carbohydrate, protein, fat, fiber, and calcium intake between intervention days. If significant differences were found, post hoc Tukey's multiple comparisons tests were conducted. Changes in VAS scores and hormonal- and glucose levels were analyzed by mixed-effects models with time point (pre to post time points) and intervention (PRO, CHO and CON) as independent variables in the fixed part of the model and participants were included in the random part of the model. Data was analyzed for main effects and any interaction between the 2 independent variables. If a significant time  $\times$  intervention interaction or main effect was found, Tukey's multiple comparisons test was conducted. The AUCs for appetite and satiety sensations were based on the interval from pre-breakfast to post-lunch on each experimental day, and were first calculated individually for each participant in a spreadsheet, using the trapezoidal rule, due to a limitation in the statistical software, before being analyzed on a group level with one-way RM ANOVAs for each sensation. If significant differences were found, post hoc Tukey's multiple comparisons tests were conducted. Data from ad libitum lunch EI, TEI, VAS scores, PASAT scores, and plasma glucose and hormonal levels are presented as the mean  $\pm$  standard error of the mean (SEM) unless stated otherwise.

## RESULTS

### **Ad libitum energy intake and total daily energy intake**

There were no differences in ad libitum EI at +180 min after consumption of the 3 breakfast test meals ( $P \geq 0.13$ ) (Figure 3A). Furthermore, the ad libitum EI was not affected by order ( $P \geq 0.61$ ). TEI intake did not differ between experimental days ( $P \geq 0.74$ ) (Figure 3B), nor did TEI differ between the days before the experimental days ( $P \geq 0.49$ ).

### **VAS scores and AUC for satiety and appetite sensations**

Baseline ratings of satiety and appetite sensations did not differ between the 3 experimental days (all  $P \geq 0.39$ ). Satiety, fullness, and satisfaction were higher after PRO and CHO, compared with CON at all time points between +10 and +170 min (all  $P \leq 0.02$ ) (Figure 4ACE). VAS scores for satiety and fullness were

higher after PRO compared with CHO at all time points between +10 and +170 min (all  $P \leq 0.02$  and all  $P \leq 0.01$ , respectively). Furthermore, satisfaction for PRO was significantly higher than after CHO at +30 ( $P = 0.03$ ) and +90 to +170 min (all  $P \leq 0.01$ ) (Figure 4E). At +180 min none of the parameters differed significantly between the experimental days (all  $P \geq 0.24$ ) (Figure 4ACE). AUC for satiety, fullness, and satisfaction were higher for PRO and CHO than for CON (all  $P < 0.001$ ) (Figure 4BDF). Regarding satiety, fullness, and satisfaction, the AUCs were 29% ( $P < 0.001$ ), 41% ( $P < 0.001$ ), and 26% ( $P < 0.001$ ) larger for PRO than CHO, respectively (Figure 4BDF).

Hunger, desire to eat, and prospective eating were lower for PRO and CHO than CON for all time points between +10 and +170 min (all  $P < 0.001$  and all  $P \leq 0.02$ , respectively) (Figure 4GJL). In addition, hunger and prospective eating were lower for PRO compared with CHO at all time points between +10 and +170 min (all  $P \leq 0.02$ ). Desire to eat was lower for PRO compared with CHO at +10 ( $P = 0.002$ ), +30 min ( $P = 0.013$ ), and +90 min ( $P < 0.001$ ), as well as between +150 and +170 min (all  $P \leq 0.02$ ) (Figure 4J). AUC for hunger, desire to eat, and prospective eating were lower for PRO and CHO compared with CON (all  $P < 0.001$ ) (Figure 4HKM). AUC for hunger, desire to eat, and prospective eating were 36% ( $P < 0.001$ ), 30% ( $P < 0.001$ ), and 31% ( $P < 0.001$ ) lower for PRO than CHO, respectively (Figure 4HKM).

