Effects of a breakfast yoghurt, with additional total whey protein or caseinomacropeptide-depleted α-lactalbumin-enriched whey protein, on diet-induced thermogenesis and appetite suppression

Rick Hursel[1]*, Lucie van der Zee[2] and Margriet S. Westerterp-Plantenga[1]

[1]*Department of Human Biology, The Nutrition and Toxicology Research Institute Maastricht (NUTRIM), Maastricht University, PO Box 616, 6200 MD Maastricht, The Netherlands*
[2]*Friesland Foods, PO Box 226, 8901 MA Leeuwarden, The Netherlands*

Previous studies have shown effects of high-protein diets, especially whey protein, on energy expenditure and satiety, yet a possible distinction between the effects of whey or α-lactalbumin has not been made. The present study assessed the effects of the addition of total whey protein (whey) or caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (α-lac) to a breakfast yoghurt drink on energy expenditure and appetite suppression in human subjects. A total of eighteen females and seventeen males (aged 20·9 (SD 1·9) years; BMI 23·0 (SD 2·1) kg/m^2) participated in an experiment with a randomised, three-arm, cross-over design where diet-induced energy expenditure, respiratory quotient and satiety were measured. Breakfasts were isoenergetic and subject-specific: a normal-protein (NP) breakfast consisting of whole milk (15, 47 and 38 % energy from protein, carbohydrate and fat, respectively), a high-protein (HP) breakfast with additional whey or a HP breakfast containing α-lac (41, 47 and 12 % energy from protein, carbohydrate and fat, respectively). Resting energy expenditure did not differ between the three conditions. HP breakfasts (area under the curve: whey, 217·1 (SE 10·0) kJ × 4 h; α-lac, 234·3 (SE 11·6) kJ × 4 h; $P<0·05$) increased diet-induced thermogenesis more compared with a NP yoghurt at breakfast (179·7 (SE 10·9) kJ × 4 h; $P<0·05$). Hunger and desire to eat were significantly more suppressed after α-lac (hunger, −6627 (SE 823); desire to eat, −6750 (SE 805) mm visual analogue scale (VAS) × 4 h; $P<0·05$) than after the whey HP breakfast (hunger, −5448 (SE 913); desire to eat, −5070 (SE 873) mm VAS × 4 h; $P<0·05$). After the HP breakfasts, a positive protein balance occurred (α-lac, 0·35 (SD 0·18) MJ/4 h; whey, 0·37 (SD 0·20) MJ/4 h; $P<0·001$); after the NP breakfast a positive fat balance occurred (1·03 (SD 0·29) MJ/4 h; $P<0·001$). In conclusion, consumption of a breakfast yoghurt drink with added whey or α-lac increased energy expenditure, protein balance and decreased fat balance compared with a NP breakfast. The α-lac-enriched yoghurt drink suppressed hunger and the desire to eat more than the whey-enriched yoghurt drink.

Obesity: High protein intake: Milk protein: Energy expenditure: Protein balance

The obese population is growing worldwide as energy intake exceeds energy expenditure and consequently more individuals are at risk of developing obesity-related diseases such as type 2 diabetes mellitus and CVD[1]. High-protein diets have been shown to induce weight loss and weight maintenance after a weight-loss period, through an appetite-suppressing effect and increased diet-induced thermogenesis (DIT), as proteins are not energy efficient[2–4]. High-protein diets may be helpful in preventing overweight or decreasing body weight in obese individuals, as they are effective for the regulation of food intake. Absorption of different proteins can vary, as proven by Boirie *et al.*[5], who showed that whey protein passes quicker through the stomach than casein. Whey protein reaches the jejunum intact in contrast to casein that coagulates under the acidic environment of the stomach. Whey protein releases peptides in the intestine after peptic hydrolysis. This process occurs slowly, which ensures that

the digestion and absorption take place over a larger length of the small intestine while descending[5–7]. As a result, whey protein has been shown to reduce short-term energy intake and affects appetite suppression through an increase in plasma amino acid levels, mainly branched-chain amino acids such as leucine[6,8]. Other mechanisms such as the utilisation of amino acids for gluconeogenesis and the thermic effects of proteins may contribute as well to the differences seen in appetite suppression[9].

