The effect of supplemental whey protein timing on postprandial glycaemia in centrally-obese males

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ABSTRACT

Consuming whey protein prior to a meal may reduce postprandial glucose excursions, however optimising timing of supplementation is important to improve its clinical utility. Thirteen centrally-obese, insulin resistant males (waist circumference: 121 (SEM 3) cm; HOMA-IR: 6.4 (SEM 1.2)) completed four experimental conditions in a single-blind, crossover design. Participants consumed mixed-macronutrient breakfast and lunch meals on all occasions, with 20 g whey protein consumed 15 min prior to (PRE), alongside (DUR) or 15 min post-breakfast (POST), or omitted (CON). Capillary glucose and plasma concentrations of insulin, triglycerides and NEFA, in addition to subjective appetite ratings, were collected for 180 min after each meal. PRE and DUR reduced post-breakfast glucose peak by 17.0 (SEM 1.9)% ($P < 0.001$) and 9.2 (SEM 2.9)% ($P = 0.046$) respectively, compared with CON. Post-breakfast glucose AUC was lower following PRE compared with POST and CON (PRE: 982 (SEM 30) vs POST: 1031 (SEM 36) and CON: 1065 (SEM 37) mmol/l x 180 min; $P \leq 0.042$), but similar to DUR (1013 (SEM 32) mmol/l x 180 min; $P = 0.77$). Insulin was lower during PRE, when compared with POST and DUR (both $P \leq 0.042$), but similar to CON. There were no between-condition differences in measures of postprandial lipaemia or appetite, and no effect of condition post-lunch. Consumption of whey protein as a preload or alongside a mixed-macronutrient breakfast reduces postprandial glucose excursions in centrally-obese, insulin resistant males. Whey consumed as a preload has superior glycaemic lowering effects. Supplementation at breakfast does not alter glycaemic responses to subsequent meals.

INTRODUCTION

Central obesity is associated with insulin resistance and an increased risk of developing type 2 diabetes and cardiovascular disease [1,2]. In those without diagnosed type 2 diabetes, postprandial glucose excursions are a stronger predictor of HbA1c than fasting glucose, and also increase the risk of cardiovascular disease [3-5]. Postprandial hyperglycaemia, no matter how brief, drives inflammation, oxidative stress and vascular dysfunction [6-9]. Thus, individuals who are centrally-obese and insulin resistant may benefit from strategies that ameliorate postprandial hyperglycaemic excursions [10].

Recent non-pharmaceutical interventional studies have sought to reduce postprandial hyperglycaemia through whey protein supplementation (for review [11]). Whey protein contains an abundant source of amino acids and bioactive peptides that are rapidly digested. These act as potent insulin secretagogues and can reduce gastric emptying, hepatic glucose production, and can increase satiety [12-16]. Despite many trials in normal-weight populations or individuals with type 2 diabetes, few studies have been conducted using centrally-obese participants without metabolic disease. This is surprising given that such individuals are likely to be exposed to elevated postprandial glycaemic excursions [17] and their associated adverse metabolic effects [18].

There are practical limitations associated with implementing pre-meal whey protein as a strategy to reduce postprandial hyperglycaemia. Firstly, studies have investigated the glycaemic response to single test meals of primarily high glycaemic index carbohydrate content [19,20], without investigating if the whey supplementation effects carry forward to subsequent meals. Secondly, dosages of whey protein administered are generally unrealistically large (45-55 g; ~200 kcal) [19,21]. Furthermore, whey protein has shown most benefit when supplemented as a preload around 30 min before the main meal [19,22], thus restricting its ecological validity when applied in free-living conditions, as this does not reflect conventional eating habits [23]. From a clinical viewpoint it is important to establish whether the beneficial effect of whey supplementation is retained at smaller doses, if the whey bolus is beneficial when consumed alongside or after the meal, and if the therapeutic effects influence glycaemia at subsequent meal times in centrally-obese insulin resistant individuals.

The purpose of this study was to investigate the effect of a realistic whey protein dose on postprandial metabolic and appetite responses in centrally-obese insulin resistant individuals, using timings and test meals that reflect habitual eating behaviours. We hypothesise that whey protein consumption will

reduce the postprandial glucose response and positively affect subjective appetite, with more favourable effects resulting from earlier supplementation.

METHODS

Participants

Centrally-obese male participants, free from metabolic disease, were recruited from the local community. Participants who met the inclusion criteria for gender (male), age (18-55 years), waist circumference (>102 cm) and physical activity level (low) were included in the study (n = 13; see Table 1 for participant characteristics). The waist circumference criterion value was based on the WHO threshold associated with the greatest risk of metabolic complications in males [24]. This was measured at the mid-point between the lower costal border and the illiocristale, according to International Society for the Advancement of Kinanthropometry (ISAK) guidelines [25]. Physical activity level was assessed using the categorical scoring method following completion of the International Physical Activity Questionnaire [26].

Ethical approval

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Faculty of Health and Life Sciences Research Ethics Committee (reference: HLSDA020415). All participants provided prior written informed consent. This study was registered at clinicaltrials.gov (NCT02658110).

Study design

Participants completed four experimental conditions in a randomised order, as part of a single-blind, crossover design. Each condition was separated by at least 3 days. On all visits participants consumed a standardised mixed-macronutrient breakfast meal, followed 180 min later by consumption of a standard lunch meal. The timing of additional protein supplementation varied by condition, with participants consuming 20 g whey protein as a preload 15 min prior to breakfast (PRE), during the breakfast meal (DUR), or 15 min post-breakfast consumption (POST). A control condition was also completed without additional protein supplementation (CON).

