Interaction between bone, the neuroendocrine system and metabolism
Limonard, E.J.

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The effect of eucaloric variation in dietary carbohydrate and fat ratios on bone turnover

Eelkje J. Limonard, Michael W. Tanck, Annemieke C. Heijboer, Johannes A. Romijn, Peter H. Bisschop

Submitted
Abstract

**Background** Diets with a high fat content reduce bone mass both in humans and rodents, but it remains unclear whether this negative effect is caused by differences in energy intake or variation in dietary carbohydrate and fat content.

**Objective** Our objective was to determine the effect of eucaloric diets with a range of fat and carbohydrate content on bone turnover.

**Design** We studied the short-term effects, i.e. 11 days, of three eucaloric diets with identical protein content (15% of total energy) and low-carbohydrate/high-fat (2% and 83% of total energy, respectively), intermediate-carbohydrate/intermediate-fat (44% and 41% of total energy, respectively), and high-carbohydrate/low-fat (85% and 0% of total energy, respectively) ratios on bone turnover in six healthy men. Bone turnover was assessed by measuring C-terminal crosslinking telopeptides of collagen type I (CTx) as a marker for bone resorption and procollagen type 1 N propeptide (P1NP) as a marker for bone formation.

**Results** CTx concentrations were higher when the dietary fat content was lower: 424±113 ng/L, 371±129 ng/L and 291±104 ng/L for the low, intermediate and high fat diets, respectively (p = 0.023). In contrast, there was no significant effect of variations in dietary carbohydrate/fat ratios on P1NP concentrations (p = 0.409).

**Conclusion** In conclusion, an eucaloric high-fat, low-carbohydrate diet decreases bone resorption and an eucaloric low-fat, high-carbohydrate diet has the opposite effect. Eucaloric variation in dietary carbohydrate and fat does not seem to affect bone formation.

Introduction

Dietary interventions are used for various purposes. In animal studies, high-fat diets are used to induce and study obesity and/or insulin resistance. In humans, diets that are low in carbohydrates, and usually high in fat, have been used to promote weight loss. Very low-carbohydrate, high-fat (LCHF) diets can be ketogenic, and are therefore also used as part of treatment for reducing seizures in children with epilepsy (1). In general diets with a high fat content have a negative effect on bone mass. In rats, a LCHF diet reduced longitudinal growth and bone mineral density as a result of impaired bone formation (2). Other studies have also demonstrated adverse effects on bone quantity and quality of high-fat diets (3-19). Children treated with a ketogenic diet show reduced growth, poor mineral status and lower bone mineral density (20, 21). Despite the substantial number of previous studies on this subject, the effect of dietary carbohydrate and fat content per se on bone metabolism has not been well established, because in most previous studies the caloric intake was also different. Caloric restriction in itself has an negative effect on bone, e.g. in subjects after bariatric surgery (22) and in patients with
anorexia nervosa (23). Interpretation of the effect of ketogenic diets on bone in patients with epilepsy is difficult, because antiepileptic drugs can also adversely affect bone health (24). It therefore remains unclear whether the previously described negative effects of high-fat diets on bone metabolism are caused by variation in dietary carbohydrate and fat content or by differences in energy ingestion and use of concurrent medication. To avoid the influence of over- and underfeeding, we determined the effect of three eucaloric diets containing an identical amount of proteins (15% of total energy) of similar composition and a wide range of fat (0 to 83% of total energy) and carbohydrate (2 to 85% of total energy) content on bone turnover markers in six healthy men.

**Subjects and Methods**

**Subjects**

Six healthy men (body mass index 21-26 kg/m2), age 29-55 years, were recruited for this study. The subjects were in good health, had no family history of diabetes, did not smoke or use any medication. All subjects were recruited among hospital employees and participated because of their special interest in this field of research. All participating subjects gave written informed consent. The study was approved by the Institutional Review Board of the Academic Medical Center, University of Amsterdam and was carried out in compliance with the Helsinki Declaration. This research is part of a larger study of which the design and results have been published previously (25, 26).

