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Title: Postprandial profiles of CCK after high fat and high carbohydrate meals and the relationship to satiety in humans

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Postprandial profiles of CCK after high fat and high carbohydrate meals and the relationship to satiety in humans
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Highlights

1) Postprandial CCK levels were higher after HF meal compared to HCHO isocaloric meal

2) There was no association between CCK levels and intensity of satiety, or meal size

3) CCK probably acts in conjunction with other peptides and the action of the stomach
Abstract

**Context:** CCK is understood to play a major role in appetite regulation. Difficulties in measuring CCK have limited the potential to assess its profile in relation to food-induced satiety. Improvements in methodology and progress in theoretical understanding of satiety/satiation make it timely for this to be revisited.

**Objective:** First, examine how physiologically relevant postprandial CCK8/33(s) profiles are influenced by fat (HF) or carbohydrate (HCHO) meals. Second, to examine relationships between postprandial CCK and profiles of satiety (hunger/fullness) and satiation (meal size).

**Participants and Design:** Sixteen overweight/obese adults (11 female/5 male) participated in a randomised-crossover study (46 years, 29.8 kg/m²) in a university research centre. Plasma was collected preprandially and for 180 min postprandially. Simultaneously, ratings of hunger/fullness were tracked for 180 min before an ad libitum lunch was provided.

**Results:** CCK8/33(s) levels increased more rapidly and reached a higher peak following HF compared to HCHO breakfast ($F_{(1,15)}=14.737, p<0.01$). Profiles of hunger/fullness did not differ between conditions ($F_{(1,15)}=0.505, p=0.488; F_{(1,15)}=2.277, p=0.152$). There was no difference in energy intake from the ad libitum meal (HF-3958 versus HCHO-3925kJ; $t_{(14)}=0.201, p=0.844$). CCK8/33(s) profiles were not associated with subjective appetite during early and late phases of satiety; nor was there an association between CCK8/33(s) and meal size.

**Conclusions:** These results demonstrate CCK levels were higher after HF meal compared to HCHO isocaloric meal. There was no association between CCK levels and intensity of satiety, or with meal size. Under these circumstances, CCK does not appear to play a unique independent role in satiety/satiation. CCK probably acts in conjunction with other peptides and the action of the stomach.
Keywords: CCK; satiety; satiation; eating behaviour; appetite
Introduction

For over 25 years, CCK has been regarded a satiety peptide whose action is critical for the short-term control of appetite [1]. The first study on the short-term control of feeding behaviour showed that CCK infused into fasted rats resulted in a dose response relationship between CCK and reductions in food intake, with no signs of toxicity [2]. The authors proposed that the exogenously administered CCK mimicked the endogenous action of CCK, thus inspiring 25 years of research aiming to clarify the effect of CCK on the control of food intake [3, 4]. It should be noted that most studies on CCK and appetite have been carried out on rats rather than humans, and the large majority of all studies have used exogenous administration rather than the measurement of endogenous CCK in response to food consumption. Three reviews [5-7] have summarised publications regarding CCK and short-term appetite control. The effect of exogenous CCK on reducing food intake (satiation) appears particularly robust, while findings on inter-meal appetite (satiety) are less consistent [5].

In using these reviews to understand the action of CCK on satiety in humans, it may be useful to keep in mind that, firstly, rat and human studies should be considered separately; secondly, exogenous administration, rather than endogenous measures of CCK precludes knowledge on circulating peptide concentrations; thirdly, some studies give rise to pharmacological rather than physiological levels of CCK.

To our knowledge, only one study has investigated the relationship between circulating plasma CCK and subjective appetite sensations in humans, although others have measured CCK and subjective appetite simultaneously without reporting correlations [8]. This study in 9 normal weight males examined plasma CCK and subjective appetite in response to a single mixed-meal (fixed quantities of beef-burger and beans, but free consumption of numerous other food items). Circulating CCK levels rose rapidly after food consumption and remained
elevated for several hours as would be expected as the food consumed is processed through the gastrointestinal tract. Correlational analyses between CCK and subjective appetite revealed that the higher the CCK profile, the lower the hunger ratings and the higher the fullness ratings. However, investigation of the inter-individual profiles in this study revealed that only 3 out of 9 (hunger) and 4 out of 9 (fullness) participants demonstrated this relationship. Another study has examined patterns of CCK and subjective appetite and reported that the patterns of change in CCK and subjective appetite do not match [9].