Pre-trial standardisation

Prior to each experimental visit participants were provided with verbal and written instructions regarding diet and physical activity control measures. For 24 hours prior to arrival participants were instructed to avoid caffeine and alcohol consumption as well as strenuous physical activity. A mixed-macronutrient meal was provided before each visit (3501 kJ; 37/19/44% energy from carbohydrate/protein/fat), and participants were instructed to consume this as their evening meal 12 hours prior to their arrival time, in order to standardise macronutrient and energy intake.

Experimental protocol

The experimental protocol is presented in Figure 1. Participants arrived at the laboratory at the same time (~08.00 hours) on each occasion following an overnight fast. After insertion of a cannula in an antecubital vein, baseline venous and capillary blood samples were collected and subjective appetite was assessed using visual analogue scales. In the PRE condition, participants consumed a 20 g whey protein beverage, with flavoured water (placebo) provided in all other conditions. After a further 15 min, breakfast was consumed in all conditions, accompanied by either a whey protein (DUR) or placebo test drink (POST and CON). At 15 min post-breakfast consumption, a further test drink was provided, with whey protein administered in the POST condition and flavoured water during all other conditions. The remainder of the protocol was identical under all experimental conditions, with blood samples and appetite ratings collected at regular intervals (Figure 1). Participants rested in a seated position for 180 min following breakfast consumption, after which a standardised lunch meal was consumed. Following a further period of seated rest (180 min), the cannula was removed and participants were able to leave the laboratory.

Test meals

At each test drink time point (15 min before, during or 15 min after breakfast) either a whey protein or placebo drink was consumed which was condition-dependent. The protein drink contained 23g whey protein isolate powder (Lacprodan SP-9225 Instant; Arla Food Ingredients Group, Viby, Denmark) mixed with 150 ml water and 0.5 ml energy-free strawberry flavouring (FlavDrops, Myprotein, UK), providing 20 g protein (87% protein content) and 343 kJ (82 kcal) energy. The placebo drink was matched for volume and taste using similarly flavoured water. All drinks were

provided in opaque bottles and no reference was made to which condition was being conducted. An additional 200 ml drinking water was administered after each test beverage to eliminate any after taste.

A standardised breakfast of rolled oats (54.0 g) with semi-skimmed milk (260 ml) and honey (42.5 g) was provided under all conditions as a semi-liquid porridge mixture. This provided 1958 kJ (468 kcal) of energy (70% carbohydrate, 17% fat, 13% protein). Participants were encouraged to consume this meal within 10 min. Porridge was selected to represent a habitually consumed breakfast food amongst the UK population [27] which provides a mixed macronutrient composition.

A standardised mixed-macronutrient lunch meal was provided in all conditions, as described previously [28]. A 125 g portion of dried fusilli pasta was cooked and combined with 170 g of a tomato-based sauce (Dolmio, Mars, USA), grated cheddar cheese (40 g) and olive oil (15 g). The resulting homogenous meal provided 3448 kJ (824 kcal) of energy (50% carbohydrate, 36% fat, 14% protein). Participants were again instructed to finish the entire meal within 10 min. Water (500 ml) was also served alongside the lunch meal, and was withheld in the post-lunch period.

Blood sampling and analysis

On arrival, a cannula (Vasofix 22G, B.Braun Melsungen AG, Germany) was inserted into a vein in the antecubital fossa while participants remained in a semi-supine position. At each sample point, 10 ml of whole venous blood was drawn into a syringe and transferred into an EDTA coated tube (Vacutainer, Becton Dickinson, USA) followed by centrifugation at 1734 *g* and 4˚C for 10 min (Allegra X-22R, Beckman Coulter, USA). Plasma was aliquoted into separate microtubes and stored at -80˚C for subsequent analysis. Sterile stylets (22G, B.Braun Melsungen AG, Germany) were inserted to keep the cannula patent between blood samples. To control for any postural changes in plasma volume participants were instructed to remain in a seated position throughout the protocol where possible.

Capillary blood samples (20 µl) were collected immediately following each venous blood draw using the finger-prick technique and processed for glucose (Biosen C_line analyser, EKF Diagnostics, UK). Additional samples were collected at 5 and 10 min post-meal in order to increase the resolution of the blood glucose curve during the period where rapid changes may occur. Venous samples were processed and analysed for concentrations of plasma insulin, NEFA and triglyceride. Insulin

concentrations were determined using a commercially available ELISA (IBL International, Hamburg, Germany), with intra and inter-assay variation (CV) of 5.9% and 8.9% respectively. NEFA and triglyceride concentrations were determined by enzymatic colorimetric assays using an automated analyser (RX Daytona, Randox Laboratories, UK) according to manufacturer instructions.

Subjective appetite

Subjective ratings of appetite were captured at baseline and at regular intervals throughout (Figure 1). The reproducibility of within-subject responses and the sensitivity to experimental manipulations for this technique have previously been established [29]. Paper-based 100 mm scales were used to record perceptions of hunger, fullness, satisfaction and prospective food consumption (PFC).

Statistical analysis

A sample size calculation was performed using a reduction in postprandial glycaemia (AUC) as the primary outcome. Based on the observed 16% reduction in glucose AUC following consumption of whey protein with a mixed-macronutrient breakfast in an overweight/obese population [30], it was calculated that a sample size of 11 would provide statistical power above 80%, with a two-sided alpha level of 0.05. To account for a 20% dropout, a minimum of 13 participants was targeted.

Serial measures of blood analytes and subjective appetite responses were used to calculate AUC using the trapezoidal method. The resultant total AUC includes all area below the response curve in order to take account of situations where concentrations fell below baseline [31]. Insulin sensitivity was assessed using the Matsuda Insulin Sensitivity Index (ISI), calculated using fasting and postprandial concentrations of plasma glucose and plasma insulin [32]. A combined appetite score (CAS), was used to combine the four aspects of subjective appetite assessment, as described previously [33].