**Diets**

The subjects were studied on three different diets, of which the sequence was randomly allocated. The diets consisted of liquid formulas and were custom-made (Nutricia, Zoetermeer, the Netherlands). The diets were eucaloric and contained identical amounts of protein (15% of total energy) and an identical protein composition. In addition to the proteins, the low-fat, high-carbohydrate (LFHC) diet contained only carbohydrates (85% of total energy); the intermediate-fat, intermediate-carbohydrate (IFIC) (control) diet contained both carbohydrate (44% of total energy) and fat (41% of total energy); and the high-fat, low-carbohydrate (HFLC) diet contained mainly fat (83% of energy) and some carbohydrate (2% of energy). The saturated: mono-unsaturated:poly-unsaturated fat ratios were 2:2:1 for all diets containing fat. The ratio mono- and disaccharides: polysaccharides was 1:1. Each diet provided 300 mg cholesterol and 15 g fiber per day. Detailed composition of the diets is shown in Table 1.
Table 1 Detailed composition of the study diets based on a daily intake of 2700 kcal

<table>
<thead>
<tr>
<th>Study diets</th>
<th>LFHC</th>
<th>IFIC (control)</th>
<th>HFLC</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Protein (g)</strong></td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><strong>Fat (g)</strong></td>
<td>1</td>
<td>122</td>
<td>249</td>
</tr>
<tr>
<td><strong>Carbohydrates (g)</strong></td>
<td>572</td>
<td>300</td>
<td>14</td>
</tr>
<tr>
<td><strong>Cholesterol (mg)</strong></td>
<td>300</td>
<td>300</td>
<td>300</td>
</tr>
<tr>
<td><strong>Fibers (g)</strong></td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td><strong>Mono- &amp; disaccharides: oligosaccharides</strong></td>
<td>1:1</td>
<td>1:1</td>
<td>-</td>
</tr>
<tr>
<td><strong>SFAs:MUFAs:PUFAs</strong></td>
<td>-</td>
<td>2:2:1</td>
<td>2:2:1</td>
</tr>
<tr>
<td><strong>Vitamin D (μg)</strong></td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td><strong>Calcium (mg)</strong></td>
<td>2440</td>
<td>2445</td>
<td>2442</td>
</tr>
<tr>
<td><strong>Phosphate (mg)</strong></td>
<td>1676</td>
<td>1681</td>
<td>1678</td>
</tr>
</tbody>
</table>

HFLC, high-fat, low-carbohydrate; IFIC, intermediate-fat, intermediate-carbohydrate; LFHC, low-fat, high-carbohydrate.
MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids.

Energy requirements for each subject were assessed by a dietician by means of a three day dietary journal. The diets supplied 138±6 kJ/kg body weight. Liquid meals with predetermined amounts of energy were taken at six fixed time points between 8:00 and 21:30 h for 11 days. In addition to the diets, the subjects were allowed to drink water ad libitum. The three diets were separated by an interval of eight to ten weeks, during which the subjects consumed their habitual diets. The investigators and subjects were not blinded to diet sequence allocation. Laboratory personnel analyzing the samples were blinded. During the study period, subjects refrained from alcohol and exercise was limited to normal daily activities. To assess compliance with the diet the respiratory quotient, which reflects the ratio of carbohydrate/fat intake (27), was determined after ten and 11 days of the diet by indirect calorimetry (Sensormedics model 2900, Anaheim, CA, USA) using the ventilated hood technique. Subjects were followed up daily to receive their diet for the next day and to assess unintended effects. No adverse events occurred during the intervention period and all diets were well tolerated by the subjects.