Whey protein itself is a constituent of milk protein (20 %), and consists of 20–25 % of α-lactalbumin. In a study by Bourthegourd *et al.*, rats that received a meal with α-lac increased their energy expenditure more than rats that were fasting or rats that received a meal containing either glucose or whole milk protein[10]. This increase in energy expenditure could in its entirety be attributed to the increase in protein oxidation, as lipid oxidation was equal with the other groups

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Abbreviations: α-lac, α-lactalbumin-enriched whey protein; DIT, diet-induced thermogenesis; HP, high protein; NP, normal protein; REE, resting energy expenditure; whey, total whey protein.

* **Corresponding author:** Rick Hursel, fax +31 43 3670976, email rick.hursel@hb.unimaas.nl

that ate a meal. Glucose oxidation was increased in rats that consumed a meal enriched with glucose, compared with the other groups. The oxidation of glucose had a protein-sparing effect, through which protein oxidation remained unchanged. The elevated protein oxidation after α-lac-enriched whey protein was the result of a rapid rise in plasma amino acid levels[10], related to the so-called 'slow' and 'fast' proteins. It remains unknown whether the increased energy expenditure after α-lac-enriched whey protein could be attributed to the total whey protein or to the enrichment with α-lac.

Therefore, the aim of the present study was to examine whether the addition of total whey protein (whey) or caseinomacropeptide-depleted α-lac-enriched whey protein (α-lac) to a normal milk protein yoghurt drink in order to achieve a specific high-protein yoghurt drink, which was served as breakfast, had an effect on energy expenditure and appetite suppression in human subjects, and whether α-lac and whey differed in these effects.

Subjects and methods

Subjects

Thirty-five healthy subjects (eighteen females, seventeen males) with a mean age of 20·9 (SD 1·9) years and with a mean BMI of 23·0 (SD 2·1) kg/m² participated in the present study. The subjects were recruited by advertisements in local newspapers and on notice boards at the university. All volunteers (*n* 94) participated in an initial screening that involved measurements of body weight and height and included the completion of a questionnaire related to eating behaviour (the Three Factor Eating Questionnaire[11]) and the completion of a questionnaire related to health, use of medication, physical activity, alcohol consumption, food allergies, smoking behaviour and daily caffeine consumption. All subjects were in good health, non-smokers, not using medication (except for contraception), at most moderate alcohol consumers and unrestrained eaters (as assessed by factor 1 of the Three Factor Eating Questionnaire). Baseline characteristics of the subjects are presented in Table 1.

Experimental design

The study had a randomised, three-arm, single-blind, cross-over design. Subjects attended the university laboratory once per week, during three consecutive weeks. They travelled by public transport or car, in order to avoid physical activity that would have increased resting energy expenditure (REE). Subjects arrived in the fasted state at 08.15 hours and were kept in time-blinded surroundings. They emptied their bladder before the test. After resting on a bed for 30 min, the REE and the substrate oxidation of the subjects were measured for 30 min by means of an open-circuit, ventilated-hood system. Gas analysis was performed by a paramagnetic oxygen analyser (OmniCal type 1155B; Servomex, Crowborough, East Sussex, UK) and an infrared carbon dioxide analyser (Omnical type 1520/1507; Servomex). Energy expenditure was calculated using Weir's formula[12]. Respiratory quotient was calculated as CO_2 produced/O_2 consumed. The subjects were lying in the supine position. After measuring the REE, subjects received one of the three breakfasts, consisting of

Table 1. Subject characteristics

(Mean values and standard deviations)

