One week of high-fat overfeeding alters bone metabolism in healthy males: A pilot study

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ABSTRACT

Objective: Short-periods of excessive consumption of indulgent high-fat foods are common in Western society, but the effect this has on bone is unknown. The aim of this pilot study was to explore how a seven-day hyperenergetic, high-fat diet affects candidate biomarkers of bone metabolism.

Research Methods & Procedures: Twelve healthy males [mean (SD): age, 24 (4) y; BMI (kg/m2), 24.1 (1.5)] consumed a 7-day hyperenergetic, high-fat diet [HE-HFD; 20.9 (0.8) MJ; 65% total energy as fat] and control diet (10.9 (2.0) MJ; 36% total energy as fat), in randomised, crossover order, with each trial separated by 3 weeks. Markers of bone formation (P1NP) and bone resorption (CTx) were measured at baseline and after 1, 3 and 7 days of each diet. Bone metabolic responses were analysed using 2-factor repeated-measures ANOVA and subsequent pairwise comparisons.

Results: There was a main effect of time ($P<0.05$), but no trial ($P=0.270$) or time-by-trial interaction ($P=0.693$) effects for plasma concentrations of CTx. Mean CTx concentrations were not different between trials (CON: 0.97 (0.39) ng/mL; HE-HFD: 1.03 (0.22) ng/mL; $P=0.225$). There was a main effect of trial ($P<0.01$), but no time ($P=0.138$) or trial-by-interaction ($P=0.179$) effects for plasma concentrations of P1NP. Mean P1NP concentrations were lower during the HE-HFD compared to CON (HE-HFD: 61.79 (26.54) ng/mL; CON: 77.89 (28.71) ng/mL; $P<0.01$).

Conclusions: A 7-day hyperenergetic, high-fat diet reduces a marker of bone formation but does not affect markers of bone resorption. This pilot study suggests that short-periods of excessive energy and fat consumption may detrimentally affect bone health.

Key words: Bone, high-fat diet, overfeeding, osteoporosis

INTRODUCTION

The effect of overfeeding and a high-fat diet on bone health is not fully understood, with both positive [1] and negative effects being reported [2-3]. A potential reason for the contrasting findings relating to a high-fat diet and bone health may be due to opposing mechanistic influences. Firstly, overfeeding and a high-fat diet can cause obesity which increases osteogenic hormones, such as leptin [4] and insulin [5], which consequently leads to increases in osteoblast differentiation and inhibition of osteoclast proliferation. In addition, the greater body mass caused by a sustained high-fat (high-energy) diet may produce higher mechanical loading of bone during weight-bearing locomotor activity, ultimately resulting in a greater bone mass [6]. Conversely, an increase in adipocytes, as a result of a high-fat (high-energy) diet may cause lipotoxicity in osteoblasts [7], leading to decreased in bone formation and subsequently bone mass. The difficultly in isolating distinct mechanistic effects make the influence of a high-fat diet on bone health currently unclear.

Acute periods (1-7 days) of high-fat overfeeding have been shown to disrupt glycaemic control [8-10] and promote an unfavourable blood lipid profile, including increased very-low-density lipoprotein concentrations [11]. These findings demonstrate that obesity is not necessarily the cause of metabolic dysfunction, as deleterious changes in metabolism can be observed before a substantial increase in body mass. Studies assessing high-fat dietary intake on bone metabolism markers have mainly been conducted in rodent models, but these studies largely indicate a negative effect on bone remodelling. Mice fed on a diet containing 60% fat for 12 weeks showed a decrease in bone formation markers and increase in bone resorption markers in comparison to a low-fat diet group [12], however energy intake was not reported which could have influenced the bone metabolic response. Similarly, mice fed a diet containing between 40-45% fat for 8-11 weeks showed an increase in bone resorption [13-15] and a decrease in bone formation [15].

High-fat diet studies in humans have largely focused on how a habitual high-fat diet influences bone health, and have reported contrasting results with evidence for increased [16] and decreased bone

mineral density [2-3]. The reason for the seemly conflicting findings may be a result of the cross-sectional nature of the studies and the assessment of population-specific differences in feeding patterns rather than dietary interventions.