A further approach to the study of CCK has involved the response to different nutrients. Consumption of fat preferentially stimulates the release of CCK [10, 11] and the primary role of CCK is thought to be to aid the digestion and absorption of fat entering the gastrointestinal tract. Previous studies have suggested that inhibition of food intake, particularly in response to intestinal administration of fat, is mediated by CCK. For example, in humans intraduodenal fat (pure corn seed oil) induced a reduction in food intake and a decrease in hunger; when loxiglumide was infused the effects of fat were abolished, thereby supporting the notion that CCK could act as a signal in short term appetite [12]. However, intraduodenal infusion of corn seed oil does not provide the same stimulus as consuming fat within normal meals and this fact may limit the interpretation of this approach. It therefore appears that the evidence for a general role for CCK in satiety or satiation still lacks critical proof. One noticeable feature of previous interpretations is that researchers have generally assumed a ubiquitous role for CCK in the adjustment of appetite in response to all foods, independent of nutritional composition. It may be questioned whether this is likely to be the case in light of CCK’s specific action in response to the ingestion and handling of fat. Of course, it should be recognised that a variety of experimental approaches and research designs may be needed to disclose the systematic relationship between the initiation of eating and the generation of physiological responses that bring the meal to an end, and then maintain inhibition over
further eating as conceptualised by the satiety cascade [13]. One procedure (out of a number of possibilities) is the presentation of controlled foods in a natural eating episode and the subsequent tracking of the profiles of hunger, fullness and the designated peptide. This strategy was employed in the present study.

The aim therefore was to investigate the role of endogenous, physiological levels of CCK in the immediate post-meal period after consumption of iso-energetic meals high in fat (low carbohydrate) or high in carbohydrate (low fat) controlled for energy content, weight (and therefore energy density) and palatability. The role of CCK in short-term appetite was assessed through quantitative measures of subjective appetite (satiety) and self-determined food intake (satiation).

**Methods**

**Participants**

Sixteen healthy overweight (9) or obese participants (7) took part (11 women, 5 men). Participants were recruited via emails and poster advertisements. Initial screening procedures were conducted within individual cubicles in our laboratory and were used to ensure the participants met the inclusion criteria of BMI between 27-38 kg/m², non-smoking, not on any medication and non-active (<1 session of moderate intensity exercise per week defined as structured exercise likely to raise the heart rate to 70% of age-predicted heart rate maximum). The overweight/obese category was used because of the relevance of gut peptides to appetite in the context of obesity and weight gain. It is important to note that this study has been published previously [14], which can be referred to for further details. At the time of this publication, we did not have the CCK data, nor did we know it was possible. Therefore, some of the data from the participants in this study were included in a previous publication, particularly the insulin/glucose and subjective appetite data [14]. However, all of the CCK
peptide data reported here are novel and have not been used in any other publication.

Participant characteristics are shown in table 1.

Ethics

The present study was conducted according to the guidelines in the Declaration of Helsinki. All procedures were approved by the NHS Leeds (West) Research Ethics Committee, UK (#09/H1307/7). Peptide analysis procedures were approved by the regional ethics committee in Stockholm, Sweden (No 2011/1956-31/2). Written informed consent was obtained from all subjects. This study obtained International Standard Randomised Controlled Trial Registry authorisation (ISRCTN47291569) in compliance with guidelines from WHO and CONSORT.

Study Design

A within-subject randomised crossover design was used. Participants visited the laboratory on two mornings (separated by at least 3 days) in a fasted state, having eaten nothing from 10pm the previous night. In addition, the night before each study visit, participants were provided with a standardised pasta meal – the participants ate ad libitum on the evening before their first test day, and then ate the same amount the night before the second test day. This ensured the participants were in the same state for both test days. Body composition was measured on one of the two mornings using air displacement plethysmography (Bodpod, Concord, CA, USA).

