A single day of mixed-macronutrient overfeeding does not elicit compensatory appetite or energy intake responses but exaggerates postprandial lipemia during the next day in healthy young men.

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ABSTRACT

Discrete episodes of overconsumption may induce a positive energy balance and impair metabolic control. However, the effects of an ecologically relevant, single-day of balanced macronutrient overfeeding are unknown. Twelve healthy men (mean(SD): age 22(2) years, body mass index 26.1(4.2) $kg \cdot m^{-2}$) completed two 28-hour, single-blind experimental trials. In a counterbalanced repeated measures design, participants consumed either their calculated daily energy requirements (Energy Balance trial, EB; 10,755(593) kJ) or were overfed by 50% (Overfeed trial, OF; 16,132(889) kJ) under laboratory supervision. Participants returned to the laboratory the next day, after an overnight fast, to complete a mixed-meal tolerance test (MTT). Appetite was not different between trials during day one (p>0.211) or during the MTT in the fasted or postprandial state (p>0.507). Accordingly, plasma acylated ghrelin, total glucagon-like-peptide-1 and total peptide YY concentrations did not differ between trials during the MTT (all p>0.335). *Ad libitum* energy intake, assessed upon completion of the MTT, did not differ between trials (EB 6081(2260) kJ; OF 6182(1960) kJ; p=0.781). Plasma glucose and insulin concentrations were not different between trials (p>0.715). Fasted non-esterified fatty acid concentrations were lower in OF than EB (p=0.005) and triglyceride concentrations increased to a greater extent on OF than EB during the MTT (p=0.009). The absence of compensatory changes in appetite-related variables after one-day of mixed macronutrient overfeeding highlights the limited physiological response to defend against excess energy intakes. This supports the concept that repeated discrete episodes of overconsumption may promote weight gain, while elevations in postprandial lipemia may increase cardiovascular disease risk.

INTRODUCTION

The increased prevalence of overweight and obesity represents a worldwide public health challenge[1] and is the result of a chronic positive energy balance achieved via a long term surplus of energy intake over energy expenditure[2]. Although long-term weight loss is achievable with lifestyle modification[3], this is notoriously difficult due to the stimulation of physiological adaptations to weight loss that favour weight regain[4]. Considering the challenges of weight loss, it remains essential to better understand the factors that may cause initial weight gain to provide guidance for prevention.

Current evidence suggests that increases in BMI during adulthood[5] are the result of discrete periods of overconsumption, rather than smaller daily energy imbalances[6–9]. Indeed, repeated episodes of overconsumption during weekends and public holidays may be sufficient to account for long-term weight gain[6,8]. To date, experimental investigations into the compensatory responses to overfeeding have primarily focussed on changes in circulating appetite-related hormone concentrations; with mixed findings likely due to differences in the duration, magnitude and composition of the dietary interventions[10–15]. Although episodes of overconsumption often occur on only one day per week and with a balanced macronutrient profile[8], there has been little investigation into the compensatory responses to this model of overconsumption. Additionally, an integrated assessment of appetite perceptions and subsequent energy intake alongside mechanistic variables (i.e., appetite-related hormones) is essential to fully understand the magnitude of compensatory responses.

Discrete periods of overconsumption may also impair metabolic control. In this regard, overfeeding with a high fat diet (≥50% increase in energy; ≥60% fat content) has consistently been shown to impair insulin sensitivity in humans[10,14,16–18]. A recent study by Lundsgaard and colleagues[18] has further advanced these findings by demonstrating opposing regulatory effects of high carbohydrate versus high fat overfeeding on central and peripheral insulin sensitivity. In this landmark study, three-days of overfeeding with a high fat diet (+75% kJ, 78% fat) improved hepatic glucoregulation but impaired muscle insulin sensitivity, whereas overfeeding with a high carbohydrate diet (+75% kJ, 80% CHO) induced hepatic insulin resistance but increased insulin sensitivity at the muscle. This evidence suggests that divergent macronutrient intakes may mediate the impaired metabolic control observed during overfeeding and it remains unclear whether short-term overfeeding with a balanced macronutrient profile would provide sufficient stimulus to induce metabolic impairments.

The primary purpose of this study was to determine whether one-day of overfeeding with a balanced macronutrient profile induces compensatory changes in appetite perceptions, appetite-

related hormone concentrations and energy intake during a mixed-meal tolerance test (MTT) the next day. The effects of overfeeding on fasted and postprandial markers of metabolic control during the MTT were also assessed. Participants were blinded to the overfeeding intervention in order to assess the physiological compensatory responses to overfeeding, while minimising the influence of psychological factors and participant bias. These findings contribute to understanding the consequences of common dietary practices and mechanisms of weight control.