Metabolic changes to a hyperenergetic, high-fat diet occur rapidly and this may lead to alterations in bone metabolism. However, an increase in body mass is often the product of chronic adherence to a high-fat, high-energy diet, which can elicit an osteogenic response due to a greater mechanical loading on bone. This makes identification of diet-specific changes in bone metabolism difficult to elucidate. In order to gain further insight into the effects of a high-fat feeding on human bone health, it is important to understand how a short-term high-fat, high energy diet affects bone remodelling, in the absence of a substantial increase in body mass. Therefore, the aim of this pilot study was to assess how a seven-day high-fat, hyperenergetic diet affects markers of bone formation and resorption.

METHODS

Participant characteristics and ethical approval

Following institutional ethical approval (R17-P144), 12 healthy males (Mean (SD); age: 24 (4) y; body mass: 76.8 (5.4) kg; BMI: 24.1 (1.5) kg/m^{-2}; body fat: 13.3 (3.0) %) provided written informed consent and completed the study. Participants were habitually active (i.e. meeting the UK physical activity guidelines), self-reported weight stable (≤ 2kg weight change in the last 6 months), non-smokers and had no known pre-existing health conditions affecting study outcomes. This manuscript presents secondary analysis of study investigating hepatokine responses to seven days high-fat, hyper-energetic diet [17]. A detailed description of the study design and metabolic responses that may be relevant to the interpretation of this study (e.g. insulin sensitivity, acylated ghrelin, peptide YY and leptin) have been published previously [17-18]. The study and this analysis was registered as a clinical trial at https://www.clinicaltrials.gov.uk (NCT03369146).

Preliminary measures

Participants attended a preliminary laboratory trial to ensure they were fully familiarised with all experimental procedures and for anthropometric measurements. Normal fasting capillary blood glucose concentration (<5.5 mmol/L) were confirmed with a point-of care analyser (CardioChek®, Polymer Technology Systems Inc, Indianapolis, USA). After this trial, participants completed a three-day weighed food record (two week days and one weekend day) and wore two accelerometers (ActiGraph GTX, ActiGraph Corp, Pensacola, USA and ActivPAL3 TM PAL Technologies Ltd, Glasgow, UK) during the subsequent week. This provided an estimate of participants' habitual dietary intake and physical activity patterns.

Study design

Participants completed two, seven-day dietary interventions (hyper-energetic, high-fat diet; (HE-HFD) and control diet (CON)) administered in a randomised, crossover order, separated by a three-week

washout period. Participants attended the laboratory at the start of each intervention (day 0), as well as 1, 3 and 7 days after commencing the dietary intervention, with a fasting venous blood sample obtained. Each visit occurred in the morning after an overnight fast (≥10 h), with participants having abstained from caffeine, alcohol and exercise for the previous 24 h. During each intervention, participants were instructed not to alter their typical physical activity patterns or begin participation in any new exercise or sport activities. Compliance with this was monitored using an accelerometer.

Dietary interventions

During the control trial, participants consumed their habitual diet for seven days and completed a second three-day weighed food record during two weekdays and one weekend day. Participants were instructed not to alter their diet from their usual intake, and compliance with this instruction was assessed by comparing each participant's food record with their baseline diet record [17].

To ensure the intended degree of overfeeding was achieved during the HE-HFD trial, individual resting energy requirements were calculated using a predictive equation [19], multiplied by a physical activity correction 1.7 to account for moderate habitual activity in physically active males [20], and increased 10% further to account for an increase in dietary induced thermogenesis. During HE-HFD, participants consumed a diet that providing 150% of estimated energy requirements, with all food consumed during HE-HFD prepared by the research team on a two-day rolling menu. Participants were permitted to consume non-energy containing beverages during the trial. Compliance with this was confirmed verbally at each study visit.

Full details on the study diets are reported in the original publication [17]. Briefly, HE-HFD provided 20.9 (0.8) MJ/day, with total energy distributed as 65.0 (0.62) % fat, 20.7 (0.4) % carbohydrate, and 14.2 (0.4) % protein. CON diet provided 10.2 (2.0) MJ/day, with total energy distributed as 35.7 (6.7) % as fat, 45.3 (8.1) % carbohydrate and 18.9 (3.3) % protein.

Biochemical analysis

Blood samples were collected into pre-chilled potassium EDTA monovettes (Sarstedt, Leicester, UK) and were centrifuged (Heraeus Labofuge 400R, Thermo Fisher Scientific, Massachusetts, USA) at 4°C for 10 min at 2383*g*. Plasma was then removed and stored at -80°C until later analysis.