Upon arrival at the research unit, an intravenous cannula was inserted into the antecubital vein in order for serial blood samples to be taken to measure glucose, insulin and CCK. One fasting blood sample and fasting appetite ratings were completed before the fixed breakfast meal was provided. On acceptance to the study, participants were allocated a number by the experimenter and randomized to start on the high fat or high carbohydrate breakfast. The
randomization was counterbalanced so that half the numbers allocated started on the high fat condition and the other half started on the high carbohydrate condition. The breakfast meals were isocaloric, weight and protein matched but differed in their fat and carbohydrate composition. Since weight and energy were matched, energy density was therefore controlled for. The breakfast was either high fat (>50% energy from fat) or high carbohydrate (<4% energy from fat). Participants were blinded to what the breakfast conditions were; both breakfasts were similar in how they were perceived and were of equal palatability assessed using visual analogue scales during piloting of the study. Rate of consumption of the breakfast was fixed (10 minutes) and therefore the same for all participants. For all meals and during the following three hours, participants stayed in the laboratory in separate cubicles to ensure no social influences took place. Participants were allowed 15 minutes to complete the ad libitum lunch meal. Water was available to participants ad libitum on the first test day, and this was matched on their second test day. Blood sampling and Visual Analogue Scale (VAS) appetite sensation measures were taken at specific time points until an ad libitum lunch meal was provided (see Figure 1 reproduced from [14]). Time points of 0 (fasting), 10, 20 30, 60, 90, 120 and 180 minutes post-breakfast were used. These timings were designed to capture the rapid changes in peptides after consumption of food and then their slower return to baseline after a 3 hour period.

Test Meals

As presented in [14], the breakfast meal consisted of yoghurt, honey and fruit with a choice of tea/coffee. The two conditions were matched for weight (685 g) and provided 2466 kJ. The breakfast meals differed as followed: (High Fat – 50.3% fat, 38.0% carbohydrate, 11.7% protein; High Carbohydrate – 3.2% fat, 83.6% carbohydrate, 13.2% protein).

On both condition days, the same ad libitum lunch meal was provided to directly measure eating behaviour after the macronutrient challenge. It was decided to use the same meal on
both days in order to assess the different macronutrient conditions on eating behaviour, if the ad libitum meal had differed on the two study days we would not be able to elucidate the effect between the different breakfasts or the different lunches. The meal consisted of tomato and herb risotto and a strawberry yoghurt. Participants were instructed to eat until they were comfortably full.

**Subjective Appetite Measures**

Visual analogue scales were used to measure subjective appetite ratings of hunger and fullness. We recently developed a Personal Digital Assistant (PDA) based system to measure various appetite ratings which has been validated against the standard pen and paper technique and an alternative handheld computer system [15]. Questions regarding the state of hunger and fullness were assessed using descriptive anchors at either end of the horizontal line (‘not at all’ to ‘extremely’). Participants placed a vertical line at some point between these anchors to describe their current level of hunger and fullness. Appetite ratings were completed immediately before and after food intake and also immediately before each blood sample as shown in figure 1.

**Blood Parameters**

Venous blood samples were collected into K$_2$-EDTA monovette tubes containing a mixture of protease inhibitors (dipeptidyl peptidase IV (DPP4) inhibitor (10µl/ml blood), aprotinin (50µl/ml blood) and pefabloc SC (50µl/ml blood)) to prevent degradation of the peptides to be measured.

Samples were drawn eight times during the morning at 0 min, post-breakfast +10 min, +20 min, +30 min, +60 min, +90 min, +120 min and +180 min for the measurement of metabolic and appetite peptide levels (see figure 1). After collection, blood samples were centrifuged
for 10 min at 4°C at 3500 rpm. Samples were immediately pipetted into eppendorf tubes and stored at -80°C until analysis.