METHODS

Participants

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures were approved by the Ethics Advisory Committee at Leeds Beckett University. Twelve healthy men were recruited for the study and written informed consent was obtained from all participants. Participants were nonsmokers, not taking medication, weight-stable for at least six months before the study, and were not dieting. The physical characteristics of participants (mean (SD)) were as follows: age 22 (2) years, body mass 82.4 (10.2) kg, body mass index 26.1 (4.2) $kg \cdot m^{-2}$, waist circumference 86.2 (8.4) cm. This trial is registered at ClinicalTrials.gov (ID: NCT03301948).

Experimental protocol

Overview

Each participant completed a screening session and two 28-hour experimental trials, separated by one-week in a single-blind counterbalanced crossover design. The initial screening session involved the collection of anthropometric measures, health screening and confirmation of the acceptability of the foods to be provided during the study.

Standardisation

Participants completed a food diary detailing all foods and drinks consumed in the 24 h before their first experimental trial and repeated this before their second trial. Alcohol, caffeine and strenuous physical activity were not permitted during this period. All trials commenced between 8am and 9am after an overnight fast of at least 10 h, and participants exerted themselves minimally when travelling to the laboratory. Verbal confirmation of adherence to these standardisation procedures was obtained at the beginning of each experimental trial.

Day one

On day one of each trial, participants visited the laboratory to consume breakfast (8am-9am), lunch (12pm-1pm) and an evening meal (5pm-6pm). All meals were prepared by the research team, consumed in isolation, and consumed at the same time of day on both trials. On one trial these meals provided the calculated energy requirements for each individual (Energy Balance trial (EB)). On the other trial, the meals were covertly manipulated to increase the energy content by 50% (Overfeed trial (OF)). Participants were required to consume all of the foodstuffs provided at each meal and this was confirmed by a member of the research team. The magnitude of overfeeding (+50% kJ) was selected to align with previous research that has investigated appetite-related and metabolic responses over more prolonged periods of five to seven days[10,14,16]. The impairments in metabolic control observed during these studies suggests that overfeeding by 50% provides a significant metabolic challenge, while we also deemed this to be a realistic target to enable covert dietary manipulation and participant blinding to the intervention.

Participants were permitted to leave the laboratory between meals but were required to remain on the university campus in order to minimise physical activity. Each participant was fitted with a SenseWear Pro3 Armband (BodyMedia, USA) upon arrival at the laboratory on day one of each trial and these were worn until arrival at the laboratory for day two of the respective trial. This was intended to discourage physical activity and was used to check that the energy expenditure of participants was matched between trials[19]. Participants returned home after consumption of the evening meal and arrived back at the laboratory the next morning having fasted overnight. Verbal confirmation of adherence to the overnight fast was obtained at the beginning of the second day of each trial for all participants.

Day two

On day two of each trial, participants arrived at the laboratory between 8am and 9am to complete a mixed-meal tolerance test. Upon arrival participants rested in a semisupine position for 5 min before a cannula (Introcan Safety; B Braun, Sheffield, UK) was inserted into an antecubital vein. A baseline blood sample and appetite visual analogue scale (VAS) were collected ~10 min after the insertion of the cannula before the participant commenced the MTT.

The MTT involved consumption of white bread (toasted), butter, strawberry jam and orange juice. The energy content of the meal was relative to each participant's estimated energy requirements by providing the same energy content as the porridge breakfast meal on day one of the EB trial (2748 (198) kJ). This approach was used to standardise energy intake for differences in body mass/composition between participants[20]. The macronutrient composition of the MTT test meal

was 60% carbohydrate, 32% fat and 8% protein, in order to increase ecological validity and provide a more 'physiological response' compared with glucose or fat only challenges[20,21].

Blood samples and appetite perceptions were collected every 30-min during the 180-min postprandial period while participants rested within the laboratory (sitting, reading or listening to music). Upon completion of the postprandial period, participants were provided with an *ad libitum* pasta meal to assess energy intake. Water intake was measured during the first trial for each participant and replicated during the second trial (505 (288) mL).

Overfeeding intervention

The meals consumed during day one of EB provided the estimated daily energy needs for each participant, which were calculated using the Mifflin–St Jeor equation[22] and a physical activity factor of 1.4 to represent the sedentary nature of experimental testing days. This approach to estimate energy requirements is consistent with previous literature[10,14,17,23] and was deemed preferable to designing the intervention based on self-report food diaries due to established concerns over the accuracy of self-report measures[24]. The energy content of all meals comprised 50% carbohydrate, 35% fat and 15% protein in accordance with the UK dietary guidelines[25]. During day one of OF, the raw weight of foodstuffs included in the meals was increased by 50%.