	Male (*n* 17)		Female (*n* 18)		Total (*n* 35)	
	Mean	SD	Mean	SD	Mean	SD
Age (years)	21·1	1·9	20·7	2·0	20·9	1·9
Body mass (kg)	80·3	8·1	66·4	6·4	73·2	10·1
Height (m)	1·85	0·07	1·72	0·07	1·78	0·09
BMI (kg/m²)	23·6	2·0	22·5	2·1	23·0	2·1
Body composition*						
FFM (kg)	66·0	6·5	47·8	4·3	56·6	10·7
FM (kg)	14·4	5·2	18·6	4·6	16·5	5·3
Body fat (%)	17·7	5·7	27·8	4·9	22·9	7·3
TFEQ†						
TFEQ F1	2·9	2·2	4·3	2·1	3·7	2·2
TFEQ F2	3·2	1·5	4·4	1·4	3·9	1·6
TFEQ F3	4·4	2·7	4·6	2·7	4·5	2·6

FFM, fat-free mass; FM, fat mass; TFEQ, Three Factor Eating Questionnaire; F1, cognitive restraint; F2, disinhibition; F3, hunger.
* Data collected before the last test day.
† The TFEQ measures three different factors of human eating behaviour.

either the control which was a normal-protein (NP) yoghurt drink with whole milk protein (Friesland Foods, Leeuwarden; 15, 47 and 38 % energy from protein, carbohydrate and fat, respectively), a high-protein (HP) yoghurt with added total whey protein (Hiprotal® 60 MP; Friesland Foods Domo®, Zwolle, The Netherlands; 41, 47 and 12 % energy from protein, carbohydrate and fat, respectively) or a HP yoghurt that contained caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (Vivinal®Alpha; Friesland Foods Domo®; 41, 47 and 12 % energy from protein, carbohydrate and fat, respectively). The 41 % energy as protein in the HP breakfasts consisted of 14 % energy from casein protein and 27 % energy from total whey protein or caseinomacropeptide-depleted α-lac-enriched whey protein. It was unknown how much extra α-lactalbumin was included in the α-lac yoghurt compared with the whey yoghurt or the NP yoghurt. Breakfasts were isoenergetic, had the same flavour (vanilla) and contained the same volume for each condition; also the HP breakfasts had the same energy density (3·2 kJ/g). Before the study started, ratings for palatability and acceptability were assessed with visual analogue scales. With an average rating of 64 mm, subjects experienced no significant differences between conditions concerning colour, taste and viscosity. The energy content of the breakfast meal was tailored to the energy requirements of each subject by calculating BMR with the Harris–Benedict equation, which uses sex, age, height and weight[13]. To estimate the total energy requirement, the BMR was multiplied with a physical activity index of 1·6, estimated by means of a computer simulation program[14]. The breakfast contained 15 % of the daily energy need. Energy content varied from 1·42 to 2·28 MJ, with an average of 1·74 MJ. During the consumption of the breakfast the hood was removed temporarily.

After the subjects had finished their breakfast the hood was placed back and the measurements continued for another 4 h, during which DIT was determined. During the measurements questionnaires concerning appetite sensations were completed every 30 min as well as immediately before and after breakfast. Subjects were not allowed to talk, laugh, move or sleep

while lying under the hood[15]. The urine collected during and after the experiment was used to determine protein turnover. Body composition was measured previous to their last test day.

Anthropometry

Body composition was measured using the ^2H_2O dilution technique. The dilution of the ^2H isotope is a measure for total body water[16]. In the evening before the last test day, the subjects ingested a dose of ^2H-enriched water (^2H_2O; Cambridge Isotope Laboratories, Inc., Andover, MA, USA) after collecting a background urine sample. After ingestion of the ^2H solution no further fluid or food consumption was permitted. The following morning, another urine sample (second voiding) was collected. ^2H concentration in the urine samples was measured using an isotope ratio mass spectrometer (Micromass Optima, Manchester, UK). Total body water was obtained by dividing the measured ^2H dilution space by 1·04 to correct for exchange of the ^2H label with non-aqueous hydrogen of body solids[17]. Fat-free mass was calculated by dividing the total body water by a hydrating factor (0·73). By subtracting fat-free mass from body weight, fat mass was obtained. Fat mass expressed as a percentage of body weight gives percentage of body fat.