Prior to analysis, plasma was thawed and an additional protease inhibitor cocktail was added (Final concentration: Sigmaprost (1x) and DPP4 inhibitor KR-62436 0.5 µM, both from Sigma-Aldrich, USA (Cat# S8820 and K4264)). Glucose was analysed by the Department of Clinical Chemistry at Uppsala University Hospital, Uppsala, Sweden. Insulin was analysed using a fluorescent multiplex ELISA using a magnetic bead based kit from Millipore, Billerica, MA, USA (Cat# HMHMAG-34K-06). The plate reader was a Luminex MagPix (Millipore) and the plate washer was a Tecan Hydroflex (Tecan, Switzerland) fitted with a magnetic holder. The inter- and intra-assay CV’s were 12.5% and 8.3%. Radioimmunoassay was used to measure cholecystokinin (sulphated CCK8/CCK33) using 125I-CCK8s tracer, recombinant human CCK8s Quality Control, anti-CCK8 Antibody and pulldown immunobeads from a commercial kit (EuroDiagnostics, Malmö, Sweden, CAT# RB302). Sulphated human CCK33 was used as standard (Phoenix Pharmaceuticals, CA, USA, CAT# 069-02). An in-house QC was also included with each run which was a plasma sample prepared and extracted in the same way as those from study volunteers (used to determine inter assay CV for actual study samples). 125I radioactivity was counted for 4 min/tube on a gamma counter (CompuGamma 1282, LKB Wallac, Finland (now owned by Perkin Elmer)). Before beginning the CCK RIA, CCK was extracted. Plasma was acidified by 0.2 M HCl (final, using 6 M stock) and incubated for at least 10 min. Samples were neutralized by NaOH and immediately diluted with two volumes of 80% EtOH (e.g., 500 µl 80% EtOH into 250 µl plasma) followed by centrifugation (1700 RCF, 15 min 4 ºC) and supernatant dried overnight at 43 ºC. Of several extraction permutations we tested, this extraction procedure yielded the highest CCK8/33(s) values, which we interpret to be optimal recovery and preservation. Samples were re-suspended and assayed in the above RIA. This assay is highly
specific – the following cross-reactivity was found (CCK8s 100.0%; CCK 33s 134.0%; CCK 8 <0.01%; CCK 4 <0.01%; gastrin-17s 0.5% and gastrin-17ns <0.01%). The inter- and intra-assay CV’s were 15.6% and 9.38%.

Statistics

Peptide concentrations and subjective appetite scores were analysed by repeated measures (2*7) ANOVA. Statistical paired t-tests were used to analyse the effect of macronutrient condition on ad libitum energy intake. There was no significant effect of gender on fasting metabolic or appetite hormone levels therefore men and women were analysed together. Due to the individual variability in blood parameters and peptide levels the change from fasting at each time point was calculated for each individual as conducted previously [14]. Relationships between CCK and subjective appetite and food intake were analysed using bivariate correlations. The postprandial period was divided into ‘early’ and ‘late’ satiety in order to identify the suppression and subsequent recovery of appetite during the inter-meal period. The ‘early’ phase was defined as the 0-60 min period and the ‘late’ phase was defined as the 60-180 min period. All data points and error bars represent mean ± SEM unless otherwise stated.

Results

The participant characteristics can be seen in table 1. The range of body weight was 69.8 – 102.2 kg.

Plasma Glucose and Insulin

These data have been reported previously, however they were considered important here to show the differences in response to the two meals. Fasting glucose and insulin levels did not differ on the two study days (glucose – 5.21 and 4.81 mmol/L for the high fat and high carbohydrate days respectively, p=0.260; insulin – 906.98 and 879.81 ng/L for the high fat
and high carbohydrate days respectively, \( p=0.653 \). For both glucose and insulin levels, there was a main effect of condition (\( F_{(1,15)}=6.200, p<0.05 \) and \( F_{(1,15)}=32.688, p<0.001 \) respectively) with the high carbohydrate breakfast causing a greater increase compared to the high fat breakfast. There was also an effect of time throughout the morning (\( F_{(6,90)}=10.720, p<0.001 \) and \( F_{(6,90)}=11.137, p<0.001 \) and a condition*time interaction (\( F_{(6,90)}=7.340, p<0.001 \) and \( F_{(6,90)}=4.171, p<0.05 \)).

**Cholecystokinin response to high fat and high carbohydrate**

Fasting CCK8/33(s) levels did not differ on the two study days (0.52 and 0.69 pmol/L for the high fat and high carbohydrate days respectively, \( p=0.451 \)). For postprandial CCK, there was a main effect of condition (\( F_{(1,15)}=10.235, p<0.01 \) and time (\( F_{(7,105)}=7.554, p<0.01 \)) and there was also a condition*time interaction (\( F_{(7,105)}=2.809, p<0.05 \)). Time-to-peak CCK was 60 minutes after the high fat breakfast and 20 minutes after the high carbohydrate breakfast. The maximum level of CCK was 5.25 and 3.27 pmol/L for the high fat and high carbohydrate condition respectively. Similar results were found when change from baseline CCK values were analysed. There was a significant effect of condition (\( F_{(1,15)}=14.737, p<0.01 \), time (\( F_{(6,90)}=4.570, p<0.01 \)) and a condition*time interaction (\( F_{(6,90)}=2.450, p<0.05 \). This demonstrated that the high fat foods created a greater rise in CCK than foods high in carbohydrate (figure 2).

