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Low-dose whey protein-enriched water beverages alter satiety in a study of overweight women☆

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ABSTRACT

Aim: To determine the effect of low-dose whey protein-enriched water beverages on postprandial satiety and energy intake (EI). *Methods*: Fifty overweight and mildly obese women were given 500 mL water-based beverages on 4 different occasions in a double blind, cross-over study. The beverages were reasonably matched for colour, flavour, sweetness and contained 0% (water control, 0 g, 8 kJ), 1% (5 g, 93 kJ), 2% (10 g, 178 kJ) and 4% (20 g, 348 kJ) whey protein by weight (ClearProtein8855™). Following a standard evening meal and breakfast, beverages were consumed 120 min before an *ad libitum* lunch at which EI was measured. Feelings associated with hunger and fullness were also measured using visual analogue scales (VAS). *Results*: 46 participants completed all 4 beverage conditions. There was a significant effect of beverage preload on hunger (beverage × time; $P = 0.0074$), where each of the 1%, 2% and 4% w/w protein beverages decreased hunger compared to the water control ($P < 0.05$). Suppression of hunger was also maintained for longer following the protein beverages (Friedman test, $P = 0.013$). Fullness (beverage × time; $P = 0.0020$) and satisfaction (beverage × time; $P = 0.0356$) were both increased by the 1% and 4% protein beverages ($P < 0.05$). EI at lunch decreased by up to 8 percent (control vs 4% protein, delta = −247 kJ, Tukey's post hoc, $P > 0.05$) when escalating protein doses were added to the water preload (water control, 3028 kJ; 1%, 3080 kJ; 2%, 2924 kJ; 4%, 2781 kJ), only partial compensation for the added energy. *Conclusions*: These low-dose, whey protein-enriched water beverages significantly altered short term postprandial satiety, however the effect was not sufficient to impact on food intake when assessed 2 h after consumption.

Introduction

Many factors have been identified as important to the control of energy intake (EI) and weight management (Blundell & Gillett, 2001; Ello-Martin, Ledikwe, & Rolls, 2005; Macht, 2008; Yeomans, 2004) including macronutrient composition (Rolls, 2000). There is a growing literature to show that high-protein (HP) foods may enhance satiety (Anderson & Moore, 2004; Bowen, Noakes, Trenerry, & Clifton, 2006; Halton & Hu, 2004; Poppitt, McCormack, & Buffenstein, 1998; Weigle et al., 2005), and play a role in weight loss (Clifton, Keogh, & Noakes, 2008; Kushner & Doerfler, 2008; Noakes, 2008; Paddon-Jones et al., 2008), and we wanted to investigate the effect of incorporating low-dose whey protein (<20 g) into water beverages. Energy-containing beverages may evoke weaker appetite and compensatory dietary responses than solid food (Crapo & Henry, 1998; Mattes, 2006; Mattes & Campbell, 2009; Mourao, Bressan, Campbell, & Mattes, 2007), and hence

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increase daily intake when added to the diet (DellaValle, Roe, & Rolls, 2005; Poppitt, Eckhardt, McGonagle, Murgatroyd, & Prentice, 1996). Certainly, whether a beverage can induce a level of satiety comparable with an energy- and macronutrient-matched solid food is not clear. In particular sugary and alcoholic beverages may be poorly recognised (Berkey, Rockett, Field, Gillman, & Colditz, 2004; Harnack, Stang, & Story, 1999; James, Thomas, Cavan, & Kerr, 2004; Ludwig, Peterson, & Gortmaker, 2001; Poppitt et al., 1996) with the potential to encourage unintentional weight gain over a prolonged period. Despite these issues of food rheology there is evidence, for example, of macronutrient specific effects such as enhanced appetite responses when volume matched, isoenergetic, mixed macronutrient (protein, fat and CHO) (St-Onge, Rubiano, & DeNino, 2004) or HP (Bowen, Noakes, Trenerry, et al., 2006) drinks replace sugar-only beverages.

On the basis of this evidence, bovine milk is a HP beverage which may be expected to enhance satiety and preload studies have indeed shown some compensation for energy derived from milk-based drinks in trials where volume of beverages consumed was an important determinant of satiety (Rolls, Castellanos, & Halford, 1998; Rolls & Roe, 2002). Recent studies comparing isoenergetic dairy drinks showed significantly less energy consumed at lunch following HP vs high-CHO (HCHO) or a low energy control (Bertenshaw, Lluch, & Yeomans, 2008, 2009), as did a beverage study of a HP skimmed milk vs an isoenergetic HCHO fruit drink (Dove et al., 2009), and a HP soup study (Bowen, Noakes, & Clifton, 2006). This however is not a universal finding in beverage preload studies (Almiron-Roig & Drewnowski, 2003; Bowen, Noakes, & Clifton, 2007; Lam, Moughan, Awati, & Morton, 2009), and protein composition may further affect satiety (Hall, Millward, Long, & Morgan, 2003; Veldhorst, Nieuwenhuizen, & Hochstenbach-Waelen, 2009). Whilst protein preloads have also been associated with changes in ghrelin, cholecystokinin (CCK) and glucagon-like peptide (GLP-1), these gastrointestinal hormones may poorly predict change in food intake (Bowen et al., 2007).