### **VAS scores for palatability sensations**

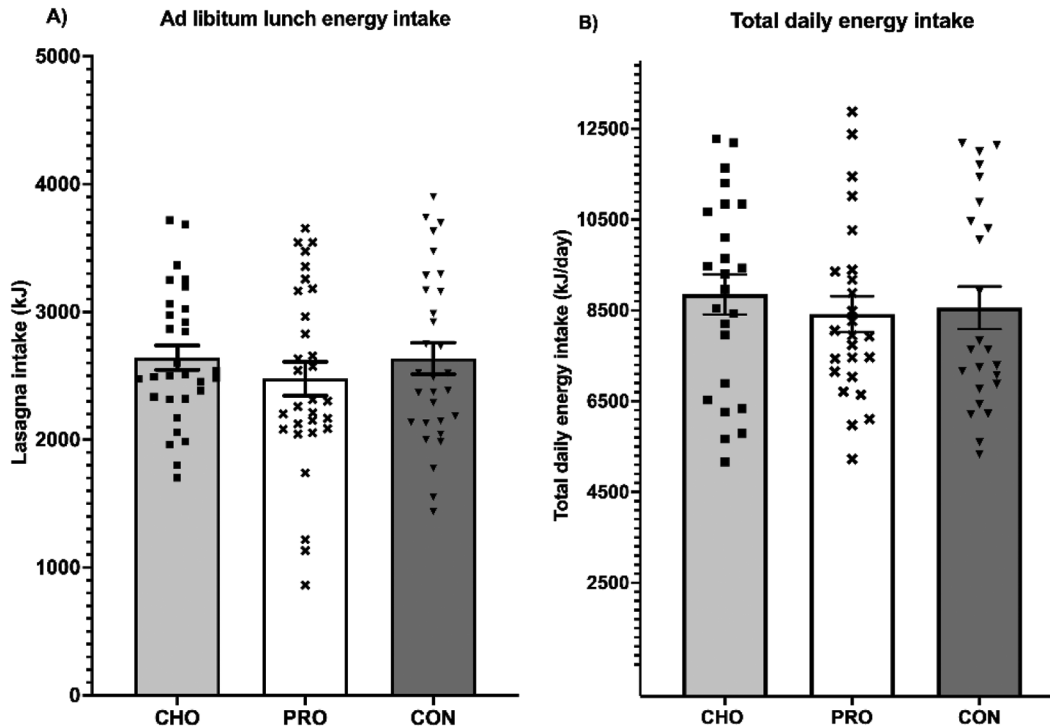
Table 3 presents the results of VAS scores assessing the palatability of breakfast and lunch meals during consumption. During breakfast meals no differences were detected for general liking of the meal ( $P = 0.53$ ), liking of appearance ( $P = 0.16$ ), smell ( $P = 0.28$ ), flavor ( $P = 0.87$ ), or texture ( $P = 0.36$ ), however, overall palatability was 29.5% lower ( $P = 0.02$ ) for PRO compared with CHO. No differences in palatability scores were detected during the ad libitum lunch meal (all  $P \geq 0.12$ ).

### **PASAT scores**

PASAT scores are presented in Figure 5. PRO was 3.5  $\pm$  1.4 ( $P = 0.03$ ) %-points higher than CON. However, the PASAT scores did not differ significantly between PRO and CHO or between CHO and CON.

### **Plasma glucose and serum insulin**

Plasma glucose and serum insulin levels are presented in Figure 6. Baseline glucose and insulin levels did not



**Figure 3.** A) Lasagna intake in kilojoules (kJ) during the ad libitum lunch and B) total daily energy intake in kJ/day for 3 separate experimental days after intake of a high-protein low-carbohydrate breakfast test meal (PRO, ©), a low-protein high-carbohydrate breakfast test meal (CHO, ∪), and a control omitting breakfast (CON, ∅). Lasagna intake was analyzed by a repeated measures one-way ANOVA and total daily energy intake was analyzed by a mixed-effects model. Data are presented as means  $\pm$  SEM. A)  $n = 30$ , B)  $n = 25$ .

differ between experimental days (all  $P \geq 0.88$  and all  $P \geq 0.65$ , respectively). From +10 to +120 min CHO and PRO plasma glucose levels were higher than CON (range: 6–44%, all  $P < 0.001$  and range: 5–23%, all  $P \leq 0.01$ , respectively). In addition, plasma glucose levels for PRO were 13% ( $P < 0.001$ ), 17% ( $P < 0.001$ ), and 15% ( $P < 0.001$ ) lower than CHO at +10, +30, and +60 min, respectively. For insulin, PRO and CHO were higher than CON at all time points from +10 to +60 min (range: 41–45%, all  $P < 0.001$  and range: 31–40%, all  $P < 0.001$ , respectively). No differences between PRO and CHO were detected, however, at +10 min there was a tendency ( $P = 0.07$ ) for PRO to be higher than CHO. At +170 min there were no differences in plasma glucose and serum insulin levels after the 3 breakfast test meals (all  $P \geq 0.06$  and all  $P \geq 0.20$ , respectively).

### Appetite-regulating gut hormones

Plasma CCK, GLP-1, and ghrelin levels are presented in Figure 7. Baseline plasma CCK, GLP-1, and ghrelin levels did not differ between experimental days (all  $P \geq 0.62$ , all  $P \geq 0.14$ , and all  $P \geq 0.99$  respectively). Mixed-effects analyses of CCK- and GLP-1 levels revealed no

significant effect of breakfast test meals (Figure 7AB). Ghrelin (Figure 7C) was higher for PRO at 170 min compared with PRO at pre ( $P = 0.03$ ) as well as CON at 10 ( $P = 0.02$ ) and 60 min ( $P = 0.02$ ). Also, CON at 170 min was higher than CON at 10 min ( $P = 0.04$ ).

### Micro- and macronutrients

The total daily dietary intake of protein, carbohydrates, fat, calcium, and fiber for all test days is presented in Table 4. Total daily dietary intake of protein was higher for PRO ( $105 \pm 5$  g/day) compared with CHO ( $+27 \pm 5$  g/day,  $P < 0.001$ ) and CON ( $+26 \pm 5$  g/day,  $P < 0.001$ ). The total daily dietary intake of carbohydrates did not differ between experimental days ( $P = 0.09$ ). In terms of dietary fat ( $P = 0.16$ ) and fiber ( $P = 0.20$ ) intake, no differences were detected. Dietary calcium intake tended to differ significantly between the experimental days ( $P = 0.08$ ), which was related to PRO showing a tendency to be higher than CHO ( $P = 0.07$ ).