The manipulation of food weights was covertly achieved by adjusting the water content of meals; cooking duration; and through the addition of thickening agents to the meals provided during EB. To avoid any *a-priori* awareness of the participants to the overfeeding intervention, this experiment was described as involving "nutrient manipulation" during recruitment and throughout the study. The blinding of participants to the true aims of the study was deemed important in order to assess the physiological compensatory responses to overfeeding, while minimising the influence of psychological factors and participant bias. All participants completed a blinding assessment upon completion of the experiment and the true nature of the intervention was discussed. The meals provided were as follows: porridge (breakfast), pasta dish and soup (lunch), rice dish (evening meal). A milkshake was provided alongside each meal which contained 837 kJ on EB and 1255 kJ on OF for all participants. The remaining energy intake was divided evenly across the three meals. The meal ingredients, preparation methods and quantities for an example participant are provided in Table 1.

Appetite, palatability and energy intake assessment

Appetite perceptions (hunger, satisfaction, fullness and prospective food consumption (PFC)) were assessed using 100-mm visual analogue scales with descriptors anchored at each end describing the

extremes (e.g. 'I am not hungry at all'/'I have never been more hungry')[26]. These measures were collected before and after each meal on day one, and in the fasted state and every 30 min during the MTT. A composite appetite score was calculated for each time point as the mean value of the four appetite perceptions after inverting the values for satisfaction and fullness[27]. Palatability ratings (visual appeal, smell, taste, aftertaste and pleasantness) were obtained for all meals immediately after consumption[26]. A composite palatability score was calculated as the mean value of the palatability subscales.

Upon completion of the 180-min postprandial period, an *ad libitum* meal was provided, consisting of penne pasta, cheddar cheese, tomato sauce and olive oil in accordance with previous research[28]. Pasta was cooked in a microwave for 13 min in unsalted water at 700 W before being mixed with the remaining ingredients and re-heated for 2 min at 700 W. The macronutrient content of the meal was 50% carbohydrate, 35% fat and 15% protein[25]. Participants consumed the *ad libitum* meal in isolation to prevent any social influences affecting food intake. Participants were provided with a bowl of the pasta meal, which was replaced by an investigator before the participant had emptied it and with minimal interaction. No time limit was set for eating, and participants were instructed to eat until 'comfortably full'. Food intake was determined as the weighted difference in food before and after eating.

Blood sampling and biochemical analyses

At each timepoint, venous blood samples were collected into one 5 mL and one 9 mL pre-cooled EDTA monovette (Sarstedt, Leicester, UK). The 9 mL monovettes were used for the determination of plasma concentrations of glucose, insulin, triglycerides, non-esterified fatty acids (NEFA), total GLP-1 and total PYY. The 5 mL monovettes were used for the determination of plasma acylated ghrelin concentrations and were pre-treated on the morning of testing, to prevent the degradation of acylated ghrelin, with a 50 µL solution of potassium phosphate buffer (PBS), P-hydroxymercuribenzoic acid (PHMB) and sodium hydroxide (NaOH). Both monovettes were spun at 1500 x *g* for 10 min at 4 °C. Plasma from the 9 mL tube was immediately aliquoted into 2 mL Eppendorf tubes prior to storage at -20 °C, whereas 1 mL of plasma from the 5 mL monovette was mixed with 100 µL of 1M hydrochloric acid[29] prior to storage at -20 °C.

Plasma glucose, triglyceride and NEFA concentrations were analysed from all blood samples photometrically with reagents from Instrumentation Laboratory (Lexington, MA) and Wako Chemicals (Dusseldorf, Germany), respectively. Insulin was analysed from all blood samples using a commercially available enzyme immunoassay (IBL, Hamburg, Germany). Plasma acylated ghrelin, total GLP-1 and total PYY concentrations were analysed using commercially available

enzyme immunoassays (SPI BIO, Montigny le Bretonneux, France; EMD Millipore, Darmstadt, Germany). Due to the plate layout of the acylated ghrelin, total GLP-1 and total PYY ELISAs, these analytes were measured at all timepoints except for 150 min. To eliminate interassay variation, samples from each participant were analysed in the same run. The within batch coefficients of variation were as follows: acylated ghrelin 3.3%, total GLP-1 3.0%, total PYY 5.1%, glucose 3.2%, insulin 4.3%, triglycerides 3.7%, NEFA 2.8%.

Statistical analyses

Data were analysed using IBM SPSS version 24 for Windows. Sphericity of the data was assessed using Mauchly's test of sphericity, with any violations corrected using the Greenhouse-Geisser method. Fasted measures and *ad libitum* energy intakes were compared using paired t-tests. The dynamic appetite, hormonal and metabolic responses to the MTT were compared using a two-way (trial x time) repeated measures ANOVA. Significant interaction effects were explored using unadjusted paired t-tests. Statistical significance was accepted at $p < 0.05$. Effect sizes are presented as Cohen's *d* and interpreted as <0.2 trivial, ≥0.2 small, ≥0.6moderate, ≥1.2 large, ≥2 very large, and ≥4 extremely large (Hopkins, 2004).