**Measurements and analytical procedures**

Each diet was consumed for a period of 11 days. At baseline, day seven and day 11 of each experimental diet, fasted blood samples were taken between 7:00 and 8:00 h to determine serum or plasma concentrations of C-terminal crosslinking telopeptides of collagen type I (CTX), a marker of bone resorption, procollagen type 1 N propeptide (P1NP), a marker of bone formation, glucose, insulin, C-peptide, leptin, insulin-like growth factor 1 (IGF-1) and free fatty acids (FFA). All blood samples were immediately centrifuged (Hettich Rotanta/RP; Détex BV, de Bilt, the Netherlands) at 3000 rpm for 10 min at 4 °C and stored at -80 °C until analysis. CTX and P1NP concentrations were measured in EDTA plasma using an immunoassay (both Cobas E601; Roche Diagnostics Corporation, Indianapolis, IN). All CTX and P1NP measurements
were performed in duplicate in the same run. Intra-assay coefficients of variation for the whole concentration range were < 3% and < 2% for CTx and P1NP, respectively.

**Statistical analysis**

The statistical analysis was carried out with SPSS for Windows (version 21.0; SPSS Inc., Chicago, IL, USA). All variables were tested for a normal distribution using graphs and the Shapiro-Wilk test (W > 0.90). Data are presented as mean and standard deviation (mean±SD). Variables that were not normally distributed are presented as median (interquartile range) and were rank transformed prior to analysis. To determine the effect of diet intervention on bone turnover, we performed linear mixed model analysis with treatment, time (linear) and their interaction, where appropriate, as fixed effects and autoregression 1 (AR1) as covariance structure. The assumptions of the model (e.g. normally distributed residuals and homogeneous variances) were checked using the (absolute) residuals. For post hoc analysis of bone turnover marker concentrations at the different time points we used linear mixed model analysis with treatment as fixed effect and compound symmetry as covariance structure. The latter model was also used to compare the metabolic parameters at day 11. All statistical tests were two-sided and a p-value of 0.05 was considered significant.

**Results**

Dietary compliance was assessed by measuring the respiratory quotient after ten and 11 days of the experimental diets. The respiratory quotient decreased from 0.85±0.07 to 0.81±0.03 to 0.73±0.04 (p = 0.004) as the ratio of dietary fat to dietary carbohydrate increased. Body weights were not significantly different after the HCLF, ICIF, and LCHF diet (79±11, 79±11, and 78±10 kg, respectively).

**Metabolic parameters**

The effect of the three diets on hormone and substrate concentrations are shown in Table 2. Glucose, C-peptide and IGF-1 concentrations were lower on the low-carbohydrate, high-fat diet compared to control diet (p = 0.004 for glucose, p < 0.001 for C-peptide and p = 0.011 for IGF-1). Plasma concentrations of free fatty acids were higher after the low-carbohydrate, high-fat diet compared to the control diet (p = 0.007). Glucose, C-peptide, IGF-1 and FFA concentrations were not different between the control and high-carbohydrate, low-fat diets. The dietary carbohydrate and fat content did not affect insulin and leptin concentrations.
Table 2  Plasma hormone and substrate concentrations after 11 days on the diets

<table>
<thead>
<tr>
<th>Study diets</th>
<th>LFHC</th>
<th>IFIC (control)</th>
<th>HFLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>5.1±0.4</td>
<td>5.3±0.3</td>
<td>4.9±0.5(^b)</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>50±14</td>
<td>49±16</td>
<td>38±15</td>
</tr>
<tr>
<td>C-peptide (pmol/L)</td>
<td>537±156</td>
<td>555±155</td>
<td>300±188(^c)</td>
</tr>
<tr>
<td>FFA (nmol/L)</td>
<td>0.36±0.16</td>
<td>0.43±0.11</td>
<td>0.75±0.21(^c)</td>
</tr>
<tr>
<td>IGF-1 (nmol/L)</td>
<td>26±5</td>
<td>25±5</td>
<td>20±5(^a)</td>
</tr>
<tr>
<td>Leptin (ng/mL)</td>
<td>3.8 (4.1)</td>
<td>3.0 (3.3)</td>
<td>3.0 (3.2)</td>
</tr>
</tbody>
</table>

HFLC, high-fat, low-carbohydrate; IFIC, intermediate-fat, intermediate-carbohydrate; LFHC, low-fat, high-carbohydrate.
FFA, free fatty acids; IGF-1, insulin-like growth factor 1.
Data are presented as mean±standard deviation or median (interquartile range).
\(^a\)p < 0.05; \(^b\)p < 0.01; \(^c\)p < 0.001 versus control.