Statistical analysis was conducted using SPSS (version 21, IBM, USA). Blood and plasma analyte concentrations and subjective appetite responses were tested for differences between conditions over time using two-way (condition x time) repeated measures ANOVA. Area under the curve was analysed using one-way ANOVA. Post hoc analysis was performed upon identification of significant main effects and the Bonferroni correction was used to correct the level of alpha for multiple comparisons. The level of statistical significance was set at $p < 0.05$ and data are presented as mean values with their standard errors.

RESULTS

Post-breakfast responses

Fasting values did not differ between conditions for all physiological and appetite variables assessed ($P > 0.05$). Blood glucose responses displayed a significant condition x time interaction effect ($P < 0.001$) in addition to main effects of condition ($P < 0.001$) and time ($P < 0.001$). Glucose peaked at 30 min post-breakfast under all conditions (Figure 2a), with the magnitude of the peak significantly reduced when whey protein was consumed as a preload or alongside breakfast compared with whey after the meal or no supplementation (PRE: 7.2 (SEM 0.3), DUR: 7.8 (SEM 0.3) vs POST: 8.6 (SEM 0.3), CON: 8.6 (SEM 0.2) mmol/l; all $P \leq 0.046$). A tendency for peak glucose to be reduced in PRE compared to DUR was also observed, however this was not statistically significant ($P = 0.076$). Compared to CON, glucose was lower in PRE at 10-45 min ($P \leq 0.017$), and in DUR at 30-60 min post-breakfast ($P \leq 0.041$). Concentrations returned to baseline levels under all conditions thereafter ($P > 0.05$). Glycaemia across the post-breakfast period was significantly lower following whey preload compared with POST and CON conditions (AUC: PRE: 982 (SEM 30) vs POST: 1031 (SEM 36) and CON: 1065 (SEM 37) mmol/l x 180 min; $P \leq 0.042$), but not significantly different from the whey with meal condition (DUR: 1013 (SEM 32) mmol/l x 180 min; $P = 0.77$; Figure 2b). Glycaemia was not significantly different from CON when whey was supplemented during ($P = 0.171$) or after ($P = 0.816$) the breakfast meal.

Postprandial plasma insulin concentrations displayed significant effects of time ($P < 0.001$) and significant condition x time interactions ($P = 0.008$; Figure 2c). At the time of breakfast consumption (0 min) insulin was elevated in PRE compared to CON ($P = 0.04$). Concentrations rose following breakfast consumption, remaining significantly elevated above baseline level at all time points between 15 and 120 min post-breakfast ($P \leq 0.007$; Figure 2c). Insulin AUC across this period showed a reduced insulin response when whey was supplemented before breakfast compared with other supplementation times (AUC: PRE: 96340 (SEM 10807) vs DUR: 112344 (SEM 10310) and POST: 121997 (SEM 15862) pmol/l x 180 min; $P \leq 0.032$; Figure 2d), and was similar to the response following breakfast without additional protein (CON: 99115 (SEM 14656) pmol/l x 180 min; $P > 0.05$). When whey was supplemented after the meal, insulinaemia was ~23% greater than CON ($P = 0.049$). Insulin sensitivity did not significantly differ between conditions during the post-breakfast period

(Matsuda-ISI; PRE: 3.8 (SEM 0.6), DUR: 2.9 (SEM 0.5), POST: 2.8 (SEM 0.4), CON: 3.3 (SEM 0.5) arbitrary units; $P = 0.161$).

Post-breakfast NEFA concentrations were significantly affected by time ($P < 0.001$) such that concentrations were immediately suppressed following breakfast consumption under all conditions, and remained significantly below baseline from 15-180 min post-breakfast ($P \leq 0.006$; Figure 2e). There was no effect of condition ($P = 0.611$) and NEFA AUC was similar between conditions across this period ($P = 0.517$; Figure 2f).

An effect of time ($P < 0.001$), but not condition ($P = 0.26$) or condition x time interaction ($P = 0.423$), was observed on appetite perceptions following breakfast. Combined appetite ratings were similarly suppressed under all conditions following breakfast consumption, reaching their nadir at 15-30 min post-consumption, the magnitude of which was not different between conditions (PRE: 14 (SEM 4), DUR: 15 (SEM 4), POST: 17 (SEM 4), CON: 20 (SEM 4) mm; $P = 0.344$; Figure 3a). Appetite subsequently increased gradually, reaching similar levels to baseline at 120-180 min post-breakfast in all conditions ($P > 0.05$). There were no significant differences in AUC between conditions for combined appetite score (Figure 3b) or individual subjective appetite components across this period ($P > 0.05$; Table 2).

Post-lunch responses

Following consumption of a standardised lunch meal, both glucose and insulin concentrations displayed a significant effect of time ($P < 0.001$), with no condition x time interactions observed ($P > 0.05$). Concentrations peaked similarly across all conditions before returning to pre-lunch levels at 60 and 90 min post-lunch for glucose and insulin respectively (Figure 4). Post-lunch AUC revealed no differences between conditions in glycaemia ($P = 0.262$) or insulinaemia ($P = 0.271$; Figure 4).

Post-lunch NEFA concentrations were not influenced by condition ($P = 0.346$), with similar AUC observed between conditions ($P = 0.587$; Figure 4f). Concentrations were moderately suppressed at 45-60 min post-lunch under all conditions, before returning to pre-lunch levels at 90-180 min (Figure 4e). Appetite was similarly affected by consumption of lunch in all conditions ($P = 0.423$). An effect of time was present ($P < 0.001$), such that there was an immediate reduction in combined appetite score following lunch, before gradually returning to pre-lunch levels at 150-180 min post-consumption across all conditions (Figure 3c). There were no significant differences observed in post-

lunch AUC for combined appetite score (Figure 3d) or its constituent components of hunger, fullness, PFC and satisfaction between conditions ($P > 0.05$; Table 2).