Results in text and tables are presented as mean (SD). Graphical representations of results are presented as mean (SEM). Appetite, hormonal and metabolic responses to the MTT are presented as line graphs within the main manuscript to display changes over time. Time-averaged area under the curve (AUC) values were calculated for these variables using the trapezoidal method, which are displayed in figures alongside the individual participant responses in the supplementary material to allow further examination of the findings.

Based on previous data from our laboratory[28], a sample size of 12 participants provided >80% power to detect a 1250 kJ compensatory increase in energy intake at the *ad libitum* meal. This calculation was performed using G*power with an alpha value of 5%[30].

RESULTS

Day one

Energy intake was 10,755 (593) kJ and 16,132 (889) kJ on the EB and OF trials, respectively. Estimated energy expenditure was 12,423 (1340) kJ and 12,450 (1679) kJ on day one of the EB and OF trials, respectively ($p = 0.917$).

Appetite was not different between trials during day one (supplementary figure 1; main effect of trial $p = 0.212$, trial x time interaction $p = 0.783$). Palatability of the meals provided on day one

were not significantly different between trials, except for the milkshake consumed as part of the evening meal which was significantly more palatable on OF than EB (p = 0.020; Supplementary Table 1). Water intake was not different between trials (EB 2003 (848) mL; OF 1876 (842) mL; p = 0.674).

Day two

Fasted measures of appetite, plasma appetite-related hormone, glucose, insulin and triglyceride concentrations did not differ between trials (all $p > 0.188$). Fasted NEFA concentrations were significantly higher in EB than OF (p = 0.005; Table 2).

Appetite changed over time ($p < 0.0005$) in response to the MTT but without any differences in the magnitude or time-course of these responses between trials (main effect of trial p = 0.720, trial x time interaction p = 0.706; Figure 1a). *Ad libitum* energy intake upon completion of the MTT was not different between trials (p = 0.781; d = 0.05; Figure 1b). Palatability of the MTT test meal was not different between trials (EB 72 (9); OF 72 (10); p = 0.885; d = 0.03). Palatability of the *ad libitum* pasta meal was not different between trials (EB 69 (12); OF 70 (11); p = 0.656; d = 0.09).

Plasma concentrations of appetite-related hormones changed over time (all $p \leq 0.021$) in response to the MTT but without any differences in the magnitude or time-course of these responses between trials (main effect of trial, all $p \geq 0.336$; trial x time interaction, all $p \geq 0.364$; Figure 2).

Plasma concentrations of glucose, insulin, triglycerides and NEFA changed over time in response to the MTT (all $p < 0.0005$) (Figure 3). There were no differences in the magnitude or time-course of these responses between trials for glucose and insulin concentrations (main effect of trial, both $p \geq 0.929$; trial x time interaction, both $p \geq 0.716$). Alternatively, plasma triglyceride concentrations diverged between trials as the duration of the postprandial period increased, resulting in a significant trial x time interaction effect (p = 0.009) but no main effect of trial (p = 0.219). A significant trial x time interaction effect was also detected for plasma NEFA (p = 0.001) due to higher concentrations in EB than OF in the fasted baseline state. In accordance with the other plasma metabolites, there was no main effect of trial for plasma NEFA concentrations (p = 0.113).

Area under the curve data and individual participant responses during the MTT are presented in the supplementary materials. There were no significant differences between trials in the AUC values for appetite, appetite-related hormone concentrations, or plasma metabolites (all $p \geq 0.175$, $d \leq 0.45$).

Blinding assessment

In response to the exit questionnaire, eight out of the 12 participants stated that they noticed a difference between meals during day one of EB and OF. Of these eight participants, only one successfully guessed that the meals differed in energy content. The remaining participants guessed that the aim of the intervention was to manipulate the sweetness of meals (two participants); the sweetness and thickness of meals (two participants); the protein and fat content (one participant); the milk and water content (one participant); and one participant declined to guess the nature of the intervention.

DISCUSSION

In the present study, we provide novel data demonstrating that one-day of overfeeding (+50% kJ) with a balanced macronutrient profile does not elicit any compensatory changes in appetite perceptions, selected appetite-related hormone concentrations, and *ad libitum* energy intake during a mixed-meal tolerance test the next day. In addition, although glucose and insulin responses were unaffected, one-day of overfeeding elicited reduced plasma NEFA concentrations after an overnight fast and elevations in postprandial triglyceride concentrations during the MTT. These findings highlight the consequences of acute overfeeding as a stimulus for the accumulation of a positive energy balance and increased levels of triglycerides as a key cardiovascular disease risk marker.

In addition to the absence of counter-regulatory appetite responses during the MTT, appetite perceptions also did not differ between trials during the day of energy intake manipulation (i.e., energy balance versus overfeeding). This observation contrasts with the established robust increases in appetite that occur during energy restriction[31–34], even with modest deficits of <800 kJ per meal[35]. Such divergent responses help to explain the ease of habitual overconsumption[6,8] and the contrasting difficulties of sustained dieting[36], especially in modern societies where energy dense, highly palatable foods are abundant and easily accessible[2]. The gradual accumulation of a positive energy balance through repeated discrete episodes of overfeeding seems plausible considering the absence of any compensatory appetite and energy intake responses during the MTT the next day. Indeed, mean values for both of these variables differed by <2 % between trials, which further highlights the limited physiological response to defend against excess energy intakes[37]. These findings emphasise the need for conscious monitoring and adjustment of food intake around such episodes of overconsumption to prevent the gradual accumulation of a positive energy balance.