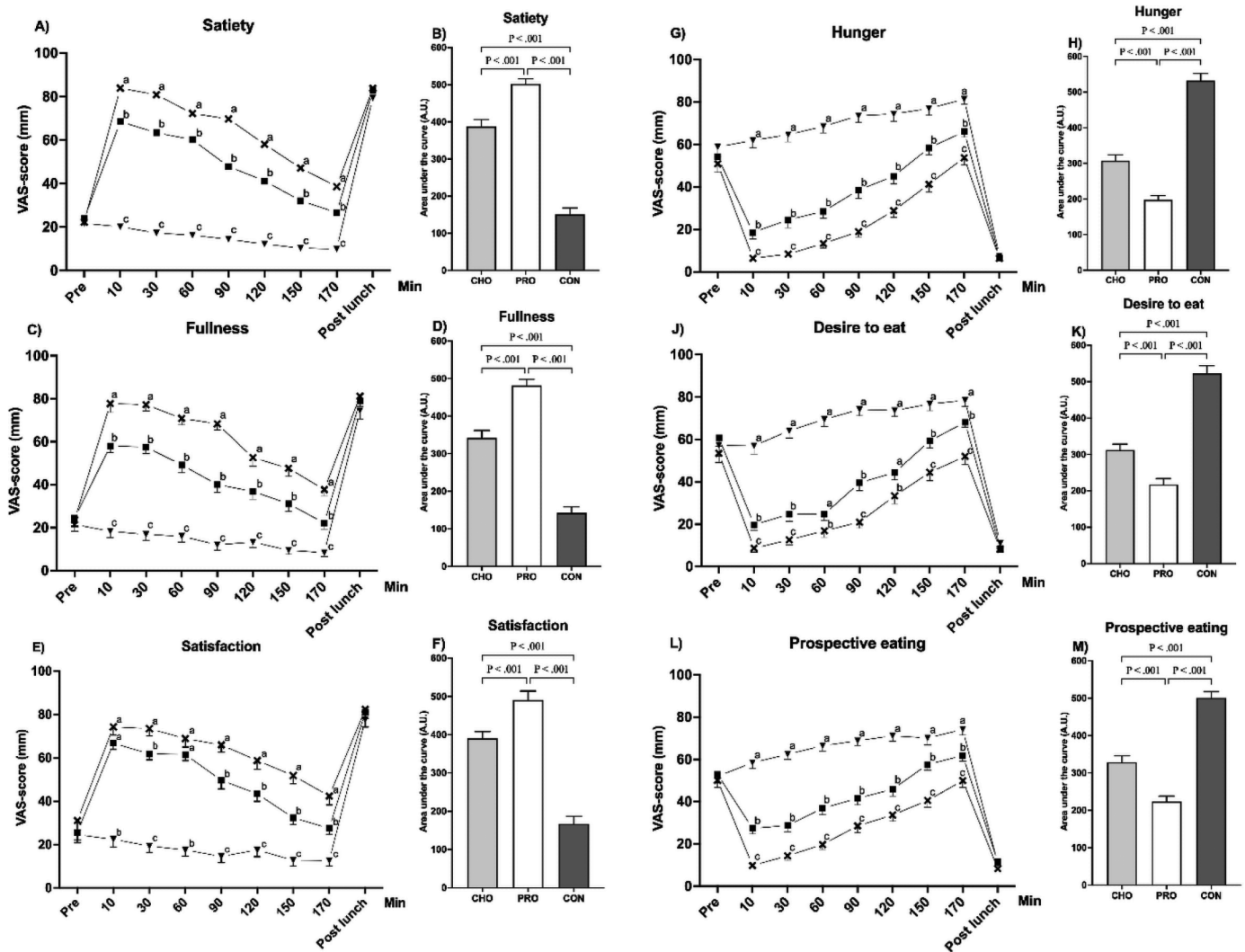


## DISCUSSION

From a real-world standpoint, our study design introduced a novel approach by examining the satiety impact of 2 breakfast options based on commercially available foods commonly consumed for breakfast in the Nordic countries. This approach allowed us to compare the effects of these breakfast choices with skipping breakfast. Our main findings were that young females with overweight to obesity felt more satiated and less hungry after a dairy-based high-protein low-carbohydrate breakfast (PRO) compared with an iso-

caloric low-protein high-carbohydrate breakfast (CHO) or no breakfast (CON). However, this was not reflected in the plasma levels of appetite- and satiety-regulating gut hormones, the ad libitum EI at lunch, and the TEI on the experimental days. Interestingly, only PRO induced an improvement in cognitive concentration 2 1/2 h after breakfast, as compared with CON.