Plasma triglyceride

Triglyceride concentrations increased similarly over the course of the experimental protocol in all conditions. Post hoc analysis of the main effect of time ($P < 0.001$) indicated that levels were elevated above baseline concentration from 60 min post-breakfast onwards ($P \leq 0.018$), and continued to rise throughout (Figure 5a). There was no effect of condition on triglyceride concentrations ($P = 0.653$), and AUC was similar between conditions for triglyceride response across the full postprandial period ($P = 0.64$; Figure 5b).

DISCUSSION

The findings of the current study demonstrate that consumption of a 20 g whey protein bolus, either before or alongside a mixed-macronutrient breakfast, attenuates peak postprandial glucose excursions in centrally-obese, insulin resistant males. Furthermore, consuming whey as a preload appears to confer the greatest beneficial effect to reducing postprandial hyperglycaemia. Moreover, post-meal whey protein consumption raises insulinaemia but without a concomitant reduction of hyperglycaemia. The beneficial effects of prandial whey protein supplementation are acute, and do not carry forward to subsequent meals.

Peak glucose excursions and glucose area under the curve were reduced when whey was supplemented as a preload or with the meal. Postprandial hyperglycaemia is a greater risk factor for CVD than increased fasting glucose in non-diabetics [3,4], where its contribution to overall glycaemia is particularly marked. Furthermore, postprandial glycaemic excursions have been established as the main causative factor in glycaemic variability in non-insulin treated individuals with impaired glucose tolerance [34], and such excursions manifest adverse metabolic effects via activation of oxidative stress and endothelial dysfunction [35]. Thus, evidence supports recommending reductions in postprandial hyperglycaemia as a relevant clinical goal in delaying or preventing the onset of type 2 diabetes [36]. Our data show that a moderate 20 g dose of whey protein offers practical utility as a meal-time aid for reducing the hyperglycaemic burden, with a post-breakfast reduction in peak glucose of 17.0% and 9.2% when whey was consumed before or alongside the meal respectively. Additionally,

a 7.3% decrease in glucose AUC across the postprandial period was observed following the whey preload, devoid of any significant change in insulin AUC. Whilst it is understood that postprandial hyperglycaemia is predictive of various complications and metabolic derangements, as described above, the clinical significance of such a reduction in glucose AUC of the magnitude shown in our study, in terms of translation to a clinical end point, remains unclear.

The mechanisms explaining the reduction in postprandial glycaemia are yet to be fully elucidated. Our data contrast with prior literature [19,21], in that the insulin concentrations were not elevated during the postprandial period with the preload supplementation strategy. However, at the onset of the breakfast ingestion, we observed raised insulin concentrations under PRE in comparison to CON (Figure 2). A reduction in the early postprandial insulin response to feeding is a characteristic associated with insulin resistance and type 2 diabetes [37], and thus the raised insulin may have contributed to clearing glucose, suppressing hepatic glucose output, and opposing the potential rise in glucagon that can occur with protein feeding. Furthermore, consuming whey as a preload is known to slow gastric emptying [19]. Thus, a combined influence of raised insulin concentrations prior to meal ingestion and a slower rate of gastric emptying could explain the superior influence of the preload strategy on reducing postprandial glycaemia. This explanation is supported by insulin concentrations that were similar between DUR and CON, yet glycaemia was significantly lower with DUR. Moreover, insulin concentrations were elevated with post-meal supplementation, without any improvement in glycaemia.

The effects of whey protein ingestion, independent of timing of consumption, were limited to glucose and insulin responses in the current study. Postprandial lipaemia was not affected by co-ingestion of whey protein with breakfast, which is in line with previous findings in normal-weight males [28] and those with and without type 2 diabetes [38], but differs from the findings of Pal *et al.* [39]. Pal and colleagues observed a 21% reduction in plasma triglyceride AUC following 45 g whey, compared with the same dose of glucose, when administered alongside a mixed-macronutrient meal in obese females. Such a disparity cannot be attributed to differences in fat content of test meals, as postprandial triglyceride was unaffected when relatively low (the present study) and high [38] fat test meals have been supplemented with prior whey protein. The amount of supplemental whey administered by Pal *et al.* however, was over 2-fold greater than both these studies, which may have influenced the observed triglyceride-lowering effect.

Subjective appetite appeared to be unaffected by whey protein supplementation, regardless of condition. Prior research has demonstrated an appetite suppressing effect of whey protein, with wide ranging protocols and participants, e.g. in overweight males after a 55 g preload [40] and with a 15 g whey bolus in type 2 diabetes [16]. In the study by King *et al.* pre-meal whey protein resulted in greater post-meal insulin concentrations compared to control, possibly playing a role in appetite suppressession [41,42]. However, in the current study the elevated pre-meal insulin during PRE, and the greater insulin during POST, did not translate into changes in appetite sensations. A mixed effect of whey on subjective appetite ratings in non-diabetic individuals has previously been shown. Several studies have reported reductions in appetite [43-45], although the exact mechanisms remain to be elucidated, while others show no effect on perceptions of appetite with [46,47] or without reductions in energy intake [48]. Future research should explore appetite sensations and energy intake following both acute and longer term use of prandial whey protein supplementation.

Whey protein at breakfast did not affect post-lunch responses across all outcomes assessed in the present study. This appears to confirm previous findings in normal-weight individuals [28], and suggests that any effects of whey protein on postprandial glycaemia are transient, and may provide rationale for researchers to investigate the supplementation of whey protein at multiple sequential meals.