To understand the effects of overfeeding on physiological regulators of appetite control, circulating concentrations of selected appetite-related gastrointestinal hormones were measured during the MTT. In accordance with the findings discussed above, the overfeeding intervention did not stimulate any changes in fasted or postprandial concentrations of the orexigenic[38] hormone

acylated ghrelin or the anorectic[38,39] hormones PYY and GLP-1. These peptides represent key markers of impaired appetite regulation in obese individuals, as depressed concentrations of PYY and GLP-1, and reduced ghrelin responses to feeding are thought to be implicated in reduced satiety and hyperphagia[40–43]. The findings from the present study support previous evidence that 3-7 days of overfeeding does not induce any changes in circulating ghrelin and GLP-1 concentrations[10,11,14]. Importantly, this also suggests that the assessments made after these longer interventions did not mask any immediate compensatory changes in hormone concentrations. Alternatively, Brøns et al.[14] reported a borderline significant increase in fasted PYY concentrations after five days of high-fat overfeeding (+50% kJ, 60% fat). Thus it seems likely that more prolonged or high fat overfeeding is required to induce compensatory changes in PYY concentrations, which accords with evidence that PYY release is more potently stimulated by fat than carbohydrate consumption[44]. Ultimately, the findings from the present study demonstrate that circulating concentrations of key appetite-related hormones do not change to provide a defence against an acute episode of overconsumption. The lack of change in these hormones also suggests that obesity-related dysfunctions in the appetite-regulating endocrine system do not occur acutely and are most likely stimulated by weight gain.

The focus of the present study on appetite, appetite-related hormones and energy intake responses precluded the additional measurement of energy expenditure during the MTT. Although this represents an important outcome to complete the energy balance equation, previous evidence suggests that mass-independent increases in resting energy expenditure and diet-induced thermogenesis do not occur during chronic or short-term overfeeding[13,45,46]. Where differences in energy expenditure have been observed in response to energy intake manipulation, these appear to be the result of changes in light-intensity activity[47], which was not permitted during the MTT in the present study. Light-intensity activities may also have been limited during the day of dietary manipulation based on the guidance to minimise physical activity levels. Nevertheless, it must be acknowledged that these activities often occur subconsciously[47] and therefore that the lack of difference between trials during the day of dietary manipulation may represent a genuine absence in compensatory movement responses. Future investigations into the free-living responses to acute overfeeding would be beneficial to further investigate these effects.

The standardisation of physical activity levels during the first day of each trial was essential for the accurate assessment of appetite-related and metabolic responses to the intervention during the MTT. However, although energy expenditure was matched between trials, estimates from SenseWear armbands were ~1650 kJ higher than the predictive equations used to calculate the feeding interventions. The extent to which these values deviated from 'true' energy expenditures is unclear

without the inclusion of a criterion measure in the current study but a recently published meta-analysis suggests that SenseWear Pro3 armbands significantly overestimate energy expenditure during sedentary activities as performed during day one of each trial[48]. Regardless of these discrepancies in energy expenditure estimates, the use of predictive equations to prescribe energy intakes is consistent with previous overfeeding interventions[10,14,17,23] and is supported by the prescribed meals inducing an appropriate degree of satiation during the trials.

One-day of overfeeding with a balanced macronutrient composition did not induce any changes in fasted or postprandial glucose and insulin concentrations during the MTT. This contrasts with previously reported impairments in glycemic control after high fat overfeeding interventions (+50% kJ, ≥60% fat) lasting for 5-7 days[10,14,16], and more extreme high fat overfeeding for a single day (+78% kJ, 68% fat)[17]. Although the shorter duration of moderate overfeeding in the present study may have reduced the stimulus for metabolic disturbance, these findings are also likely to reflect the impact of overfeeding with a balanced macronutrient composition. In this regard, recent evidence demonstrated that whole body insulin sensitivity is reduced after three days of high fat overfeeding but increased after three days of high carbohydrate overfeeding[18]. These differences appeared to be primarily mediated by changes in substrate oxidation at the muscle, which highlights the importance of divergent macronutrient intakes for stimulating short-term changes in glycemic control.