The increased sensation of satiety and the reduced sensation of hunger in response to PRO as compared with CHO or CON, are aligned with our hypothesis. Similarly, others have observed enhanced satiety after eating protein-rich breakfast meals compared with iso-



**Figure 4.** Ratings of sensations in visual analog scales (VAS), and corresponding areas under the curve (AUC), for satiety (A & B), fullness (C & D), satisfaction (E & F), hunger (G & H), desire to eat (J & K), and prospective eating (L & M) at specific time points throughout 3 separate experimental days after intake of a high-protein low-carbohydrate breakfast test meal (PRO,  $\odot$ ), a low-protein high-carbohydrate breakfast test meal (CHO,  $\nu$ ), and a control omitting breakfast (CON,  $\theta$ ). Different superscripted letters (a, b, and c) at individual time points indicate statistical differences between interventions ( $P < 0.05$ ). Data was analyzed using a mixed-effects model with time point and intervention (PRO, CHO and CON) as fixed factors and participant as random factor. If significant time point  $\times$  intervention effects were observed Tukey's multiple comparisons tests were performed to elucidate effects between interventions at specific time points. AUCs were compared by one-way repeated measurements ANOVAs with Tukey's correction applied. Data are presented as means  $\pm$  SEM  $n = 30$ .

caloric control meals, especially if the protein content was above 30 g of protein (Leidy et al., 2015). Nevertheless, in contrast to our study, most previous studies have tested the effect of test meals specifically designed for the experiment (Boelsma et al., 2010; Kung et al., 2018; Leidy & Racki, 2010; Veldhorst et al., 2009) or with a higher energy content (3–4 MJ) (Belza et al., 2013; Nielsen et al., 2018) than normally consumed for breakfast in the Nordic countries (Gibney et al., 2018). Boelsma et al. reported no difference in appetite and satiety sensations in the 4 h after either a high-protein low-carbohydrate breakfast or an isocaloric high-carbohydrate low-protein breakfast in a group of young males with normal-to-overweight (Boelsma et al., 2010). The discrepancy between the findings of Boelsma et al. and our findings may be related to BMI status (normal-to-overweight vs. overweight-to-obese). Substitution of classic carbohydrate-rich breakfast meals (e.g., bread and cereals) with protein-rich foods (e.g., high-protein dairy) may more effectively enhance satiety sensations in obese compared with normal-weight individuals. This is supported by the observation that people with obesity seem to have depressed sensitivity to the anorexigenic hormone insulin (Flint et al., 2007; Hwang et al., 2017) and the finding that adults with obesity and Type 2 diabetes mellitus show a blunted rise in brain glucose during hyperglycemia (Hwang et al., 2017). The latter observation was linked to a reduced feeling of fullness (Hwang et al., 2017). Another likely explanation for Boelsma et al. (2010) not showing a positive effect of enhanced protein content on satiety is likely because they served liquid meals compared with primarily solid foods in our study. A liquid meal does not

suppress appetite to the same extent in the hours after a meal as a semi-solid meal, which is related to lower gastric retention (Mackie et al., 2013). Therefore, as a strategy to prevent further weight gain in populations with overweight, it is more relevant to improve the satiating effect of semi-solid meals, as done in our study, rather than focusing on liquid meal interventions.

Even though we observed clear differences in satiety and appetite sensations after the 3 breakfast test meals, the ad libitum lunch EI and TEI on experimental days did not differ significantly between breakfast test meals. This is consistent with the generally reported discrepancy between changes in satiety and appetite sensations in response to different test meals and EI during a subsequent ad libitum meal (Belza et al., 2013; Boelsma et al., 2010; Veldhorst et al., 2009). In a review by Leidy et al., it was reported that ~71% (17/24) of the included studies found positive changes in appetite ratings in response to high- versus low-protein test meals (Leidy et al., 2015). However, only ~18% (3/17) of these studies reported an aligned reduction in the EI of a subsequent ad libitum meal served 2–4 h after the test meal. In general, discrepancies between changes in satiety and appetite sensations and changes in subsequent meal EI may be explained by an inherent flaw in the fixed-meal-times study design. The timing of test meals in our study was utilized to reflect free-living conditions; however, it may be hypothesized that a relationship between changes in satiety and appetite sensations and reduced subsequent meal EI could be detected if participants were allowed to consume the subsequent meal when they wanted to, instead of at a fixed time point. It may also be proposed that par-

**Table 3.** Ratings in Visual analog scales (VAS) regarding palatability of breakfast- and lunch meals

	PRO <sup>1</sup>	CHO <sup>2</sup>	CON <sup>3</sup>	P-value
Breakfast test meal (n = 30)				
General liking of meal	48.4 ± 4.2	57.1 ± 3.1	— <sup>4</sup>	0.525
Liking of appearance	40.4 ± 4.3	52.6 ± 3.1	—	0.155
Liking of smell	51.8 ± 4.4	62.6 ± 3.4	—	0.273
Liking of flavor	52.8 ± 4.5	58.6 ± 2.6	—	0.873
Liking of texture	48.2 ± 4.7	57.9 ± 3.5	—	0.360
Overall palatability	38.9 ± 4.6 <sup>a</sup>	55.2 ± 4.0	—	0.020
Ad libitum lunch meal (n = 30)				
General liking of meal	68.6 ± 3.8	69.9 ± 2.9	71.1 ± 3.3	0.608
Liking of appearance	52.8 ± 4.6	54.5 ± 4.0	54.5 ± 4.0	0.871
Liking of smell	77.1 ± 2.8	79.1 ± 2.6	81.2 ± 2.3	0.121
Liking of flavor	68.5 ± 3.7	70.7 ± 3.0	67.9 ± 3.5	0.500
Liking of texture	66.7 ± 4.0	72.1 ± 3.5	70.1 ± 4.1	0.143
Overall palatability	61.2 ± 5.0	63.1 ± 4.4	59.5 ± 4.6	0.430

<sup>1</sup>PRO, high-protein low-carbohydrate breakfast test meal.