Since there is some evidence that whey may be more satiating than other dairy proteins (Hall et al., 2003; Luhovyy, Akhavan, & Anderson, 2007; Veldhorst et al., 2009), we enriched water beverages with increasing doses of whey protein isolate (Clear-Protein8855^{TM}, Fonterra Co-operative Group Ltd., New Zealand). The aim of this study was to determine the effect of low-dose whey protein-enriched water beverages on satiety and EI. We hypothesised that the addition of whey protein to a 500 mL water beverage would enhance satiety and decrease subsequent intake, and wanted to determine whether low incremental doses of 1%, 2% or 4% (w/w) protein would be sufficient to generate a measurable effect on appetite regulation when compared with a no-protein, no-energy water control. Flavoured water beverages are popular and commonly consumed, and in this trial we assessed the effects of adding 5, 10 and 20 g whey protein to a 500 mL flavour- and volume-matched water beverage by measuring subjective appetite sensations and subsequent food intake over a single day in a group of overweight and mildly obese women.

Methods

Participants

Fifty overweight and mildly obese but otherwise healthy women (BMI 24–33 kg/m^2) aged 18–45 years were recruited via local newspaper and poster advertisement, and via a consumer research recruitment company. Participants were non-smokers, had no history of cardiovascular disease, diabetes, or any other significant metabolic, endocrine or gastrointestinal disease, and were not taking any medications which may have had any effect on appetite or weight regulation throughout the trial period. Participants were selected to be unrestrained eaters as defined by a score of $\leq$12 on the cognitive restraint scale of the 3 Factor Eating Questionnaire (Stunkard & Messick, 1985). Other exclusion criteria included pregnancy and/or breast-feeding, participation in an active diet program or loss/gain of >5 kg body weight within the last 6 months. Hypersensitivities or allergies to any foods or ingredients included in the study, as well as dislike and/or unwillingness to consume items listed as study foods (evening meal, breakfast, *ad lib* lunch), unwilling/unable to comply with study protocol, or current participation in another clinical intervention trial were also exclusions.

Participants were asked to complete questionnaires detailing their dietary behaviours on the day prior to each study visit. They were given a daily diary to take home and completed menstrual cycle questionnaires at each visit to provide information on their menstrual phase at each study visit. Written informed consent was obtained from each participant and ethical approval obtained from the Northern Regional Ethics Committee X, Auckland, New Zealand. The Clinical Trial registration number was ACTRN12609000723280.

Procedures

This was a 4 condition study in which protein-supplemented water beverages were given to the participants mid-morning following a standard evening and breakfast meal. It was a double blind, cross-over study carried out at the University of Auckland Human Nutrition Unit (HNU). Each beverage preload was separated by a washout period of at least 3 days. There were no restrictions for the participants between study visits other than to refrain from consuming alcohol and undertaking prolonged vigorous physical activity on the day before test visits. The women were asked to consume the standard dinner meal provided by the HNU between 6 and 8 pm on the evening prior to each test day, and to arrive at the Unit fasted on the morning of the test. These details were verified in detail upon arrival. Participants arrived at the Unit at 8:30 am on the test day and were weighed lightly clad and given 150 mL of water to drink before breakfast. At 9 am participants were given a standardized breakfast and were required to consume all items within 15 min. Immediately after completion of breakfast, a 150 mL drink of decaffeinated black tea/coffee or water was also given. At 11 am, 120 min after the breakfast, the 500 mL test beverages were served. Beverages were served chilled from covered bottles into opaque drinking glasses to minimise visual comparisons between preloads. Participants were required to consume the beverage within 15 min. At 1 pm, a further 120 min after the test beverage, the *ad libitum* lunch meal was served. Each item of the lunch was provided in moderate excess (estimated as between 4 and 6 typical individual portions based on standard serving sizes), and participants were asked to stop eating when they felt comfortably full. Distractions were kept to a minimum by seating participants within individual dining booths with no reading material, mobile phone or other items. Background music was employed to dampen the sound of cutlery, crockery and the noises of eating. Participants were asked to remain in their booths for 30 min until 1.30 pm. The daily protocol is outlined in Fig. 1.

Protein-enriched water beverages

Participants received each of the beverages in random order over 4 visits. Beverage preloads comprised (i) water control, 0% w/w protein, (ii) 1% w/w protein, (iii) 2% w/w protein, (iv) 4% w/w protein. Glycomacropeptide (GMP) content of this whey protein was low, at ~0.9% of total protein. Energy content of the preloads increased in parallel with protein content such that the compositions were: 0% control (0 g protein, 8 kJ), 1% (5 g protein; 93 kJ), 2%

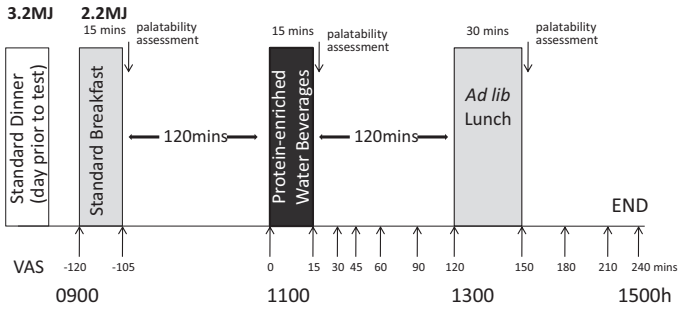

Fig. 1. Daily protocol.

(10 g protein; 178 kJ) and 4% (20 g protein; 348 kJ) whey protein as shown in Table 1. The preloads were matched for volume and were sweetened and flavoured to mask the increase in protein content. The flavours used were a combination of nature-identical mango, peach and vanilla, plus masking flavour. The beverages were coloured with sunset yellow to replicate the appearance of a tropical fruit (mango/peach) drink. Trace amounts of each were added to the 500 mL. The artificial sweeteners were sucralose and acesulfame K. The flavour of the products was matched as closely as possible prior to the study using a consumer panel of 50 women (data not shown).