**Subjective Appetite Ratings**

The two breakfast conditions produced similar profiles of subjective appetite measured through hunger and fullness. There was no effect of macronutrient condition on changes in hunger levels throughout the morning (\( F_{(1,15)}=0.505, p=0.488 \) or a condition*time interaction (\( F_{(6,90)}=0.540, p=0.645 \). There was an effect of time (\( F_{(6,90)}=33.387, p<0.001 \) with hunger being suppressed immediately following food consumption before a gradual rise 30 minutes
post-meal consumption until the lunch meal. Similarly, there was no difference in the effect of the two meals on fullness levels ($F_{(1,15)}=2.277$, $p=0.152$) and no condition*time interaction ($F_{(6,90)}=1.240$, $p=0.306$). However, there was a significant effect of time ($F_{(6,90)}=30.615$, $p<0.001$) with both breakfasts stimulating an immediate rise in fullness levels before a steady decline until the lunch meal (figure 3 reproduced from [14]).

**Relationship between CCK and Subjective Appetite**

Using the change from baseline, there was no correlation between CCK8/33(s) and hunger in either the early or late satiety phases after the high fat (Early Phase: $r=0.346$, $p=0.189$ and Late Phase: $r=0.468$, $p=0.068$) or high carbohydrate (Early Phase: $r=0.405$, $p=0.120$ and Late Phase: $r=0.135$, $p=0.619$) breakfast condition. No relationship between CCK8/33(s) and fullness was found during early or late satiety phases (range $r=0.000$ to $r=-0.235$, $p>0.05$) (see figure 4).

**Relationship between CCK and Satiation (at the ad libitum meal)**

*Ad libitum* energy intake was measured 3 hours after consumption of the breakfast meals. There was no effect of the high fat or high carbohydrate pre-load on subsequent energy intake from a standardised meal (3958 (±333) versus 3925 (±356) kJ; $t_{(14)}=0.201$, $p=0.844$). No relationship between CCK8/33(s) and satiation (meal size) was found. The average rise in CCK8/33(s) (change from baseline CCK) was not associated with energy intake on the high fat ($r=-0.140$, $p=0.958$) or high carbohydrate ($r=-0.225$, $p=0.402$) condition days (see figure 5).

**Discussion**

The results of the present study show a significantly raised postprandial CCK response to meals high in fat compared to meals high in carbohydrate. However, the additional rise in CCK in response to the high fat breakfast did not result in a greater post-meal suppression of
appetite measured through hunger, fullness or *ad libitum* energy intake. In addition, no association between CCK or any measure of short-term appetite control was found, when tested for either high fat or high carbohydrate conditions. Under these experimental circumstances, we have not been able to detect any relationship between CCK and the postprandial effect of food on appetite— in contrast to GLP-1, insulin and ghrelin [14].

One issue arising from these data is how to interpret the plasma levels of CCK following consumption of the fat and carbohydrate meals. It is apparent that the different meals exert differing effects on the release of CCK and this is consistent with the known action of CCK in managing the consequences of dietary fat in the intestine. However, one weakness of this approach is that the measurement of CCK only begins once eating has come to an end. Therefore, any action of CCK arising from early ingestion of food and continuing during eating itself cannot be assessed. (However it should be noted that in practice it is extremely difficult to measure CCK during a meal in humans.) How can these data be reconciled with earlier reviews proposing a positive relationship between CCK and satiety [5, 6], in which it is proposed that CCK is likely to have a specific role in appetite control?