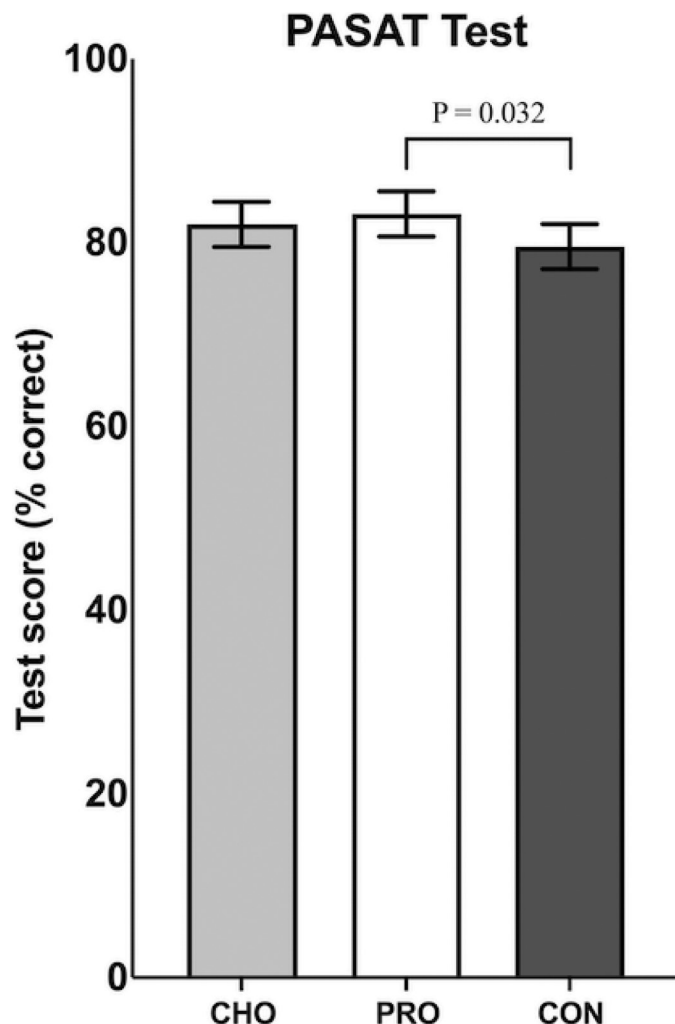
<sup>2</sup>CHO, low-protein high-carbohydrate breakfast test meal.

<sup>3</sup>CON, control omitting breakfast.

<sup>4</sup>VAS-sheets were not completed during the CON breakfast.

<sup>a</sup>Mixed-effects models showed significant difference from CHO ( $P < 0.05$ ).

Data are presented as means ± SEM n = 30.



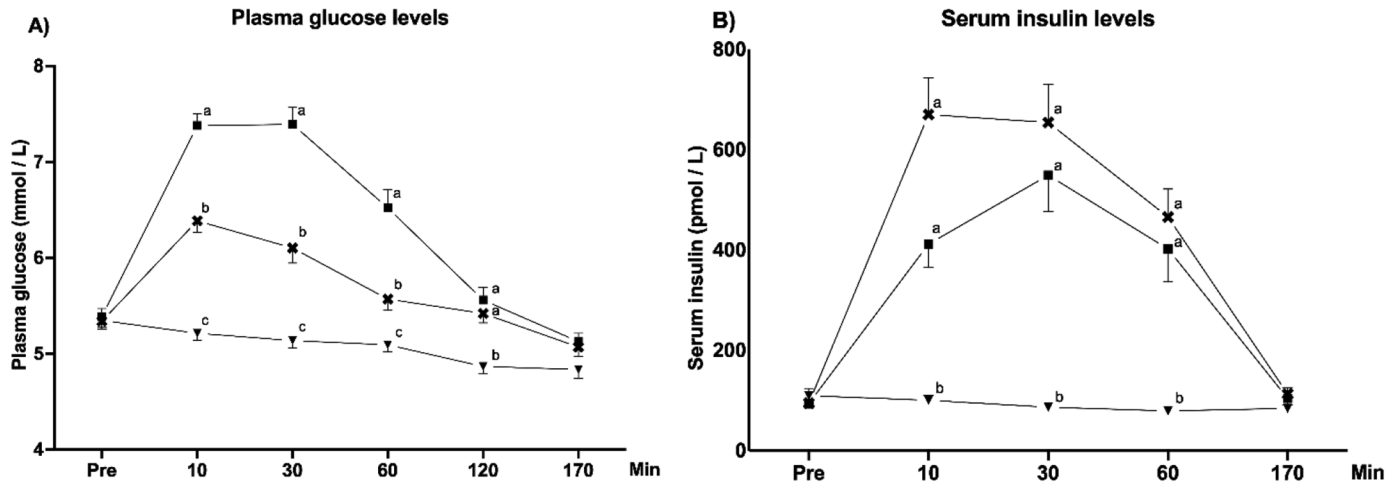
**Figure 5.** Paced Auditory Serial Addition Task (PASAT) scores on 3 separate experimental days after intake of a low-protein high-carbohydrate breakfast test meal (CHO, gray bar) a high-protein low-carbohydrate breakfast test meal (PRO, white bar), and a control omitting breakfast (CON, black bar). PASAT results were adjusted for an order effect and analyzed by a mixed-effects model. Data are presented as means  $\pm$  SEM  $n = 30$ .