Visual analogue scales

Visual analogue scales (VAS) were used according to the standard methodology of Flint, Raben, Blundell, and Astrup (2000). The following questions were used: "How hungry do you feel?/How full do you feel?/How satisfied do you feel?/How much do you think you can eat now?" and were anchored on the left by "I am not hungry/I am not full at all/I am completely empty/nothing at all" and "I am as hungry as I have ever been/I am totally full/I cannot eat another bite/a large amount" on the right. A set of scales rating how thirsty, energetic and relaxed the participants felt was included as a distraction from the main outcome. Ratings of nausea were also recorded. Participants were asked to mark their responses by placing a vertical line across the 100-mm horizontal scale. VAS measurements were collected on 3 occasions prior to the presentation of the beverages; fasted pre-breakfast ($t = -120$ min), immediately post-breakfast (-105 min) and immediately pre-treatment ($t = 0$ min). Once the beverage was given VAS were recorded at 15 min intervals over the following hour ($t = 15, 30, 45$ and 60 min), and then recorded every 30 min for the remainder of the day ($t = 90$, 120, 150, 180, 210 and 240 min). Area under the curve (AUC) for hunger and fullness were calculated as the AUC of net change in hunger and fullness as measured over the 0–120 min immediately following beverage consumption: $AUC_{\text{delta 0–120 min}}$. Immediately after the standard-breakfast, the test beverage and the lunch meal, participants also rated the pleasantness, visual appeal, smell, taste, aftertaste and overall palatability of each meal/beverage on separate 100-mm VAS ($t = -105$, 15 and 150 min).

Standardized meals and ad libitum lunch

Participants were provided with a standardized dinner meal on the evening prior to each study day and a standardized breakfast meal on the morning of the study with the aim of normalizing feelings hunger and fullness across the conditions immediately prior to the beverage preload. Evening meals were prepared by the metabolic cook at the HNU, frozen and distributed several days prior to the test. They comprised a beef or lamb casserole served with rice, and a lemon dairy dessert with fruit. The energy content of the evening meal was 3.2 MJ with a fixed macronutrient composition of 24 en% fat, 29 en% protein, and 47 en% CHO. Participants were asked to complete the meal by 8 pm, to eat from the meal until they felt comfortably full, and to refrain from eating or drinking anything other than water with the meal. The standard breakfast meal comprised toast with butter and jam/marmalade, a banana, and a glass of orange juice. The energy content was 2.2 MJ and macronutrient composition 12 en% fat, 7 en% protein and 80 en% CHO.

The *ad libitum* lunch consisted of a restricted item buffet-style meal with a hot chicken or ham rice-based flan, salad leaves with oil-based dressing, canned peaches, carrot and raisin loaf, and bottled water (Table 2). Prior to the study, it had been established with each participant that the items provided in the lunch were acceptable as meal choices. In an attempt to avoid over-consumption the variety of items presented was limited. Each meal item was provided in moderate excess and was weighed before and after the meal for calculation of energy and macronutrient intake. The energy and macronutrient composition

Table 1
Composition of the 4 beverage preloads.

	Control	1% w/w protein	2% w/w protein	4% w/w protein
Energy (kJ)	8	93	178	348
Protein (g)	0	5	10	20
Fat (g)	0	0	0	0
Carbohydrate (g)	0	0	0	0
Volume (mL)	500	500	500	500
Flavouring	[a]	[a]	[a]	[a]
Colouring	[b]	[b]	[b]	[b]
Sucralose/ acesulfame K	[c]	[c]	[c]	[c]

All beverages reasonably matched for volume, flavour and colour during prior sensory testing.
w/w, weight for weight.
[a] Flavoured with nature-identical (synthetic) mango, peach and vanilla, plus masking flavour.
[b] Coloured with sunset yellow to replicate the appearance of a tropical fruit (mango/peach) drink.
[c] Artificial sweeteners sucralose and acesulfame K.

Table 2
Energy and macronutrient composition of foods and beverages served at the *ad libitum* lunch.

Food and beverage item	Portion size (g)	Energy (kJ)	Protein (g)	Fat (g)	CHO (g)
Chicken or ham flan	1090	5570	90.14	54.94	113.14
Salad leaves, plus dressing	75	95.4	1.5	0.26	3.32
Canned peaches	542	975.6	2.71	0.54	51.49
Carrot and raisin loaf	193	2817.8	9.46	20.8	110.1
Water, bottled	1500	0	0	0	0

of each of the lunch items were calculated using the dietary program FoodWorks™ (Professional Edition, Version 5, 1998-2007; Xyris Software). Compensation for the energy consumed within each protein beverage was calculated on each occasion as the decrease in EI at lunch relative to the zero protein control (decrease in EI at the *ad lib* lunch relative to control/energy content of the test beverage, presented as a percentage). Complete compensation is represented by a score of 100%, i.e. the additional energy consumed with the beverage would elicit a matched decrease of energy at the lunch meal.