Questionnaires

The appetite questionnaire was composed of visual analogue scale (in mm) questions on subjective feelings of hunger, fullness, appetite suppression, thirst and desire to eat. Opposing extremes of each feeling were described at either end of a 100 mm horizontal line, and subjects marked the line to indicate how they felt at that moment.

Statistical analysis

Data are presented as mean values and standard deviations or standard errors. Data were analysed using SPSS 11 (SPSS, Inc., Chicago, IL, USA). A three-factor repeated-measures ANOVA including a Scheffé *F post hoc* test was used to determine a possible difference between breakfast effects on DIT and appetite ratings. Factor DIT, which expresses DIT after correction for body size, was calculated as follows: (REE + DIT)/REE. The level for establishing significant differences was taken at $P < 0.05$.

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects were approved by the Medical Ethical Committee of Maastricht University Medical Centre. Written informed consent was obtained from all subjects.

Results

No adverse events occurred, as no subjects reported any feelings of discomfort after consuming the yoghurt drinks. No different effects for men or women were observed, so these data were analysed together. Average energy intake was equal in all three conditions (1·74 (SD 0·25) MJ). There were significant treatment effects ($P < 0.05$) for energy
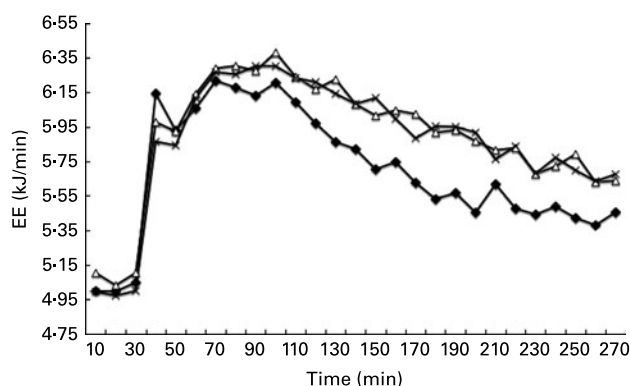

Fig. 1. Energy expenditure (EE; kJ/min) during the entire measurement for the placebo (−♦−), total whey protein (−△−) and caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (− × −) conditions in thirty-five subjects (eighteen women and seventeen men). Resting EE was measured during the first 30 min, followed by a breakfast and another 4 h of measuring. Values are means. Data were analysed by repeated-measures ANOVA.

expenditure after consumption of the yoghurt drinks. REE did not differ between the three conditions before consuming the whey, α-lac or NP yoghurt drinks (5·08 (SD 0·89), 4·99 (SD 0·83) and 5·02 (SD 0·80) kJ/min, respectively) (Fig. 1). On average, across the 4 h post-breakfast period, total energy expenditure increased significantly compared with baseline measurements in all three conditions (5·99 (SD 0·93), 5·97 (SD 1·02) and 5·77 (SD 0·96) kJ/min; $P < 0.001$). Areas under the curve for energy expenditure increased significantly after the HP yoghurt drink with whey and after the HP yoghurt drink with α-lac compared with after the NP yoghurt drink (217·05 (SE 10·00) kJ × 4 h ($P < 0.05$); 234·30 (SE 11·61) kJ × 4 h ($P < 0.01$); 179·71 (SE 10·87) kJ × 4 h ($P < 0.05$), respectively) (Fig. 2). No sex differences were seen after comparing the increase of DIT in percentages between all conditions, between males and females. This was also supported after correction for body size by means of calculating factor DIT, where the increase in energy expenditure remained significantly different between whey and placebo ($P < 0.05$) as well as α-lac and the NP yoghurt drink ($P < 0.001$). Total energy expenditure was positively related to fat-free
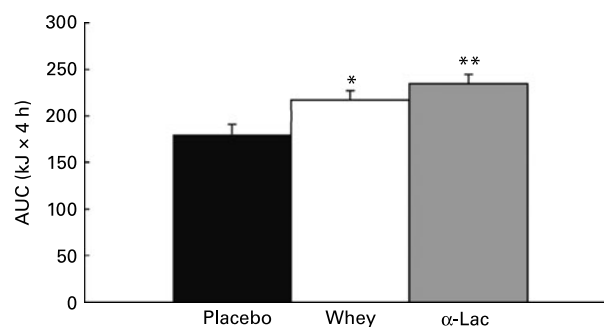