Although markers of glycemic control did not differ between trials, the overfeeding intervention induced significant elevations in postprandial triglyceride concentrations during the MTT. This is an important outcome considering that postprandial lipemia is an established independent risk factor for cardiovascular disease[49,50]. Furthermore, the divergence in triglyceride concentrations between trials towards the end of the 180-min postprandial period suggests that this effect is likely to continue in response to subsequent feeding[51]. The mechanisms underlying the observed elevations in postprandial lipemia are unclear but are likely to relate to the increased consumption of absolute amounts of carbohydrate and fat. In this regard, increased insulin release during high carbohydrate overfeeding has been suggested to exaggerate postprandial lipemia by increasing VLDL-TG production and/or decreasing hydrolysis of circulating triglycerides due to reduced muscle lipoprotein lipase activity[18]. This potential role of elevated insulin concentrations during the day of dietary manipulation is also supported by the observed lower fasted concentrations of plasma NEFA after the day of overfeeding. Alternatively, increases observed after high fat overfeeding have been suggested to be the result of increased storage and subsequent release of triglycerides within the enterocyte pool[17]. While further research is required to elucidate the mechanisms of this effect, these findings demonstrate that even short-term episodes of overfeeding with habitual macronutrient distributions can exert negative effects on metabolic control.

The present study has provided novel insights into the effects of an ecologically relevant episode of energy overconsumption on appetite-related and metabolic responses. Nevertheless, some limitations must be acknowledged. First, the blinding of participants to the overfeeding intervention may have prevented the occurrence of psychologically-driven compensatory responses and subsequent reductions in energy intake during the MTT. Although this is feasible, the aim of this study was to isolate the physiological responses to excess energy consumption, which required the removal of potential psychological influences. The lack of counter-regulatory physiological changes in response to the overfeeding intervention highlights the ease with which energy overconsumption can occur, especially considering the increased prevalence of eating away from the home[52] and limited awareness of required portion sizes more generally[53]. Second, although this is the first study to investigate the effects of one-day of mixed-macronutrient overfeeding, these findings must be extended to investigate the consequences of repeated bouts of such overconsumption. Evidence from chronic overfeeding interventions suggests that additional compensatory responses are unlikely to occur with repeated exposure[46] but future investigations remain essential to confirm the role of repeated discrete episodes of overconsumption in the accumulation of a positive energy balance. Third, the population sample for this study comprised young healthy men in order to investigate the consequences of dietary practices for potential weight gain and metabolic impairments in a presently healthy population. However, although the prevention of weight gain has been highlighted as a major public health priority[54], these findings may not generalise to women or obese participants. Future investigations in these populations would be beneficial, especially in obese participants to further understand the effects of dietary manipulation on energy balance and weight control.

In conclusion, this study has demonstrated that one-day of overfeeding with a balanced macronutrient profile does not elicit any compensatory changes in appetite perceptions, selected appetite-related hormone concentrations, and *ad libitum* energy intake during a mixed-meal tolerance test the next day. Appetite perceptions during the day of overfeeding were also unaffected. Taken together, this absence of compensatory appetite-related responses to an ecologically relevant overfeeding protocol supports the concept that repeated discrete episodes of overconsumption may promote weight gain. Increases in postprandial triglyceride concentrations during the day after overfeeding further emphasises the risks of acute dietary excess. These findings highlight the need for dietary awareness and conscious compensatory behavioural adjustments should episodes of overconsumption occur.

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Figure 1. Composite appetite score (a) and *ad libitum* energy intake (b) during a mixed-meal tolerance test after a day of supervised feeding in accordance with estimated energy requirements (Energy Balance trial; ●; solid line) or 50% overfeeding (Overfeed trial; ○; dashed line). Values are mean (SEM); lines in panel b represent individual participants. n = 12.

Figure 2. Plasma acylated ghrelin (a), total GLP-1 (b) and total PYY (c) concentrations during a mixed-meal tolerance test after a day of supervised feeding in accordance with estimated energy

552 requirements (Energy Balance trial; ●; solid line) or 50% overfeeding (Overfeed trial; ○; dashed
553 line). Values are mean (SEM), *n* = 12.

554 **Figure 3.** Plasma glucose (a), insulin (b), triglyceride (c) and non-esterified fatty acid (d)
555 concentrations during a mixed-meal tolerance test after a day of supervised feeding in accordance
556 with estimated energy requirements (Energy Balance trial; ●; solid line) or 50% overfeeding
557 (Overfeed trial; ○; dashed line). Values are mean (SEM), *n* = 12. *Significant difference between
558 trials determined via two-way ANOVA and post-hoc paired t-test analysis of a significant trial x
559 time interaction ($p < 0.05$).

560 **Table 1.** Ingredients, preparation methods and example quantities for the meals provided during
561 day one of the energy balance and overfeed trials.