Participants simply are accustomed to eating a meal of a certain size, whereby, habits overruled differences in the satiating effects between the breakfast test meals. Finally, our study participants scored moderate-to-high on the modified DEBQ, indicating an externally conditioned disturbed eating behavior (Table 1); possibly influencing the results related to the ad libitum lunch EI and TEI, as the participants may have constrained themselves because their food behavior also seem to be affected by other factors than their sensations of satiety and hunger.

The plasma levels of the appetite- and satiety-regulating gut hormones, CCK, GLP-1, and ghrelin showed no time  $\times$  intervention effects. A review concluded

that studies investigating high- versus low-protein meals showed divergent findings in terms of hormonal responses. However, no studies have shown effects on satiety hormones in favor of low- compared with high-protein meals (Leidy et al., 2015), whereas favorable effects have been reported in some studies after eating high- compared with low-protein meals (Belza et al., 2013; Leidy et al., 2013). Divergent findings may be explained by differences in the content and type of protein, but also differences in meal timing, the macronutrient composition of meals, the use of liquid vs. solid meals, and the energy content (Leidy et al., 2015). Belza et al. demonstrated the potential impact of the postprandial time frame on the appetite-regulating gut hormones (Belza et al., 2013). They found that postprandial ghrelin levels were diminished during the initial 30–150 min following a high-protein breakfast, in contrast to after a normal-protein or medium-high-protein breakfast. However, this effect was not observed at the 180 and 240-min marks following the test meals. The later observations could indicate the timing of the ad libitum lunch might influence the actual EI. Nevertheless, Belza et al. (2013) reported a positive effect of a high-protein meal on the satiety hormone CCK was more pronounced 2–4 h after the meal compared with 0–2 h. Still, no dose-dependent effect of the breakfast protein content was observed on EI at a lunch meal after 4 h. The diverging effects of protein intake on the different appetite-regulating hormones underline that regulation of EI is complex and measurements of single hunger- or satiety-regulating hormones may not predict sensations of hunger or satiety and the following EI.

Interestingly, we found that postprandial plasma insulin levels were elevated to a similar extent after intake of a high-protein low-carbohydrate breakfast compared with a low-protein high-carbohydrate breakfast, even though the plasma glucose levels were more elevated 10–60 min after the low-protein high-carbohydrate breakfast test meal compared with the high-protein low-carbohydrate breakfast test meal. The latter finding is not surprising based on the meal composition of low compared with high carbohydrates. Supporting our findings of a comparable insulin response, Boelsma et al. (Boelsma et al., 2010) reported no differences in the total AUC for insulin when comparing low- and high-protein breakfasts. However, Belza et al. observed significantly lower plasma insulin concentrations 30 and 150 min after consumption of a high-protein breakfast (88.4 g protein) compared with after isocaloric normal- (24.3 g protein) or medium-high protein breakfast meals (44.5 g protein) in a group of young males with overweight to obesity (Belza et al., 2013). The discrepancies between our findings and those of Belza et al. (2013) are likely explained by differences in the