Fig. 2. Areas under the curve (AUC) for energy expenditure after the placebo, total whey protein (Whey) and caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (α-Lac) conditions in thirty-five subjects (eighteen women and seventeen men). Values are means, with standard errors represented by vertical bars. Mean value was significantly different from that for the placebo condition: *$P < 0.05$, **$P < 0.01$ (repeated-measures ANOVA).
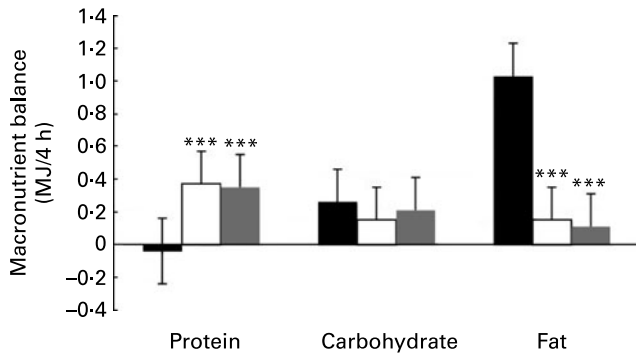
Fig. 3. Macronutrient balances for the placebo (■), total whey protein (□) and caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (▨) conditions in thirty-five subjects (eighteen women and seventeen men). Values are means, with standard deviations represented by vertical bars. *** Mean value was significantly different from that for the placebo condition ($P < 0.001$; repeated-measures ANOVA).

mass (whey, r^2 0.81, $y = 0.08x + 1.54$; α-lac, r^2 0.76, $y = 0.08x + 1.24$; NP, r^2 0.78, $y = 0.08x + 1.26$; $P < 0.001$), as was REE (whey, r^2 0.75, $y = 0.07x + 0.96$; α-lac, r^2 0.78, $y = 0.07x + 1.12$; NP, r^2 0.78, $y = 0.07x + 1.24$; $P < 0.001$), respectively, for all conditions.

Subjects were in positive energy balance during the 4 h post-treatment, and energy balance was significantly different between the HP conditions and the NP condition (whey, 0.67 (SD 0.14) MJ/4 h; α-lac, 0.68 (SD 0.16) MJ/4 h; NP, 1.25 (SD 0.21) MJ/4 h; $P < 0.001$). No significant differences in respiratory quotient were seen between the three conditions at baseline (whey, 0.87 (SD 0.08); α-lac, 0.86 (SD 0.06); NP, 0.87 (SD 0.06); NS) and post-treatment (whey, 0.88 (SD 0.07); α-lac, 0.87 (SD 0.05); NP, 0.86 (SD 0.04); NS). Figure 3 shows the separate macronutrient balances. The protein and fat balances were significantly different between the NP and the HP conditions. Fat balance was lower after the HP breakfasts compared with the NP breakfast (α-lac, 0.11 (SD 0.26) MJ/4 h; whey, 0.15 (SD 0.29) MJ/4 h v. placebo, 1.03 (SD 0.29) MJ/4 h; $P < 0.001$) and protein balance was