Statistical analysis

VAS data assessing the palatability of the standard breakfast, the 4 beverage conditions on a single occasion immediately after they were consumed, and the *ad lib* lunch was analysed using repeated measures Linear Mixed Model ANOVA (SAS: PROC MIXED, SAS version 9.1, SAS Institute Inc, Cary, NC, USA, 2002–2003). VAS data assessing postprandial feelings of hunger, fullness and other satiety indicators throughout each study visit were also analysed using repeated measures Linear Mixed Model ANOVA. The participant, the dietary preload, the study period and the study day were included in the procedure, in addition to the beverage/time interaction which addressed whether the trajectory over time during the study period differed between conditions (beverage × time). Energy and macronutrient intake data from the *ad lib* lunch meal also analysed using repeated measures Linear Mixed Model ANOVA, included the stage of menstrual cycle in the procedure. Where the ANOVA was significant, Tukey's post hoc analysis was used for comparisons between conditions. Time to return to pretreatment baseline for VAS hunger and fullness was analysed using the Friedman nonparametric procedure (SPSS version 16.0, SPSS Inc, Chicago, Il, USA, 2007), i.e. to assess the possibility of preloads resulting in 'suppression of hunger' and 'fuller for longer' across beverage conditions. Analyses were performed both as intention to treat (ITT) and per Protocol for participants who completed all arms of the study. No participants were replaced due to drop out after they had entered the study and completed the first study period. Missing data was assumed missing at random and no data imputation was performed. Statistical significance was set at a level of 0.05. Participant characteristics are presented as mean, standard deviation (mean, SD). Efficacy endpoints of VAS and EI are presented as mean, standard error of the mean (mean, SEM).

Results

Participants

Of the 50 women randomized into the trial, 46 completed all 4 beverage conditions [control, $n = 48$; 1% protein, $n = 48$; 2% protein, $n = 46$; 4% protein, $n = 48$]. The women were on average young and overweight or mildly obese but otherwise healthy, with a mean age of 32.5 years (7.8 SD) and mean BMI of 27.6 kg/m^2 (2.5 SD). The mean restraint score of the group was 6.9 (3.5 SD). Four women withdrew from the trial following completion of at least 1 study visit due to prior history of mango allergy, inadequate time to complete the trial, bereavement, and difficulty in complying with study protocol, respectively. Baseline characteristics of the 50 female participants are shown in Table 3. Body weight and BMI did not change significantly throughout the 3 months that the trial was conducted in this group of women, indicating that major changes in either diet or lifestyle had not occurred as a result of inclusion in a nutrition study. Review of the menstrual diaries and questionnaires allowed each woman to be assigned to either the follicular or luteal phase of the menstrual cycle at each study visit.

Table 3
Characteristics of the female participants.

	Mean	SD	Range
$n = 50$			
Age (years)	32.5	7.8	18–45
Restraint (units)	6.9	3.5	1–12[a]
Weight (kg)	74.9	10.4	58–100
Height (m)	1.6	0.06	1.5–1.8
BMI (kg/m^2)	27.6	2.5	24–33
Waist (cm)	81.8	8.2	66–103
Hip (cm)	104.9	7.1	91–122
WHR	0.78	0.06	0.64–0.91
SBP (mmHg)	111.5	11.1	88–146
DBP (mmHg)	68.1	8.2	52–86

Restraint assessed using cognitive restraint scale of the 3 Factor Eating Questionnaire (Stunkard & Messick, 1985).
BMI, body mass index; Waist, waist circumference; Hip, hip circumference; WHR, waist: hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure. $n = 50$, all measurements recorded at screening visit.
[a] 1 subject enrolled with restraint score 14.

There was no significant difference between phase of cycle across the 4 beverage conditions, and follicular:luteal (F:L) stage of the cycle was reported by 20:26 (F:L), 17:29, 15:30 and 18:28 participants at each of the control, 1%, 2% and 4% protein conditions. Two women could not be classified by phase. Phase of menstrual cycle was not found to influence the major outcomes of EI or VAS appetite ratings between the 4 beverage conditions in this trial.

Visual analogue scales (VAS)

Palatability of the beverage preloads

The 4 beverages were formulated to be closely matched for flavour, sweetness and appearance, however the 4% protein beverage had lower ratings for pleasantness, taste and palatability ($P < 0.05$), and higher ratings for aftertaste compared with each of the other beverages ($P < 0.05$). There were no differences in either visual appeal or smell. There was also no significant difference between beverages for ratings of nausea immediately following consumption of each of the 4 beverages ($P > 0.05$). Throughout the trial, ratings for nausea did not increase by more than 10 mm on any of the 4 regimens.

Hunger, fullness, thoughts of food, satisfaction and thirst

The mean VAS ratings for hunger, fullness, thoughts of food and satisfaction measured throughout each study day on each of the 4 study arms are shown in Fig. 2. Analyses were performed as both ITT ($n = 50$) and completers only ($n = 46$) groups, and it was confirmed that there was no difference in outcomes between the two analysis methods. There was a significant difference in both hunger and fullness between the 4 beverage preloads when analysed during the 120 min immediately following the preload (ANOVA beverage × time: hunger: $P = 0.0011$; fullness $P = 0.0003$) and during the complete 6 h study period including the post-lunch measures (ANOVA beverage × time: hunger: $P = 0.0074$; fullness: $P = 0.0020$). Post-hoc pairwise comparisons identified the greatest difference in hunger and fullness to occur between the control and 4% protein beverages at 90 min ($P < 0.05$). AUC calculations of change in hunger and fullness over the 2-h post-beverage period (net $AUC_{delta\ 0–120\ min}$) confirmed that participants felt less hunger ($P = 0.0055$) and greater fullness ($P = 0.0026$) when whey protein was added to the water beverage. The histograms presented in Fig. 2 show the tendency towards a dose response, such that hunger $AUC_{delta\ 0–120\ min}$ decreased and fullness $AUC_{delta\ 0–120\ min}$ increased, as 1%, 2% and 4% protein was added to the water beverage, however in this group of 50 participants the differential effects of dose were not detected as significant. Whilst hunger significantly decreased