To the authors' knowledge, this was the first study to assess the effect of timing of whey protein consumption on post-meal glycaemia in a non-diabetic population. The methodology was strengthened by efforts to increase the ecological validity of findings, including administering a dose of protein that could realistically be supplemented alongside a meal, and timings of supplementation that would not inconvenience individuals wishing to carry out this strategy to reduce glycaemic excursions. Care was also taken to use foods that were typical of those consumed at breakfast and lunch meals across the population. Further examination of the findings, and associated mechanisms underpinning them, is however limited. The measurement of circulating concentrations of amino acids and incretin hormones, as well as rates of gastric emptying, may have been beneficial in this regard. Whilst the aim of the present study was to investigate the effect of whey protein supplementation and its timing, irrespective of the mechanisms of action, consideration should be given to incorporating these measures into future work.

In summary, consumption of whey protein alongside a mixed-macronutrient meal attenuated postprandial glucose excursions in centrally-obese insulin resistant males. In addition, as hypothesised, consumption of whey as a preload had a similar effect on peak glucose, also attenuating

glycaemia over the subsequent 3 hours, with a simultaneously reduced insulin response. Reductions in postprandial glycaemia may be beneficial in preventing or delaying the progression from normal to impaired glucose tolerance or type 2 diabetes. Postprandial lipaemia and subjective appetite, contrary to the original hypothesis, were not affected by the supplementation of 20 g whey protein or its timing of consumption. The benefits of whey supplementation are acute and future research should explore glycaemic responses and macro and micronutrient intake following long-term supplementation.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Authorship

D. M. A., P. L. S. R., D. J. W. and E. J. S. designed the research; D. M. A. conducted the research; D. M. A. analysed the data; P. L. S. R., D. J. W. and E. J. S. contributed to writing the paper; D. M. A. wrote the paper. All authors read and approved the final manuscript.

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FIGURES

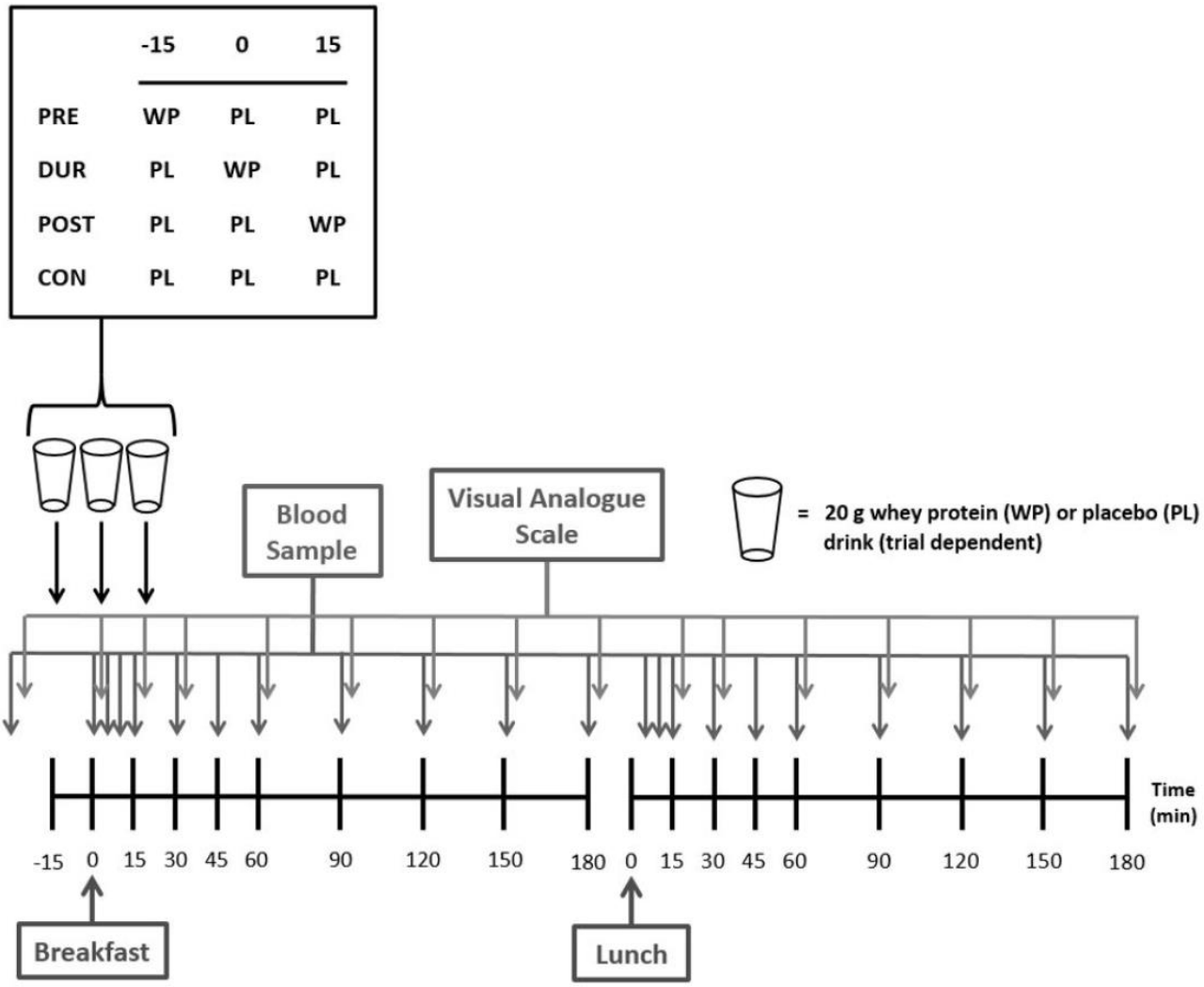


Figure 1. Schematic representation of experimental trials.