	Energy Balance	**Overfeed**	**Preparation methods**
Milkshake	**837 kJ**	**1255 kJ**	
Whole milk	179.4 mL	269.2 mL	Guar gum mixed with the water (EB). All ingredients combined and shaken to mix.
Single cream	3.6 mL	5.4 mL	
Maltodextrin	17.9 g	26.9 g	
Whey protein isolate	1.3 g	2.0 g	
Vanilla flavouring	5 drops	5 drops	
Guar gum	1.3 g	n/a	
Water	100 mL	n/a	
Breakfast	**2,571 kJ**	**3,857 kJ**	
Porridge oats	57.6 g	86.4 g	Porridge cooked in a microwave at 700 W for 3-min (EB) or 2-min (OF) after combining all ingredients.
Whole milk	111.4 mL	167.1 mL	
Single cream	61.5 mL	92.2 mL	
Double cream	12.5 mL	18.7 mL	
Maltodextrin	28.8 g	43.2 g	
Whey protein isolate	11.5 g	17.3 g	
Water	141.6 mL	n/a	
Lunch	**2,571 kJ**	**3,857 kJ**	
Pasta dish	*1,286 kJ*	*1,929 kJ*	Pasta cooked in a microwave at 700 W for 15-min (EB) or 7.5-min (OF) before combining with the remaining ingredients.
White spaghetti	51.8 g	77.7 g	
Green pesto	21.6 g	32.4 g	
Butter	4.3 g	6.5 g	
Whey protein isolate	4.3 g	6.5 g	
Water	362.6 mL	233.1 mL	
Soup	*1,286 kJ*	*1,929 kJ*	Guar gum mixed with the water (EB). Soup cooked in a microwave for 2-min at 700 W after combining all ingredients.
Tomato soup	126.9 g	190.3 g	
Single cream	49.3 g	74.0 g	
Yoghurt	84.6 g	126.9 g	
Maltodextrin*	23.1 g	38.1 g	
Vegetable stock cube	One cube	One cube	
Tomato ketchup*	10 g	n/a	
Guar gum	2.3 g	n/a	
Water	143.1 g	n/a	

Evening meal	**2,571 kJ**	**3,857 kJ**	
White rice	79.8 g	119.7 g	Rice cooked in a microwave at 700 W for 15-min (EB) or 7.5-min (OF) before combining with the remaining ingredients.
Butter	26.6 g	39.9 g	
Chicken slices	46.5 g	69.8 g	
BBQ sauce	33.2 g	49.9 g	
Whey protein isolate	6.6 g	10.0 g	
Water	415.0 mL	398.6 mL	

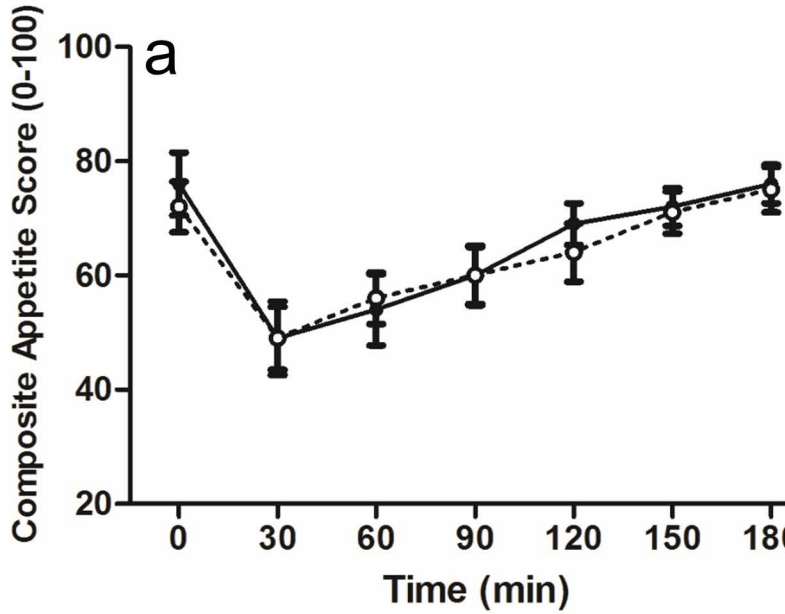
*Note that a small proportion of maltodextrin was replaced with tomato ketchup in the energy balance trial when preparing the soup dish. This was deemed necessary to ensure blinding of the meals and this did not alter the macronutrient composition of the meal. The energy content of all meals comprised 50% carbohydrate, 35% fat and 15% protein. Differences in preparation methods are denoted as EB (energy balance trial) and OF (overfeed trial) to describe the specific procedures for each trial.

Table 2. Fasted appetite perceptions, plasma appetite-related hormone concentrations and metabolite concentrations after a day of supervised feeding in accordance with estimated energy requirements (Energy Balance trial) or 50% overfeeding (Overfeed trial).