It is apparent that plasma levels of CCK continue to rise (and therefore should be stronger) at the time when the suppression of hunger is weakening and hunger is being rehabilitated. A similar pattern is true for fullness. This dissociation suggests to us that the plasma levels of CCK following the end of consumption are not related to postprandial appetite control. One way to explain the effect of CCK (and consistent with our initial expectations) is to consider that the early release of CCK when food is first ingested is acting on satiation via a neural mechanism mediated through the vagus nerve. We recognise that this process would not be detected by the design of our study. This explanation would acknowledge the dominant and accepted explanation concerning CCK and satiety. It is possible that the postprandial levels of CCK do not add much to the intensity of satiety but rather manage the processing of nutrients.
in the intestine. This would separate the satiety function of CCK from the nutrient management effect.

A limitation of the present study was that gastric emptying was not measured; it is possible that the rise in CCK was sufficient to delay gastric emptying, but the fact that the breakfasts were identical for weight and volume may have resulted in gastric emptying being very similar, it is difficult to elucidate this without direct measurement. In addition, it could be argued that the *ad libitum* test meal used should be high in fat in order to more sensitively investigate the effect of CCK on satiation. There is currently a paucity of research in this area. The current study has indicated that the postprandial CCK profile is a very sensitive indication of the amount of fat in a meal, but this response does not appear to be related to changes in hunger or fullness, or to subsequent food intake. Also, it could be argued that the isocaloric breakfast provided was a limitation and that this provided a different percentage of each participant’s energy requirements (the range was 26.1 – 46.9%). However, when controlling for this variable, it did not affect the outcome in this study, but it should be a consideration when designing future studies. A further potential limitation to be addressed is the technique of measuring eating behaviour in humans. We have recently discussed the issue of laboratory versus free living studies [16] and decided that a two-item buffet was the most applicable for this experimental design and of the environmental and cultural norms of the study population. In addition, others have assessed the validity of buffet-style ad libitum meal tests [17] concluding that there was high reproducibility of energy and macronutrient intake.

In summary, the data presented here on profiles in the after meal period (not during the meals) were not able to throw light on the accepted view of CCK exerting a major action on satiation (meal termination). Considering the process of postprandial satiety (operating in the post-ingestive period) we suggest it is likely that there is a nutrient specific, cumulative action of several appetite hormones that contribute to short-term appetite control, and this
approach appears promising [18]. Our study has shown that different profiles of peptides –
elicited by different nutrients – can bring about the same degree of appetite suppression [14].
It seems most plausible that satiety (post-meal inhibition) is brought about by a number of
peptides acting conjointly with the same degree of satiety being achieved by several different
combinations of peptides; such profiles in conjunction with gastric distension (a full stomach)
produce the psychological sensation of fullness with an absence of hunger. It is an interesting
proposition that subjective appetite may respond to the behavioural action of food intake
whereas appetite peptide responses reflect a coordinated physiological response to the
ingestion and subsequent transit of nutrients. It is clear from previous data from classic
studies that CCK plays a role in appetite control by bringing a meal to an end. It is
interesting to consider whether the nutrient-induced levels of CCK in blood which are clearly
measured after eating has finished, continue to exert an effect on hunger or appetite control
after the meal is over.

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DLW, PH and EN provided essential reagents and materials; CG, GF and JEB analysed data;
CG, GF and JEB wrote manuscript. All authors discussed results/interpretation and approved
the final manuscript. No authors declare a conflict of interest.

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Pubmed Indexing - Gibbons, Caudwell, Finlayson, Webb, Hellström, Näslund, Blundell
References


Figure Captions

Figure 1 Schematic diagram to illustrate the study design and timings of the measurements (BS – blood sampling, VAS – visual analogue scale, BF – breakfast, L – lunch)
Figure 2 to show the postprandial profiles of absolute and change from baseline CCK levels after consumption of a high fat and high carbohydrate breakfast meal in overweight/obese participants (n=16)
Figure 3 to show the postprandial profiles of hunger and fullness levels after consumption of a high fat and high carbohydrate breakfast meal in overweight/obese participants (n=16)
Figure 4 to show the lack of relationship between CCK and hunger on both high fat and high carbohydrate condition days and during early and late phases of satiety in overweight/obese participants (n=16)
Figure 5 to show the lack of relationship between CCK and *ad libitum* energy intake on both the high fat and high carbohydrate condition days in overweight/obese participants (n=16)
Table

Table 1 to show the participant characteristics

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