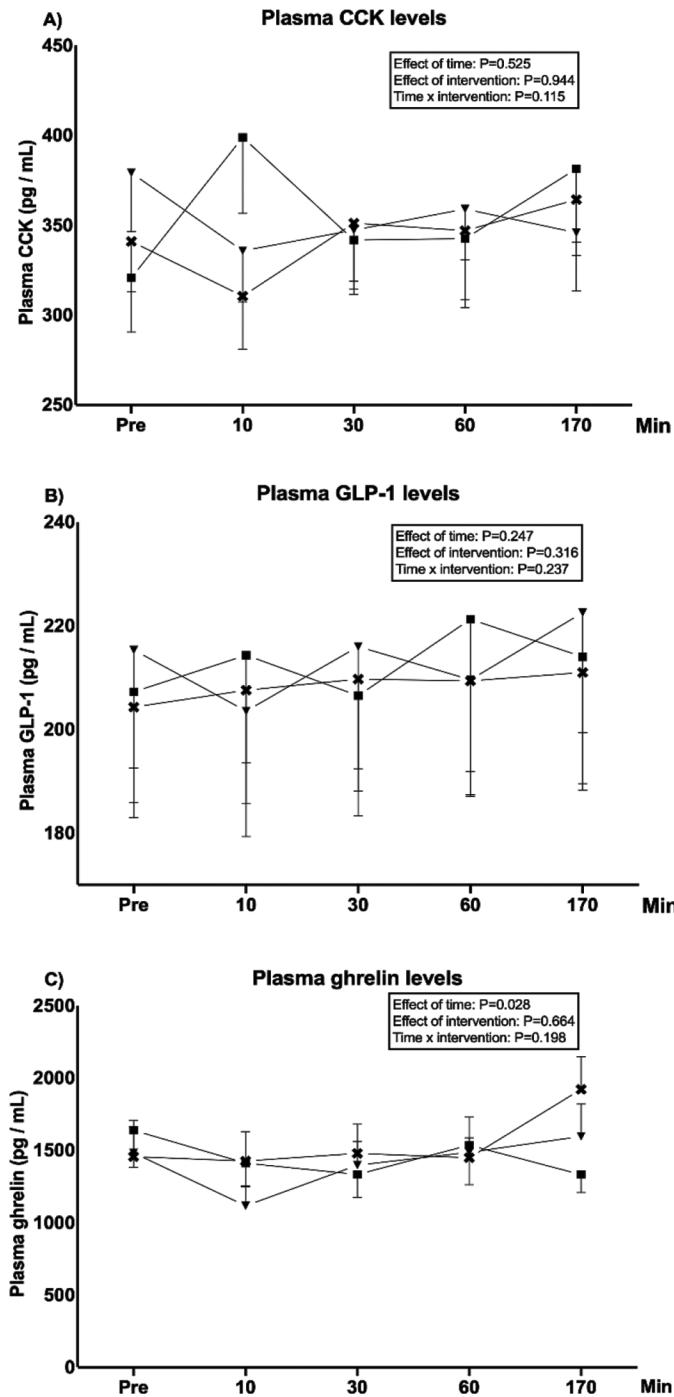


**Figure 6.** Plasma glucose and serum insulin levels at specific time points throughout 3 separate experimental days. Plasma glucose levels (A) (mmol/L) and serum insulin levels (B) (pmol/L) before breakfast consumption (Pre) and 10, 30, 60, 120 (only plasma glucose), and 170 min after breakfast consumption on 3 separate experimental days. Breakfast test meals included a high-protein low-carbohydrate breakfast test meal (PRO, ◻), a low-protein high-carbohydrate breakfast test meal (CHO, ◉), and a control omitting breakfast (CON, ◊). Different superscripted letters (<sup>a</sup>, <sup>b</sup>, and <sup>c</sup>) at individual time points indicate statistical differences between interventions ( $P < 0.05$ ). Data was analyzed using a mixed-effects model with time point and intervention (PRO, CHO and CON) as fixed factors and participant as random factor. If significant time point  $\times$  intervention effects were observed Tukey's multiple comparisons tests were performed to elucidate effects between interventions at specific time points. Data are presented as means  $\pm$  SEM. Glucose  $n = 30$ . Insulin  $n = 25$ .

insulinotropic effect of the protein sources, differences in subject characteristics, or a greater difference in carbohydrate content between the test meals in Belza et al. (2013) (33 g versus 75 g and 95 g carbohydrate in the isocaloric high-, medium-, and normal-protein breakfast meals, respectively). The primary protein sources in the breakfast meals of Belza et al. (2013) were ham and eggs, whereas those in our study were based on dairy protein. Certain types of proteins, especially dairy proteins, have been shown to have insulinotropic properties (Comerford & Pasin, 2016; Manders et al., 2014; Veldhorst et al., 2009). The insulinotropic effect of protein ingestion seems to be more pronounced in type-2 diabetics than in healthy individuals (Comerford & Pasin, 2016; Nuttall et al., 1984). Since BMI and insulin sensitivity are closely related, it might suggest that the higher average BMI (BMI 30.0) in our study compared with Belza et al. (2013) (BMI 25.8) has stimulated a greater insulinotropic effect of protein ingestion in our study, however, this is speculative.

Performance in the cognitive concentration test was improved 2 1/2 h after ingestion of the high-protein low-carbohydrate breakfast, but not the low-protein high-carbohydrate breakfast, compared with omitting the breakfast meal. To the best of our knowledge, the cognitive effects of acute protein intake in young females with overweight to obesity have not been previously investigated. However, our findings align with previous findings in healthy non-obese adults (Muth & Park, 2021). Muth and Park reported in a review that

a short-term (3 weeks) high protein intake (3.0 g/kg BW) was associated with a short-term enhancement of reaction time in a demanding cognitive function test when compared with a normal protein intake (1.5 g/kg BW). Young, healthy men have been reported to improve their performance across a comprehensive battery of cognitive tests in response to a very high-protein breakfast meal (~76 g) with low carbohydrate content (Fischer et al., 2002). This improvement was in contrast to a more balanced breakfast meal with high-protein content (~47 g) and normal carbohydrate level, as well as in comparison to a standard-protein breakfast (~19 g) with a high carbohydrate component. The suggested mechanism behind the association between a high protein-to-carbohydrate ratio in meals and enhanced cognitive performance has been related to increased plasma concentrations of amino acids, especially tyrosine and tryptophan, which are involved in the metabolism of the neurotransmitters dopamine and serotonin (Muth & Park, 2021). Furthermore, the branched-chain amino acid leucine, which is highly abundant in dairy products, has been shown to enhance glucose sensing in the brains of healthy and obese humans (Comerford & Pasin, 2016), thereby potentially influencing brain function. Nevertheless, in healthy humans, acute carbohydrate intake has also been proposed to improve cognitive function by increasing plasma insulin levels, thereby increasing brain glucose uptake (García et al., 2021). Interestingly, Hwang et al. (Hwang et al., 2017) showed a lower glucose uptake in the brains of obese