Fig. 4. Changes in desire to eat (mm visual analogue scale; VAS) after the placebo (−○−), total whey protein (−▲−) and caseinomacropeptide-depleted α-lactalbumin-enriched whey protein (α-lac) (−×−) conditions in thirty-five subjects (eighteen women and seventeen men). Values are means, with standard errors represented by vertical bars. * Mean value for the α-lac condition was significantly lower than that for the total whey protein condition ($P < 0.05$; repeated-measures ANOVA). † Mean value for the α-lac condition was significantly lower than that for the placebo condition ($P < 0.05$; repeated-measures ANOVA).

higher in the HP conditions compared with the NP condition (α-lac, 0.35 (SD 0.18) MJ/4 h; whey, 0.37 (SD 0.20) MJ/4 h v. placebo, −0.04 (SD 0.16) MJ/4 h; $P < 0.001$).

Baseline values of the measured variables from the appetite questionnaires (hunger, fullness, satiety and desire to eat) were not significantly different between the conditions. The results and the areas under/above the curve of the visual analogue scales are presented in Fig. 4 (desire to eat) and Table 2. The area above the curve was significantly higher after the α-lac condition compared with the whey condition, for hunger and desire to eat ($P < 0.01$). No differences were seen between the placebo and α-lac conditions or between the placebo and whey conditions concerning areas above the curve.

Discussion

Consumption of a high-protein breakfast with either added whey or α-lac is more thermogenic than consumption of a normal-protein breakfast containing milk protein. The results of the present study confirmed that high-protein breakfasts enhance thermogenesis more compared with a normal-protein breakfast[18]. The NP breakfast contained milk protein, which consists of 80% casein and 20% whey. Milk protein has a higher energy efficiency due to casein, which has the lowest cost for ATP production (8.2 kJ/ATP) compared with α-lac and whey (8.6 and 8.4 kJ/ATP)[19]. Much research has already been conducted concerning the effect of high-protein diets on DIT in comparison with normal-protein diets, in the long-term as well as in acute studies[2,3,20–25]. Moreover, effects of high-protein diets on weight loss and weight maintenance have been shown, through the thermogenic effect and preservation of fat-free mass, following a relatively high-protein diet, which is the main determinant of REE[3]. Together, this guarantees sustained thermogenesis on a high-protein diet. The positive protein balance after the HP conditions indicate that anabolism was taking place, which may support the preservation of fat free mass[26]. However, more amino acids are available after the HP breakfasts compared with the NP breakfast, which will cause an increase in protein oxidation in the postprandial and post-absorptive state. As a consequence, the daily protein balance will probably be less positive. The low fat content of the HP conditions compared with the NP condition resulted in a fat balance that was reduced, which means that less fat was stored after the HP conditions. The present study was performed under acute conditions; therefore subjects were not adapted to the HP breakfast. This might also explain some of the effects observed with the HP meals compared with the NP meal and particularly the reduction in fat balance.

An explanation for the thermogenic effect of α-lac may be that it is a complete protein. The effect of proteins on DIT is dependent on the quantity of protein, the rate of absorption and thereby increasing the level of amino acids in the bloodstream and also the concentration of essential amino acids, which determines whether a protein is 'complete' or 'incomplete'. α-Lac is a well-balanced protein, which contains high concentrations of indispensable amino acids such as cystine, lysine and tryptophan[27]. Not all amino acids are used for protein synthesis and those that remain are oxidised, causing an increase in energy expenditure. Costs for ATP production
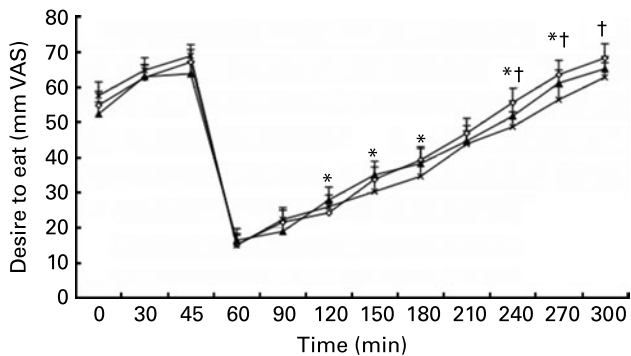
Table 2. Area under (or above) the curve of desire to eat, hunger, appetite suppression and fullness (mm VAS × 4 h) as rated by thirty-five subjects (eighteen women and seventeen men)