C-terminal telopeptide of type I collagen (CTx) and procollagen type 1 N-terminal propeptide (P1NP) were selected as markers of bone resorption and formation, as recommended by the International Osteoporosis Foundation and the International Federation of Clinical Chemistry (Vasikaran *et al.,* 2011). Plasma concentrations of CTx (Immunodiagnostic Systems, West Bolden, UK) and P1NP (Novatein Biosciences, Massachusetts, USA) were measured by commercially enzyme-linked immunosorbent assays. Within-batch coefficient of variation (CV) for CTx was <4.5% and P1NP was <12.5%.

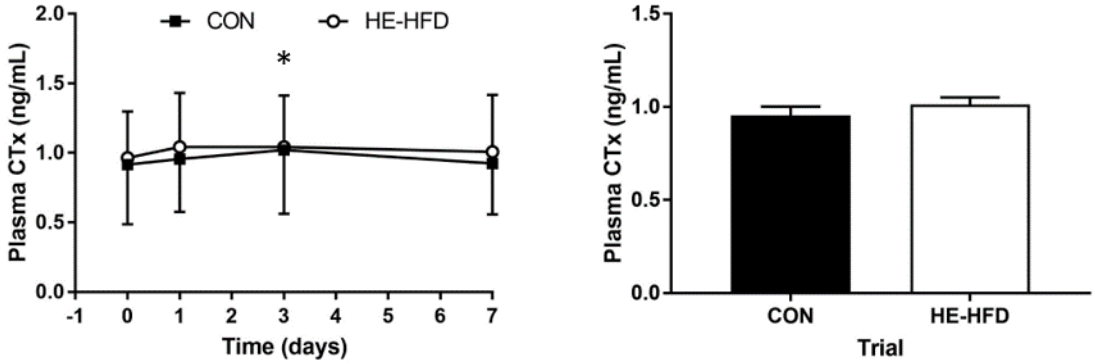
Statistical analysis

The data reported in this manuscript are secondary outcomes from a previous trial (Willis et al. 2020), so was not informed by a formal power calculation. Data were analysed using the software package IBM SPSS Statistics for Windows version 26 (IBM Corporation, New York, NY, USA). Data was checked for normality using a Shapiro-Wilk test and determined to be normally distributed. Repeated measures ANOVA were used to evaluate time, trial and time-by-trial interactions, and Bonferroni-corrected *post-hoc* paired t-tests were conducted, where interaction effects were observed. Statistical significance was set at $P<0.05$. Data are presented as mean (SD).

153 **RESULTS**

154 *CTx*

155 There was a main effect of time (*P*<0.05), but no trial (*P*=0.270) or time-by-trial interaction (*P*=0.693)
156 effects for plasma concentrations of CTx. Compared to baseline, plasma CTx concentrations were
157 greater at 72 h (0.94 (038) vs. 1.03 (0.41) ng/mL; *P*<0.05) and tended to be greater at day 1 (0.94 (038)
158 vs. 1.00 (0.38) ng/mL; *P*=0.092) during both trials. Mean plasma CTx concentrations over the 7-day
159 period were not different between trials (CON: 0.97 (0.39) ng/mL; HE-HFD: 1.03 (0.22) ng/mL;
160 *P*=0.225).



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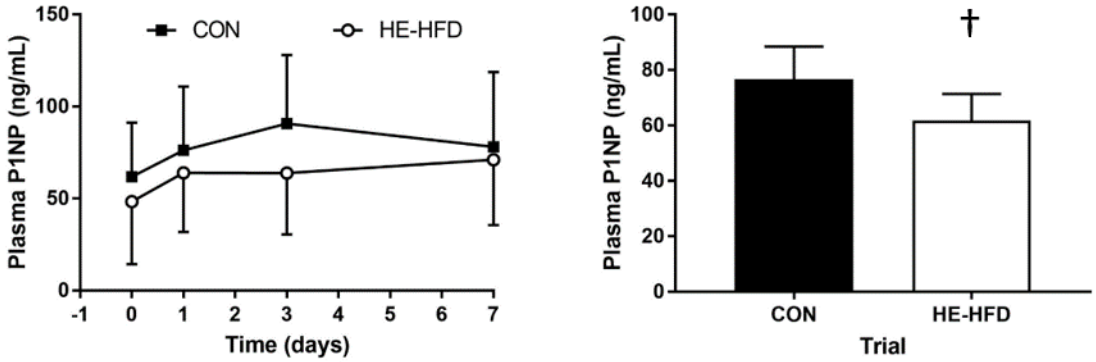
162 **Figure 1:** C-terminal telopeptide type 1 collagen (CTx) concentrations at each time point (left panel)
163 and average concentrations over the intervention period (right panel), during control (CON) and
164 hyperenergetic high-fat diet (HE-HFD). Data are mean with error bars representing standard deviation.
165 * indicates a time point is significantly different to baseline (day 0) for both trials.