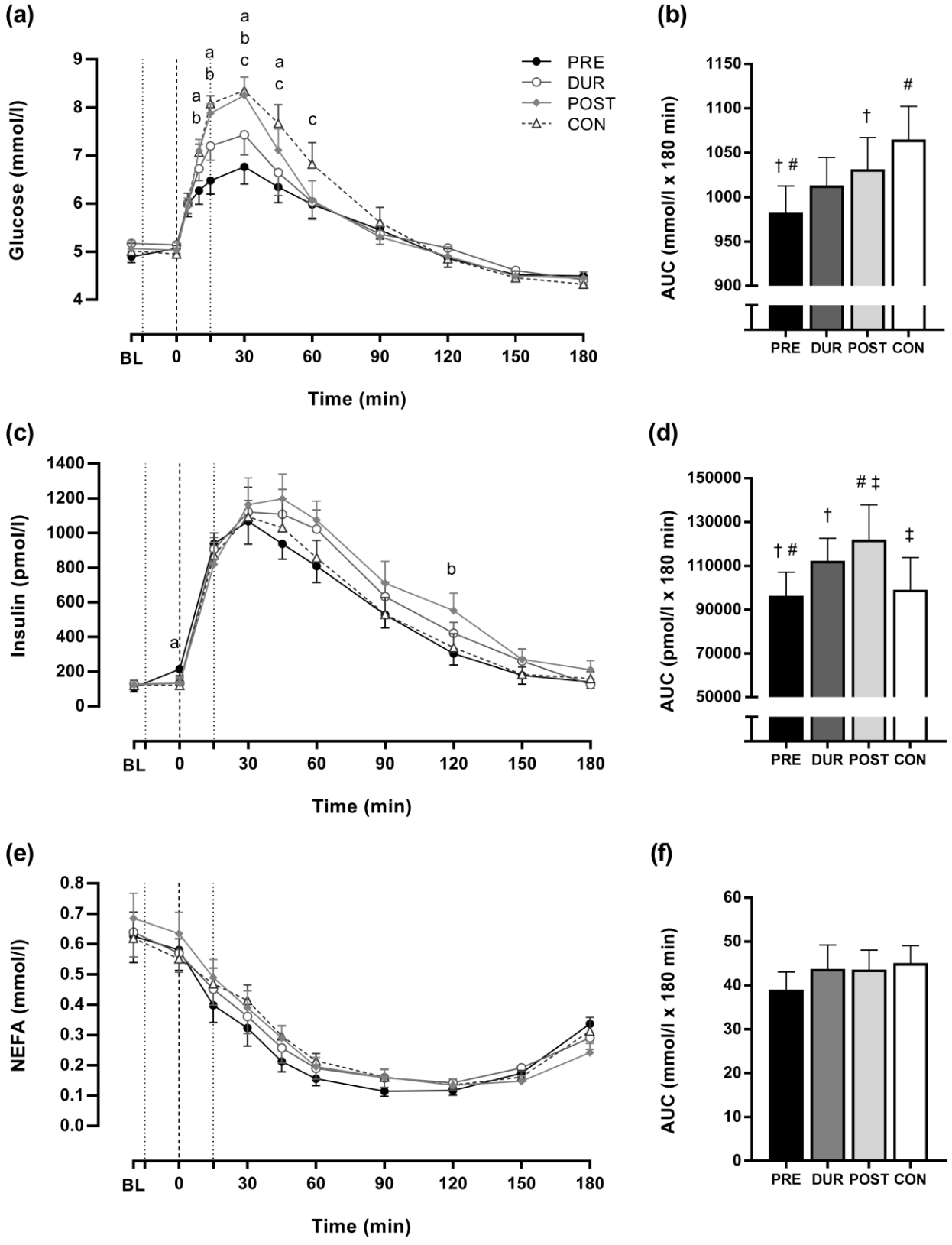


Figure 2. Time course changes in glucose (a), insulin (c) and NEFA (e) concentrations and area under the curve for glucose (b), insulin (d) and NEFA (f) during the post-breakfast period (0-180 min). Significant differences ($P < 0.05$) between conditions at individual time points are defined as follows:

a, PRE vs CON; b, PRE vs POST; c, DUR vs CON; black dotted line indicates time of breakfast consumption; grey dotted lines indicate time of whey protein consumption during PRE and POST conditions. Bars sharing a common symbol differ significantly from one another ($P < 0.05$). Time course data were analysed using two-way (condition x time) repeated measures ANOVA. AUC data were analysed using one-way repeated measures ANOVA. Data are presented as mean with SEM. BL, baseline.