	Energy Balance	**Overfeed**	***p***	***d***
Composite appetite score (0-100)	76 (19)	72 (15)	0.508	0.19
Plasma acylated ghrelin ($pg.mL^{-1}$)	179.8 (351.3)	188.9 (364.4)	0.189	0.03
Plasma GLP-1 (pM)	50.4 (20.7)	47.4 (15.4)	0.405	0.17
Plasma PYY ($pg.mL^{-1}$)	99.0 (49.1)	105.4 (71.6)	0.602	0.11
Plasma glucose ($mmol.L^{-1}$)	4.8 (0.5)	4.9 (0.4)	0.347	0.31
Plasma insulin ($\mu IU.mL^{-1}$)	31.3 (13.8)	32.9 (14.4)	0.442	0.11
Plasma triglyceride ($mmol.L^{-1}$)	0.98 (0.32)	0.98 (0.21)	0.940	0.02
Plasma non-esterified fatty acids ($mmol.L^{-1}$)	0.49 (0.12)	0.37 (0.14)	0.005	0.90

Values are mean (SD), $n = 12$.

Figure 1



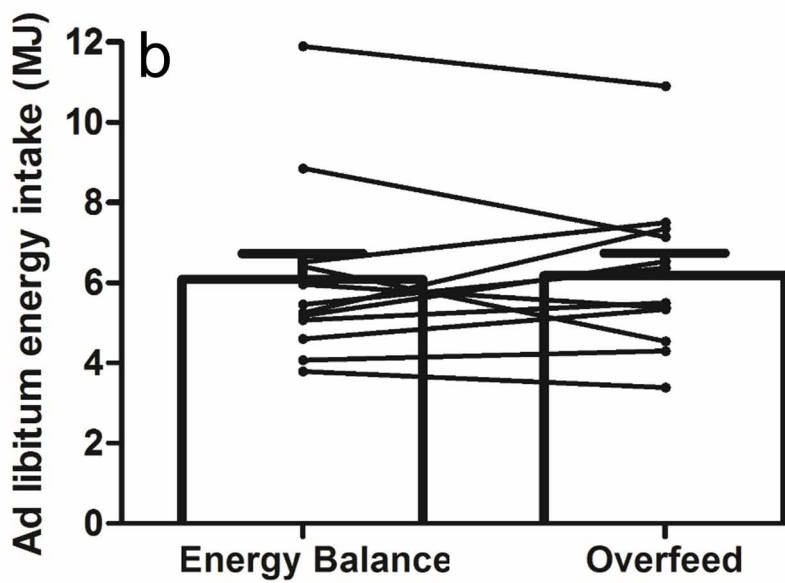


Figure 2

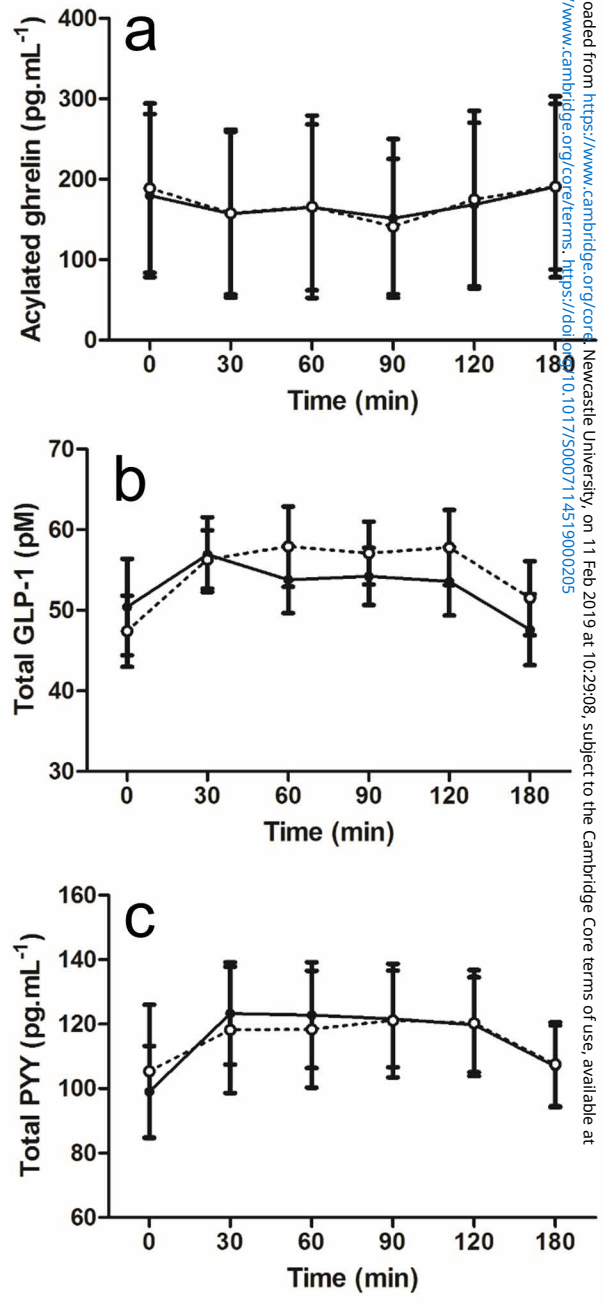


Figure 3