**Figure 7.** Plasma cholecystokinin (CCK), glucagon-like peptide-1 (GLP-1), and ghrelin levels at specific time points throughout 3 separate experimental days. Plasma concentrations (pg/mL) of CCK (A), GLP-1 (B), and ghrelin (C) before breakfast consumption (Pre) and 10, 30, 60, and 170 min after breakfast consumption on 3 separate experimental days. Breakfast test meals included a high-protein low-carbohydrate breakfast test meal (PRO,  $\odot$ ), a low-protein high-carbohydrate breakfast test meal (CHO,  $\triangle$ ), and a control omitting breakfast (CON,  $\square$ ). Boxes show p-values related to the effects of time, intervention, and time  $\times$  intervention from mixed-effects models. Data are presented as means  $\pm$  SEM  $n = 25$ .

non-diabetic and diabetic humans in response to standardized glucose exposure (hyperglycemic clamp) when compared with healthy humans. The latter observation may suggest that the positive effect of glucose ingestion on cognitive performance might be impaired in populations with overweight to obesity, and could explain why we did not observe improved performance in the cognitive concentration test after the carbohydrate-rich meal compared with breakfast skipping.

A general limitation of our study was that the findings cannot necessarily be extrapolated to populations with other characteristics. In terms of the specific limitations of our study, the results from the modified DEBQ indicated that our participants may have constrained themselves when eating on the experimental days instead of following their sensations of satiety and hunger. In a group of less restricted eaters, we might have observed a better alignment between the hunger and satiety sensations and the subsequent meal EI and the TEI during the experimental days. However, we aimed to recruit a representative group of young females with overweight to obesity since the risk of further weight gain is highly relevant in this specific population in perspective to reducing the risk of further weight gain. We acknowledge that the dropout percentage is high, however, when comparing characteristics (age and BMI) of the completers ( $24.2 \pm 2.2$  years;  $30.0 \pm 3.5$  kg/m<sup>2</sup>) and drop-outs ( $24.2 \pm 3.3$  years;  $30.1 \pm 5.8$  kg/m<sup>2</sup>) there are no differences, thereby, we do not think it has affected our results. In addition, the timing of the subsequent ad libitum meal was determined beforehand. The EI at lunch and TEI during the experimental days might have been different if there had been no food restrictions during the first hours following the breakfast test meals. We find it relevant to look into the timing of the effects of the meals on top of the overall analyzes as people may eat when feeling hunger or when food is available at an earlier time point after breakfast in a real-life scenario. However, we acknowledge that a limitation of this approach is that it results in a large number of statistical analyzes, whereby, we must interpret statistical significance with caution. Nevertheless, despite the above-mentioned limitations, we consider our study design to be strong because we utilized a robust blinded-crossover study design, based on our breakfast test meals on typical commercially available foods in the Nordic countries, matched the energy content, energy density, fat content, and dietary fiber content of the breakfast test meals, and finally, we included a reasonable sample size.

In conclusion, eating a dairy-based high-protein low-carbohydrate breakfast reduces hunger and enhances satiety in the subsequent hours in young females with overweight to obesity when compared with a low-protein

**Table 4.** Daily dietary macro- and micronutrient intake on the experimental days

Nutrient <sup>1 2</sup>	Experimental day			P-value
	PRO <sup>3</sup>	CHO <sup>4</sup>	CON <sup>5</sup>	
Protein intake (g/day)	105 ± 5 <sup>a</sup>	77 ± 4	79 ± 6	<0.001
Carbohydrate intake (g/day)	220 ± 14	257 ± 15	228 ± 16	0.098
Fat intake (g/day)	71 ± 5	81 ± 6	86 ± 6	0.163
Fiber intake (g/day)	18 ± 1	19 ± 1	16 ± 2	0.201
Calcium intake (mg/day)	930 ± 60	721 ± 91	791 ± 96	0.082

<sup>1</sup>g, grams.<sup>2</sup>mg, milligrams.<sup>3</sup>PRO, high-protein low-carbohydrate breakfast test meal.<sup>4</sup>CHO, low-protein high-carbohydrate breakfast test meal.<sup>5</sup>CON, control omitting breakfast.<sup>a</sup> Mixed-effects models showed significant difference from CHO and CON ( $P < 0.05$ ).Data are presented as means ± SEM  $n = 25$ .

high-carbohydrate breakfast or omitting breakfast. In addition, cognitive performance before lunch improved after the high-protein low-carbohydrate breakfast as compared with no breakfast. However, EI at lunch and TEI for the remainder of the day did not differ significantly between the breakfast test meals, which might indicate that the effect of the meal on the regulation of EI is only effective in the first couple of hours. On the other hand, small non-significant differences in TEI may add up and have a significant effect on body composition after a prolonged period. Therefore, long-term studies are needed to investigate further how the composition of breakfast meals influences body composition and health parameters.

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Author contributions M.H., L.B.D., and B.V.A. conceived and planned the experiment. M.H., L.B.D., D.Z.K., and K.N. carried out the experiments. D.Z.K. and K.N. analyzed data under the guidance of M.H. M.H., D.Z.K., L.B.D., B.V.A., and K.N. contributed to the interpretation of the results. D.Z.K. took the lead in writing the manuscript. All authors provided critical feedback and helped shape the research, analyzes, and manuscript.

Competing interests The authors declare that they have no competing interests.

Supplementary Files [https://figshare.com/articles/online\\_resource/Supplementary\\_Files/24042975/5](https://figshare.com/articles/online_resource/Supplementary_Files/24042975/5)

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