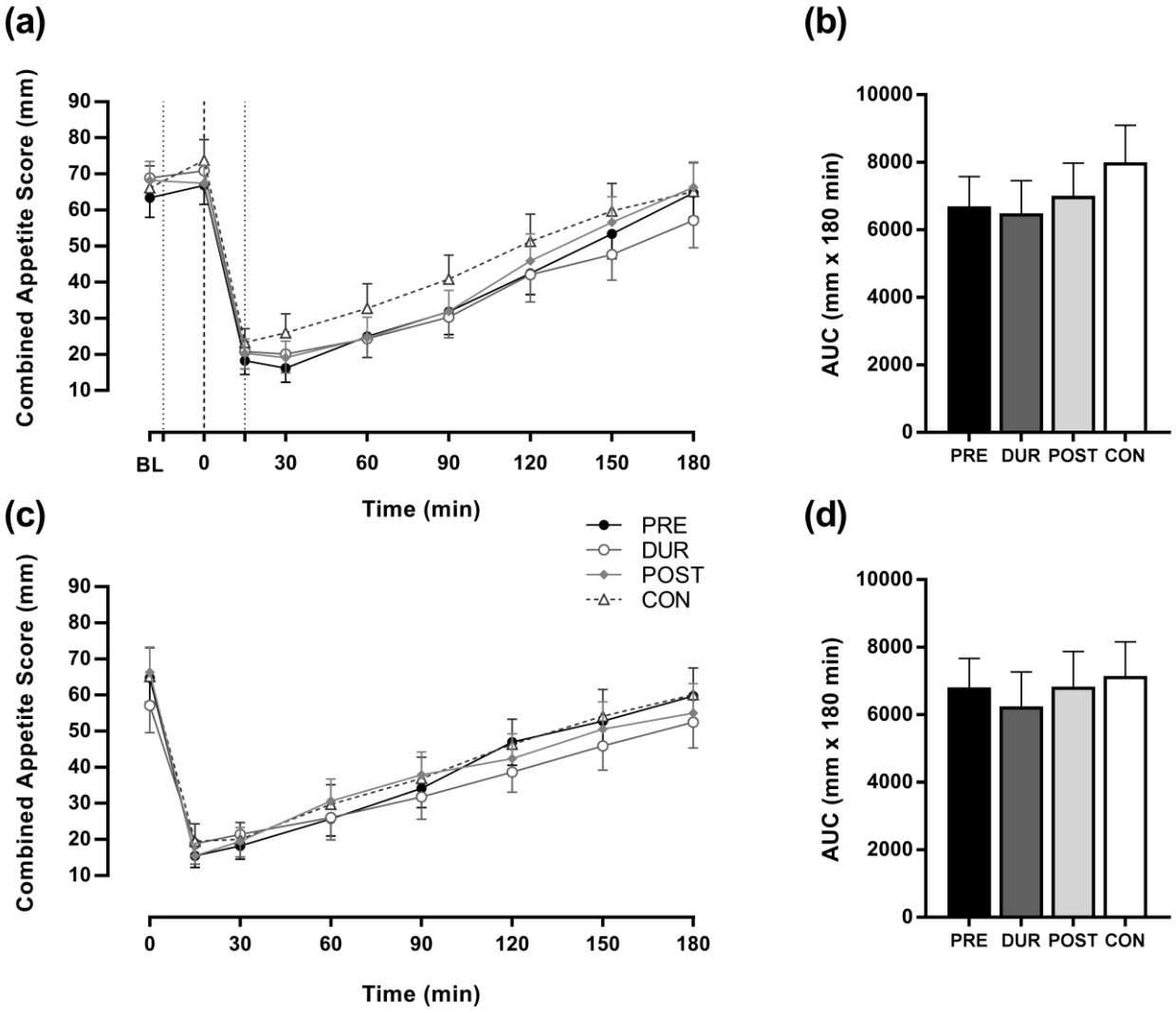
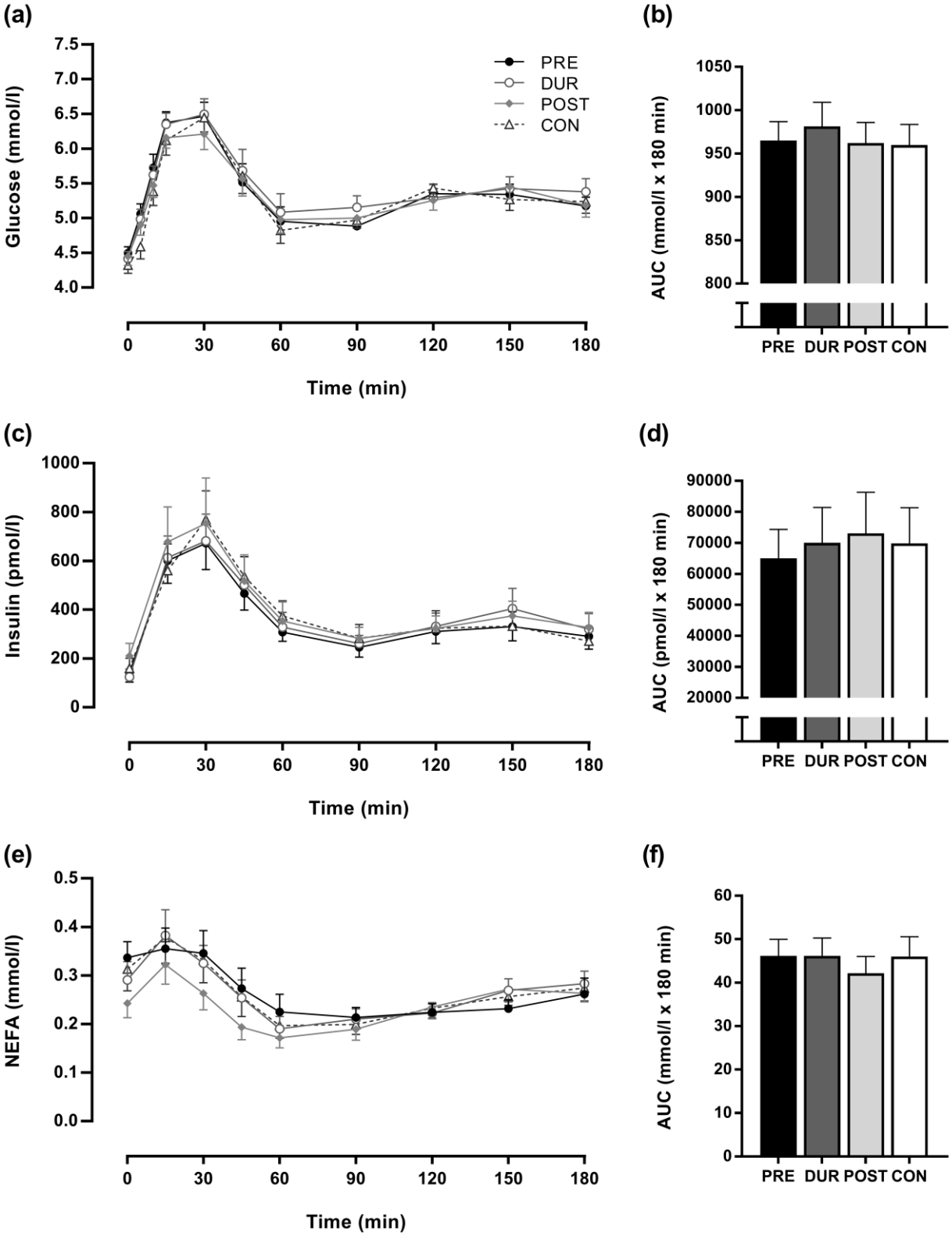