(Mean values with their standard errors)

	Placebo (NP)		Whey (HP)		α-Lac (HP)	
	Mean	SE	Mean	SE	Mean	SE
Desire to eat	−5589·2	840·3	−5070·4	873·8	−6750·6**	805·5
Hunger	−6013·5	824·6	−5448·0	913·0	−6627·4*	823·2
Appetite suppression	7324·9	834·8	6175·5	842·0	6583·5	730·2
Fullness	7023·4	811·3	6245·6	840·7	6584·1	763·3

NP, normal-protein breakfast; Whey, total whey protein yoghurt; HP, high-protein breakfast; α-Lac, caseinomacro-petide-depleted α-lactalbumin-enriched whey yoghurt.

Mean value was significantly lower than that after the Whey (HP) breakfast: * $P<0·05$, ** $P<0·01$ (repeated-measures ANOVA).

are higher after α-lac compared with whey and milk protein (8·6 v. 8·4 v. 8·3 kJ/ATP), which means that α-lac is not as energy efficient as whey and milk protein, i.e. the higher the cost for ATP production, the more thermogenic the protein[19]. There are hardly any studies that have investigated the thermogenic properties of whey and α-lac. The study mentioned before from Bouthegourd et al. showed that whey enriched with α-lac caused an increase in DIT in rats, but in the present study whole milk protein had a comparable effect size[10].

Appetite scores showed that the HP breakfast with α-lac suppressed hunger significantly more than the HP breakfast with whey and the NP breakfast. No suppressive effect on hunger of the HP breakfast with whey compared with the NP breakfast was seen. The suppressive effect of hunger by α-lac may be attributed to the fast rise in amino acid levels in plasma, as Mellinkoff stated in his aminostatic theory in 1956 that elevated concentrations of plasma amino acids create a appetite-suppressing signal[28]. Furthermore, α-lac contains high concentrations of leucine and lysine, which are ketogenic amino acids[27]. It is known that hunger is suppressed during a ketogenic state[29]. However, compared with total whey protein there is not much difference between concentrations of ketogenic amino acids[27]. Nieuwenhuizen et al. found the same hunger-suppressive effect in a study where they compared α-lac with gelatin, and with gelatin with added tryptophan[30]. They concluded that tryptophan, which is present in high concentrations in α-lac and often seen as a modulator of appetite regulation via serotonin, was not involved in the suppressive effect. Neither involved were the ratio between tryptophan and large neutral amino acids or total amino acids, glucagon-like peptide-1 or ghrelin responses[30]. In a study by Veldhorst et al. the lowering effect of α-lac on energy intake was also demonstrated. Energy intake at lunch, after a breakfast with α-lac, was 19 % lower than after a breakfast with whey[18].

Furthermore in a rodent study, Pichon et al. showed that energy intake was reduced after a high-protein diet with whey compared with a normal-protein diet with milk protein[31]. Diepvens et al. found that after a breakfast with whey protein subjects were more satiated than after a breakfast with milk protein[32]. Bowen et al.[33] and Hall et al.[7] have studied the effects between casein and whey, the constituents of milk protein. Both studies found different results, as only Hall et al.[7] could demonstrate that energy intake was lower after a whey preload compared with a casein preload[7,33].

Summarising, HP breakfasts increased thermogenesis more than a NP breakfast, which can be attributed to the amount of protein but also to the higher bioactivity of α-lac and whey compared with milk protein. Furthermore, α-lac suppressed hunger more than whey and placebo. In conclusion, the addition of α-lac, i.e. whey, to a NP milk protein yoghurt drink increases diet-induced thermogenesis; additional α-lac also suppresses hunger and the desire to eat.

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