166 *P1NP*

167 There was a main effect of trial (*P*<0.01), but no time (*P*=0.138) or trial-by-interaction (*P*=0.179) effects
168 for plasma concentrations of P1NP. Mean plasma P1NP concentrations over the 7-day period were
169 lower during HE-HFD compared to CON (HE-HFD: 61.79 (26.54) ng/mL; CON: 77.89 (28.71) ng/mL;
170 *P*<0.01).



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172 **Figure 2:** N-terminal propeptide (P1NP) concentrations at each time point (left panel) and average
173 concentrations over the intervention period (right panel), during control (CON) and hyperenergetic
174 high-fat diet (HE-HFD). Data are mean with error bars representing standard deviation. † indicates a
175 significant difference between trials.

176

DISCUSSION

This pilot study shows that a seven-day hyperenergetic high-fat diet was associated with lower average concentrations of bone formation marker P1NP, when compared to a control group, while no differences were shown in bone resorption marker CTx. These findings suggest that the consumption of a hyperenergetic high-fat diet, even for a short period, might have a deleterious effect on bone formation.

Short-term hyperenergetic high-fat diets are commonly consumed during festive periods, such as Christmas, Thanksgiving and New Year, as part of a typical 'westernised' diet [21]. The present study assessed how a 7-day hyperenergetic, high-fat diet providing an average of 20.9 MJ/day and 365 g/day of fat (65% of energy), affected bone formation and resorption markers, compared to a 7-day control diet estimated to provide an average of 10.9 MJ/day and 102g of fat/day (~35% of energy). Bone formation marker PN1P was 21% lower over the trial period during the hyperenergetic, high-fat diet trial, compared to the control trial. The reduction in bone formation as a result of a high-fat diet is in line with previous studies in humans and animal models. In a population of race walkers, Heikura et al., [22] showed bone formation to decrease -14% following 3.5 weeks of high-fat feeding when compared to a high carbohydrate group. Similarly, low-carbohydrate high-fat diets fed ad libitum for 8-12 weeks has also been shown to reduce bone formation [12;15] and down regulate genes associated with bone formation in growing mice [23]. The data from the current study extends these findings as a reduction in a biological marker of bone formation was observed from a high-fat diet without concurrent carbohydrate restriction. The reason for a high-fat diet having a deleterious effect on a marker of bone formation could be due to adipogenesis causing the inhibition of osteoblastogenesis [24] or osteoblast dysfunction and apoptosis caused by lipotoxicity [7]. Combined, these findings suggest that short-term adherence to high-fat diets have a negative influence on bone metabolism, but the implications of this on long-term bone health have not been determined. Future

studies should adopt radiological scanning techniques to gain a greater understanding of the effects of a short-term high-fat diet on bone health.

Several possibly interconnected theories have been proposed to explain how a high-fat diet influences bone metabolism. It has been suggested that the accumulation of adipocytes elevates circulating cytokines, such as TNF-alpha, IL-1 and IL-6 [25]. This causes the subsequent differentiation of osteoclasts by up-regulating RANK-RANKL binding, ultimately leading to an increase in bone resorption. A 184% increase in bone marrow adiposity has been shown following a 12-week high-fat diet in mice coupled with a decrease in trabecular bone mass and cortical thickness [26]. It is thought this is due to the preferential recruitment of pre-adipocytic cells from bone marrow mesenchymal stem cells in response to excessive calorie consumption, which leads to a decrease in osteoblast recruitment causing restricted osteoblastogenesis and a subsequent decrease in bone formation [27]. Chronic studies exploring associations between overfeeding and/or high-fat diets with bone health have reported contradictory results [1-3;16]. A limitation with these studies is the difficultly in separating diet-specific effects from the anthropometric consequences of long-term adherence to a hyperenergetic or high-fat diet. These diets typically cause an increase in body mass resulting in an osteogenic response that may be due to a higher mechanical loading being exerted on the bone [6].