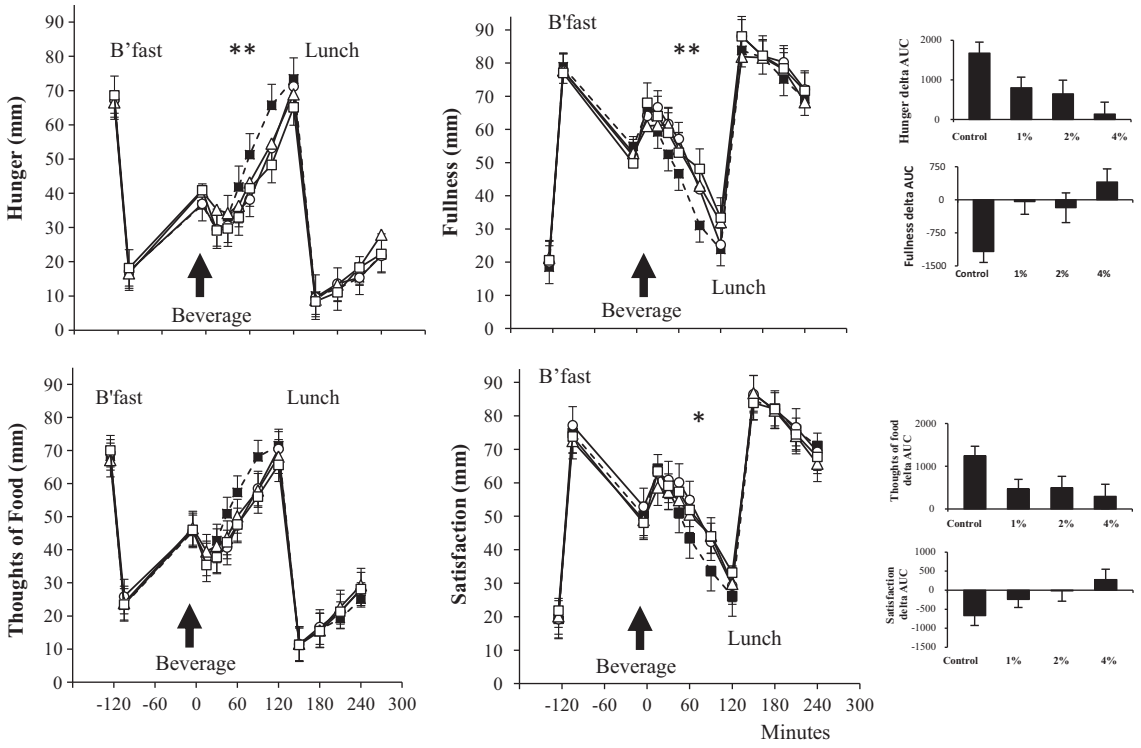

Fig. 2. Mean (SEM) visual analogue scale (VAS) subjective scores for hunger, fullness, satisfaction and thoughts of food for water control (■, $n = 48$), 1% w/w protein (○, $n = 48$), 2% w/w protein (△, $n = 46$) and 4% w/w protein (□, $n = 48$) beverages. Area under the curve histograms for 0–120 min are shown in RH plots ($AUC_{delta\ 0–120\ min}$). Beverage preloads were consumed at 0 min and the *ad libitum* lunch at 120 min. The time-by-beverage interaction was statistically significant for hunger, fullness ($P < 0.01$, 0–120 min) and satisfaction ($P < 0.05$, 0–120 min). Tukey's post hoc revealed significant differences for control vs 1%, 2%, and 4% w/w ($P < 0.05$) for hunger and for control vs 1%, and 4% w/w ($P < 0.05$) for fullness.

relative to the water control for all 3 protein doses ($P < 0.05$) and fullness was increased following the 1% and 4% protein beverages ($P < 0.05$), there were no differences between the 3 protein doses ($P > 0.05$). Suppression of hunger was also maintained for longer on the protein supplemented beverages (Friedman test, $P = 0.013$). The mean (SEM) time taken to return to baseline levels of hunger following the 11 am beverage was 24 (4), 36 (5), 31 (5) and 49 (6) minutes for the control, 1%, 2% and 4% protein preloads respectively. Whilst the length of time that participants felt full tended to increase with protein content ('fuller for longer') from 37 (4) to 48 (6), 46 (7) and 58 (6) minutes respectively, this was not a significant dose-related effect. Larger subject numbers may have allowed greater discrimination of this effect.

The addition of whey protein to water also significantly affected perceived feelings of satisfaction during the 2 h after the beverage (beverage × time; $P = 0.035$) (see Fig. 2), with greater satisfaction reported following all protein beverages. Again, however, there were no differential effects on satisfaction between any of the 1%, 2% or 4% protein beverages. A similar pattern was observed for thoughts of food but the decrease induced by protein enrichment was weaker (beverage × time; $P = 0.093$). Change in satisfaction and thoughts of food during the 2 h following the preloads (net $AUC_{delta\ 0–120\ min}$) confirmed these effects ($P < 0.05$) with no significant dose effects amongst the 3 protein preloads. Thirst was unaffected by preload (beverage × time; $P = 0.1338$, data not shown). Mean (SEM) reported nausea immediately following the test beverage was 12 (3) mm, 13 (2) mm, 9 (2) mm and 16 (3) mm for the control, 1%, 2% and 4% protein drinks respectively. This represented an increase of nausea rating of <10 mm in all groups between the pre- and post-beverage VAS (delta mean, SEM: control: 5, 0.1 mm; 1%: 7, 0.2 mm; 2%: 4, 0.1 mm; 4%: 9, 0.2 mm). Since changes in appetite ratings are likely to be driven in part by postprandial sensory responses, particularly should adverse responses to the protein addition arise, the repeat measures VAS data were reanalysed for individuals who reported little nausea (<30 mm) and significant nausea (>30 mm) immediately after the beverage preloads. There was no significant effect on the main outcome variables in these 2 subgroups.