Figure 3. Time course changes and area under the curve for combined appetite score during the post-breakfast (a-b) and post-lunch (c-d) postprandial periods. Black dotted line indicates time of breakfast consumption; grey dotted lines indicate time of whey protein consumption during PRE and POST conditions. Time course data were analysed using two-way (condition x time) repeated measures

ANOVA. AUC data were analysed using one-way repeated measures ANOVA. Data are presented as mean with SEM. BL, baseline.



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585 **Figure 4.** Time course changes in glucose (a), insulin (c) and NEFA (e) concentrations and area under
586 the curve for glucose (b), insulin (d) and NEFA (f) during the post-lunch period (180-360 min). Time

course data were analysed using two-way (condition x time) repeated measures ANOVA. AUC data were analysed using one-way repeated measures ANOVA. Data are presented as mean with SEM.

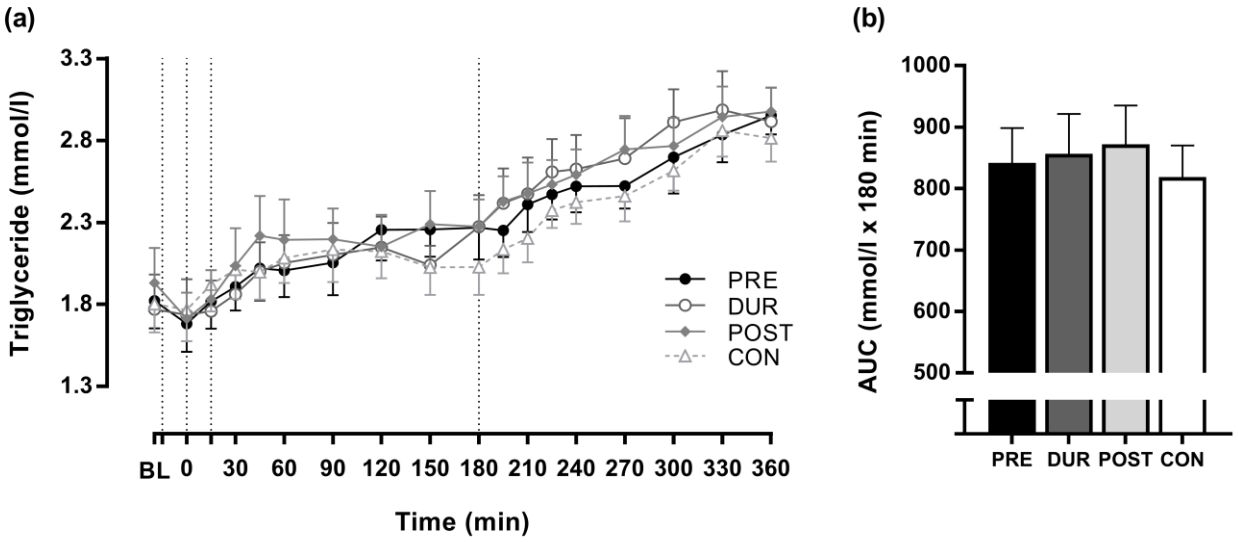

Figure 5. Time course changes (a) and area under the curve (b) for plasma triglyceride concentrations throughout the full experimental protocol (0-360 min). Black dotted line indicates time of breakfast consumption; grey dotted lines indicate time of whey protein consumption during PRE and POST conditions. Time course data were analysed using two-way (condition x time) repeated measures ANOVA. AUC data were analysed using one-way repeated measures ANOVA. Data are presented as mean with SEM. BL, baseline.

TABLES

Table 1. Participant characteristics (mean values with standard errors, n = 13).

	Mean	SEM
Characteristics		
Age (years)	39	3
Body mass (kg)	117.9	3.7
Stature (cm)	181.3	2.8
BMI (kg/m^2)	36.0	1.1
Waist circumference (cm)	121.3	2.6
Waist/hip ratio	1.00	0.01
Waist/height ratio	0.67	0.02
Fasting variables		
Blood glucose (mmol/l)	5.0	0.1
Plasma insulin (pmol/l)	122.0	24.8
HOMA-IR	6.4	1.2
Plasma triglyceride (mmol/l)	1.91	0.17

Fasting values are presented as mean of fasting samples for each main trial.

Table 2. Area under the curve for components of subjective appetite during post-breakfast (0-180 minutes) and post-lunch (180-360 minutes) postprandial periods (mean values with standard errors, n = 13).

		Area under the curve							
		PRE		DUR		POST		CON	
		Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Hunger (mm x 180 min)	Post-breakfast	6033	801	6070	911	6461	887	7641	1095
	Post-lunch	6046	734	5651	954	6378	1012	6649	929
Fullness (mm x 180 min)	Post-breakfast	11350	885	11553	964	11107	965	9963	1139
	Post-lunch	11347	824	11980	974	11130	1080	11003	1007
PFC (mm x 180 min)	Post-breakfast	7303	1034	6642	1086	7448	1094	8761	1288
	Post-lunch	7499	1069	6882	1187	7423	1165	7707	1201
Satisfaction (mm x 180 min)	Post-breakfast	11203	929	11194	966	10767	969	10411	982
	Post-lunch	10941	881	11548	1018	11313	979	10767	980

PFC, prospective food consumption. AUC data were analysed using one-way repeated measures ANOVA.