Short-term dietary manipulation studies in humans are, therefore, important to isolate the diet-specific effects without causing a substantial increase in body mass. Although the present study was only 7-days in duration, previous studies have shown that 5 days of high-fat dieting is sufficient to achieve metabolic adaptation, including an increase in fat and decrease in carbohydrate oxidation, along with elevated plasma non-esterified fatty acid (NEFA) concentrations during fed-state exercise [28]. Elevated plasma NEFA concentrations can cause lipotoxicity, which can lead to a decrease in bone formation in 1-3 days through the increase in mitochondrial and peroxisomal metabolism and increased endoplasmic reticulum stress, causing apoptosis [29]. This could be a mechanistic explanation for the reduction in bone formation marker P1NP observed in the present study. However,

it should be acknowledged that several previous studies have not observed changes in fat oxidation or plasma non-esterified fatty acid concentrations in the overnight-fasting state, and plasma triglyceride concentrations are consistently decreased after 7-days of high-fat overfeeding [17;30]. However, in these cases, measurements were conducted in the overnight fasted state, when a natural increase in plasma NEFA concentrations and fat oxidation would be anticipated, as this is typically the longest period of fasting within a 24 h period [31]. Bone metabolism markers respond rapidly to feeding [32], and as such, the relative increase in fat oxidation and plasma NEFA concentrations after 5-days of high-fat dieting observed by Burke et al. [28] in the fed state may more accurately depict how bone metabolism responds to high-fat dieting. In any case, the time course of lipotoxicity occurring in response to a high-fat diet in humans warrants further investigation.

There was no effect of a hyperenergetic high-fat diet on CTx, a marker of bone resorption, in the present study. It has previously been reported that a high-fat and low-carbohydrate diet caused an increase in bone resorption following a 3.5 week high-fat diet in race walkers [22]. There is also evidence that carbohydrate consumption post-exercise is important in the regulation of bone metabolism. Hammond et al., [33] found that low-carbohydrate/high-fat feeding after a morning exercise session, increased bone resorption marker CTx, compared to high-carbohydrate/low-fat feeding. Moreover, a high-energy availability, low-carbohydrate/high-fat diet (60 kcal/kg FFM) resulted in similar CTx concentrations to a low-energy availability diet (20 kcal/kg FFM), indicating that carbohydrate, rather than energy, is a primary regulator of bone resorption. The effect of carbohydrate on bone resorption is further demonstrated by CTx being lower in a group consuming an 8% carbohydrate solution before and during prolonged exercise (Treadmill running, 120 min at 70% of VO2max) compared to a placebo, while P1NP did not change between conditions [34]. Although not fully elucidated, carbohydrate-induced regulation of insulin, which has been shown to induce acute changes in bone metabolism [35], may explain the associated effects on bone resorption. In the present study, absolute carbohydrate intake was similar between trials [17], and it is likely that both of these diets elicited a sufficient insulin response in this healthy male cohort. Therefore, if

carbohydrate content of the diet and/or insulin (or insulin-dependent glucose uptake) are important mediators of bone resorption, it is likely that the insulin response to these diets was not substantially different between trials, which may explain why no differences in CTx were observed between trials in the present study. These findings may indicate that the potential for a high-fat diet to negatively effect on bone resorption may be dependent on whether the increase in fat intake is achieved by decreasing the carbohydrate content of the diet. However, longer term trials are required to confirm this.

Exercise has a well-established osteogenic effect on bone health [36] and is known to influence the markers of bone metabolism used in the present study [37]. No differences were shown in accelerometer monitored activity/exercise and participants were instructed not to engage in new exercise activities. However, the specific day in which the physical activity took place and the magnitude of the loading patterns the participants undertook was not recorded. Both these factors have the potential to influence markers of bone metabolism [38], however as exercise did not change over the duration of the study the authors are confident that this did not influence bone marker responses.

CONCLUSION

This pilot study indicates that a seven-day hyperenergetic high-fat diet was associated with lower average concentrations of bone formation marker P1NP in young healthy males, while no significant changes were shown in the bone resorption marker CTx. These preliminary data suggests that a hyperenergetic high-fat diet has a deleterious short-term effect on bone metabolism through a reduction in bone formation. Larger-scale, longer-term studies are warranted to determine the long-term implications of a hyperenergetic high-fat diet on bone metabolism.

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