Ad libitum lunch

Mean EI and the contributions made by CHO, fat and protein at the *ad lib* lunch are presented for each preload in Fig. 3. Despite the effects on hunger and fullness, there was no significant decrease in

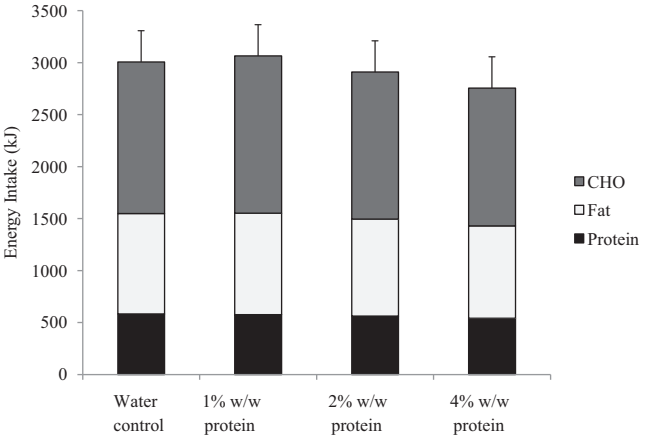

Fig. 3. Mean (SEM) EI and macronutrient consumption at the *ad libitum* lunch for each beverage. The greatest decrease relative to the water control occurred following the 4% protein beverage but this was not statistically significant (delta = −247 kJ, −8 percent, Tukey's post hoc, $P > 0.05$). 46 women completed all 4 arms of the study (water control, $n = 48$; 1% w/w, $n = 48$; 2% w/w, $n = 46$; 4% w/w, $n = 48$).

ad lib EI when the water beverage was supplemented with increasing doses of whey protein (Tukey's post-hoc, control vs 1%, 2%, 4% protein, all $P > 0.05$). Mean (SEM) intake at lunch was 3028 (157) kJ, 3080 (143) kJ, 2924 (133) kJ, and 2781 (134) kJ on each of the control, 1%, 2% and 4% protein beverages respectively ($P = 0.034$). Relative to the control beverage, the greatest decrease in food intake at lunch occurred following the 4% protein drink where EI fell by 247 kJ (−8%, Tukey's post-hoc, $P > 0.05$). The 4% beverage contained an additional 340 kJ as whey protein, hence compensation for this added beverage energy at the lunch meal was only partial. Total EI for beverage and *ad lib* lunch combined was 3036 kJ, 3172 kJ, 3102 kJ, 3129 kJ for control, 1%, 2% and 4% beverages respectively. Compensation for the additional beverage energy at lunch was in turn – 60 percent, 61 percent and 73 percent for the 1%, 2% and 4% protein beverages, hence the greatest compensation occurred at the highest protein dose. The score was negative for the 1% beverage since there was a failure to compensate for any portion of the beverage energy and participants ate more at lunch than they did in response to the no-added-protein water control. This increase in EI following the 1% beverage in turn led to a significant difference in energy consumed at the *ad lib* lunch between 1% and 4% protein treatments of 299 kJ (Tukey's post hoc, $P = 0.028$).

Discussion

Whilst there is evidence that dietary proteins may evoke a stronger satiety response than either fats or CHOs, these effects are primarily in studies manipulating solid foods. Whether there is a similar nutrient hierarchy when in beverage form is not well established although evidence of better compensation for HP vs HCHO (Bertenshaw et al., 2008; Bowen, Noakes, Trenerry, et al., 2006; Dove et al., 2009), and mixed macronutrient compared with sugar only beverages (St-Onge et al., 2004) does exist. Beverages may evoke weaker appetite and compensatory responses than energy matched solid foods (Crapo & Henry, 1998; Mattes & Campbell, 2009; Mattes, 2006; Mourao et al., 2007) although mechanisms underpinning effects of food rheology remain little explored. Since flavoured water beverages are a commonly consumed item and whey proteins have been shown to enhance satiety (Hall et al., 2003; Veldhorst et al., 2009), we were interested to investigate the effects of low-dose incorporation of whey proteins into water beverages.

The main findings of this study were (a) the addition of 1, 2 and 4% w/w of whey protein to a 500 mL water beverage significantly decreased feelings of hunger, maintained suppression of hunger for longer, and increased feelings of fullness when compared with a water control; (b) increasing the dose from 1 to 4% tended towards a greater suppression of hunger and enhanced fullness, but these were trends only; (c) despite altered perceptions of hunger and fullness, there was limited modulation of eating behaviour such that EI at the lunch meal presented 120 min after the beverage was not significantly decreased on any of the protein supplemented beverages when compared with the control (maximum 8% decrease), and d) there was only partial compensation at lunch which increased with protein dose up to a maximum of 73 percent when the 4% protein beverage was consumed, but no beverages resulted in a full (100%) compensatory decrease at lunch for the energy consumed within the beverage 2 h prior. Interestingly, a recent study of 34 overweight and obese men and women also reported an 8% decrease in EI ($P = 0.04$) at an *ad lib* lunch meal in a 2 arm study which compared isoenergetic high and low protein skim milk and fruit juice beverages (30). An 8% decrease in our current study however did not represent a significant change in EI, which in light of similar intakes of ~2.5–2.8 MJ at the *ad lib* outcome meal between the two studies, is likely a consequence of greater variability in response to the beverages in our current trial of overweight women.

It has become clear from the many preload studies published that modulating eating behaviour may be considerably more difficult than eliciting a change in sensations of appetite. Appetitive sensations do not always link energy need and/or desire with intake and it is not uncommon for favourable (or adverse) changes in hunger ratings to be followed by unresponsive eating behaviour at the subsequent test meal (Flint et al., 2000; Mattes, Hollis, Hayes, & Stunkard, 2005; Mattes, 1990; Mourao et al., 2007). Reasons for this are many and varied but may be a consequence of experimental design. In a preload study there is little that the investigator can do with respect to the design of the trial which may bias the reported ratings of appetite, providing standard methods are adhered to, such as the use of a 100 mm line and collection of repeat measures postprandially. Although it is clear that VAS in turn are not always predictive of changes in food intake induced by preload challenges (Borzoei, Neovius, Barkeling, Teixeira-Pinto, & Rossner, 2006; Mourao et al., 2007), they exhibit a reasonable degree of reliability and validity and are sensitive to experimental manipulation (Stubbs, Johnstone, O'Reilly, & Poppitt, 1998). Conversely, presentation of a meal from which a subject is asked to eat freely is beset with methodological issues that may alter outcome. These include, but are not limited to the: prior experience of the participant, environment in which lunch is served, eating alone or in a social setting, number of repeat treatments in a cross-over trial, choice/type/variety/palatability/portion size of foods offered, timing of the meal, interval between preload and lunch, and the covert or overt nature of the experiment. It is difficult to ascertain whether any of these variables may have influenced the outcome in our current trial where 2 h after a beverage preload we presented a moderate choice lunch meal containing both savoury and sweet items to participants eating in individual lunch booths, with instructions to continue eating until they felt comfortably full.

Methodological differences may help to explain the varied results observed in trials investigating the effects of protein beverages on food intake, although upon review of these studies no clear pattern emerges. Table 4 summarises methods employed in 13 previous studies with variable outcomes. Two prior studies were in accord with our present findings that protein beverages were unable to significantly modulate food intake compared with a water control (Almiron-Roig & Drewnowski, 2003; DellaValle et al., 2005), whilst 4 studies have shown no effect vs an isoenergetic CHO beverage (Bowen et al., 2007; Burton-Freeman, 2005; Harper, James, Flint, & Astrup, 2007; Lam et al., 2009). Conversely, 6 studies have shown suppression of EI when HP is compared with either (HCHO) or zero/low energy control beverages (Anderson & Moore, 2004; Bertenshaw et al., 2008; Bertenshaw et al., 2009; Bowen, Noakes, Trenerry, et al., 2006; Bowen, Noakes, & Clifton, 2006; Dove et al., 2009). Whilst preload timing may be an important determinate of response to the beverage challenge, significant changes in EI have been observed following both short (e.g. 30 min, Bertenshaw et al., 2008; Bertenshaw et al., 2009) and long (e.g. 180 and 240 min, Bowen, Noakes, Trenerry, et al., 2006; Bowen, Noakes, & Clifton, 2006; Dove et al., 2009) time intervals. Conversely, lack of response to the preload challenge has been reported following short 30 min time intervals (Harper et al., 2007; Lam et al., 2009). In these beverage studies, response is not clearly driven by dose either since high dose trials had no effect (Bowen et al., 2007; Lam et al., 2009), whilst lower dose proteins may elicit a change in food intake (Bertenshaw et al., 2009; Dove et al., 2009).

There remains debate as to the ability of VAS to predict subsequent food intake, and the importance of being able to successfully modulate perceptions of hunger without favourably altering EI at subsequent meals (Blundell et al., 2009). It is notable

Table 4
Protein beverage studies. The primary outcome measure in each of these studies was *ad libitum* energy intake (EI).

	Participants	Beverages	Preload	Study outcome
(1) No effect on EI				
(i) Protein vs water control				
Poppitt et al. [current study]	Women, $n=50$ overweight	Water beverages: 5 g (93 kJ), 10 g (178 kJ), 20 g (348 kJ) whey protein; water control (0 g, 8 kJ)	120 min	Increased VAS-rated satiety on high-protein vs water control, but no significant difference in EI between protein beverages and water control
(ii) Protein vs water control vs CHO				
Almiron-Roig and Drewnowski (2003)	Men and women, $n=32$	Beverages: skim-milk, orange juice, cola, 1036 kJ; water control, 0 kJ	135 min	Increased VAS-rated satiety on high-protein vs water control, but no significant difference in EI between high-protein, high-CHO or water control
DellaValle et al. (2005)	Women, $n=44$, lean and overweight	Beverages: water control; diet cola; cola (0 g prot, 40 g CHO); orange juice (3 g prot, 38 g CHO); 1% milk (12 g prot, 18 g CHO); ~650 kJ	0 min; co-presentation	No significant difference in VAS-rated satiety or EI between high-protein (1% milk), high-CHO (cola, OJ) or water control [note: total intake of preload + lunch was significantly greater for energy vs no energy beverages]
(iii) Protein vs CHO				
Harper et al. (2007)	Men, $n=22$, lean	Beverages:cola (53 g CHO); chocolate milk (13 g protein, 36 g CHO); 900 kJ	30 min	Increased VAS-rated satiety on high-protein, but no significant difference in EI between protein and CHO beverages
Bowen et al. (2007)	Men, $n=28$, obese	Beverages: 50 g whey protein, fructose, glucose; 25 g whey + 25 g fructose; 1100 kJ	240 min	Increased VAS-rated fullness on whey vs fructose (not glucose), but no significant difference in EI between protein and CHO beverages
Burton-Freeman (2005)	Men and women, $n=20$, lean and overweight	Beverages: high-CHO (1 g prot, 59 g CHO) vs high-protein [whey, whey-GMP (26 g prot, 33 g CHO)] vs GMP isolate (2 g prot, 58 g CHO); 1000 kJ	75 min	Increased VAS-rated satiety on whey-protein in women (not men), but no significant difference in EI between protein and CHO beverages
Lam et al. (2009)	Men and women, $n=50$	Milkshakes: CHO (10 g prot, 59 g CHO); WPI (45 g prot); WPI + GMP (43 g prot); WPI–GMP (46 g prot); ~1300–1700 kJ	30 min	Increased VAS-rated fullness on WPI-GMP vs CHO, WPI, WPI + GMP, but no significant difference in EI between protein and CHO beverages
(2) Significant effect on EI				
(i) Protein vs water control vs CHO				
Bertenshaw et al. (2008)	Men, $n=18$, lean	Dairy fruit drink: water control (40 kJ); low-CHO (16.5 g CHO, 330 kJ), high-CHO (72.8 g CHO), high-protein (37.7 g prot); 1250 kJ	2 preloads: 120 min, 30 min	EI lower following protein vs CHO and control; no effect on VAS-rated satiety
(ii) Protein vs CHO				
Anderson and Moore (2004)	Men, $n=13$, lean	Beverages: high-CHO [sucrose] vs high-protein [egg albumin; whey; soy protein. 0.65 g/kg body weight (~45 g)]	60 min	EI lower following whey and soy protein vs egg albumin and sucrose
Bowen, Noakes, Trenerry, et al. (2006)	Men, $n=19$, lean and obese	Chocolate milk: whey protein (55 g), casein protein (55 g), lactose (56 g), glucose (56 g); 1000 kJ	180 min	EI lower following both protein treatments vs glucose (not lactose)
Bowen, Noakes, and Clifton (2006)	Men, $n=72$, lean and obese	Beef soup: whey protein (50 g), soy protein (50 g), gluten (50 g), glucose (50 g); 1100 kJ	180 min	EI lower following all protein treatments vs glucose
Bertenshaw et al. (2009)	Men, $n=28$, lean	Dairy fruit drink: low (9 g prot), medium (17 g prot), high (35 g prot), 1155 kJ; low-CHO (16.5 g CHO, 330 kJ)	30 min	EI lower on protein (dose response) vs low-energy CHO; no effect on VAS-rated satiety
Dove et al. (2009)	Men and women, $n=34$, overweight	Skim milk (25 g prot, 36 g CHO); fruit drink (<1 g prot, 63 g CHO)	240 min	EI lower following high-protein vs CHO
(3) EI not measured				
(i) Protein vs CHO				
St-Onge et al. (2004)	Men and women, $n=20$, lean overweight	Beverages: sugar only (150 g CHO), mixed nutrient (100 g CHO + 25 g prot + 10 g fat); 2510 kJ	N/A	Decreased VAS-rated hunger and increased fullness following MN vs CHO

that in our current trial increasing the protein content of the beverages, even at these relatively low protein doses, significantly decreased hunger and increased fullness relative to the water control. It is clear from the studies reviewed in Table 4 that by far the majority of beverage studies show increased VAS-rated feelings of satiety when protein beverages are consumed, but commonly these acute changes are not translated into changes in eating behaviour at a later meal (Almiron-Roig & Drewnowski, 2003; Bowen et al., 2007; Burton-Freeman, 2005; Harper et al., 2007; Lam et al., 2009). Achieving a negative energy balance is of course central to successful weight loss yet achieving a change in eating behaviour within the laboratory setting can be challenging. Whether individuals alter their eating behaviour in response to changes in sensations of hunger, fullness, satisfaction and other appetite parameters is also under debate since it becoming apparent that 'hedonistic' eaters who consume in response to pleasure rather than physiological hunger may be widespread (Blundell, Stubbs, & Golding, 2005; Mela, 2006). In this study we investigated the effects of protein beverages in women who were overweight yet reported relatively low restraint, but who may be poor responders to physiological cues. It is important to realise, however, that this is the target group in which putative 'satiety-enhancing beverages' must be efficacious to have any public health worth. Whether the modulations in hunger sensations observed in this trial may have been sufficient to alter eating behaviour during the 2 h period between the beverage and the ad lib test meal, i.e. prevent pre-lunch snacking, may be worthy of investigation. In addition, whilst we reviewed menstrual cycle by diary and questionnaire in this study, since menstrual cycle may affect appetite regulation and food choice (Buffenstein et al., 1995), we did not perform serum hormone profile analyses to confirm follicular or luteal status which must be considered a limitation of the design.

It is likely that stronger effects than those observed in our study would be found if the protein dose in the beverage was increased. Low doses of 5, 10 and 20 g in a 500 mL volume were chosen in this study to enable whey protein to be incorporated into a water beverage. Consumption of bottled waters has increased exponentially worldwide over the past 5 years and now represents a significant portion of the commercial beverage industry. Achieving a low energy water-based protein beverage that is palatable to weight conscious consumers was one of the aims of this trial. Doses greater than 4% w/w can of course readily be incorporated into more viscous beverages and such studies have successfully shown proteins to maintain at least some of the increased satiety observed in HP foods (Almiron-Roig & Drewnowski, 2003; Bertenshaw et al., 2008). Whether an effect on EI would have been observed had the lunch been served closer to the preload beverage can also be debated. In conclusion, in this dose escalation study there was evidence that when compared with a water control, a low-dose whey protein-enriched water beverage consumed mid-morning had a positive effect on immediate postprandial measures of satiety, however the effects were short-term and not sufficient to significantly impact on subsequent food intake when measured 2 h later.

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