Bone turnover markers
Baseline concentrations of CTx (bone resorption) and P1NP (bone formation) were not different between the diets (LFHC 476±119 ng/L, IFIC 456±145 ng/L and LFHC 476±94 ng/L, p = 0.886 and LFHC 54±15 μg/L, IFIC 52±12 μg/L and HFLC 55±11 μg/L, p = 0.743, respectively). A decrease in CTx concentrations was observed in all three diets with a significant faster decrease for the HFLC diet compared to the control and the LFHC diet (p = 0.020 and p = 0.009, respectively. LFHC vs control: p = 0.337) (Figure 1A). At day 11, CTx concentrations were significantly lower on the HFLC (291±104 ng/L) (p = 0.002) and significantly higher on the LFHC diet (424±113 ng/L) (p = 0.02) compared to the control diet (371±129 ng/L) (Figure 1A). There was no significant effect of dietary carbohydrate or fat content on P1NP concentrations (p = 0.409) (Figure 1B).
Figure 1 The effect of a low-fat, high-carbohydrate (LFHC, closed dots), intermediate-fat, intermediate-carbohydrate (IFIC/control, open dots) and high-fat, low-carbohydrate (HFLC, closed squares) diet on C-terminal crosslinking telopeptides of collagen type I (CTx), a marker for bone resorption (A) and procollagen type 1 N propeptide (P1NP), a marker for bone formation (B) at day 0 (baseline), 7 and 11. Data are presented as change from baseline values (mean±standard error of the mean). * p < 0.05 and ** p < 0.01 versus control.
Discussion

In this study we demonstrated that an eucaloric high-fat, low-carbohydrate diet decreases bone resorption, while an eucaloric low-fat, high-carbohydrate diet has the opposite effect. Bone formation was not affected by dietary fat/carbohydrate ratios.

Several studies have investigated the effects of high-fat/low-carbohydrate hypocaloric diets on bone. A study in 30 obese subjects (15 low-carbohydrate diet subjects and 15 controls) did not find a difference in bone turnover marker concentrations at three months between the subjects on a low-carbohydrate diet and the controls (28). In another study in 307 overweight subjects on a low-carbohydrate diet, a decrease in both hip and spine bone mineral density was observed after 24 months (29). A comparable effect was demonstrated in a study in six obese adolescents treated with a combined low-fat, low-carbohydrate ketogenic diet for five months (30). In the abovementioned studies, the subjects investigated were obese and the hypocaloric diet caused them to lose a significant amount of their body weight during the study period. Therefore, these studies do not allow do draw any conclusions on the effect of carbohydrate and fat on bone.

To date only one study, by Brown and colleagues (31), investigated the effect of diet macronutrient composition on bone. Thirty two lean trained cyclists were randomly assigned to receive either a eucaloric high-carbohydrate (HC, 15% of total energy from fat and 69% from carbohydrate) or a eucaloric moderate high-fat (HF, 50% of total energy from fat and 37% from carbohydrate) diet for a period of 12 weeks. Total body bone mineral density increased significantly within HF, but nonsignificantly in HC. The observed increase in bone mineral density in the high-fat diet group, is compatible with the decrease in CTx concentrations in our study, indicating reduced bone resorption.

When studying the effects of carbohydrate and fat intake, carbohydrates and fats can simply be added to or removed from the standard diet, as has been frequently done before. In the present study we used three diets with identical energy content and identical amounts of proteins. Therefore, the observed effects on bone turnover markers in our study can only be attributed to changes in carbohydrate/fat ratios and not to caloric deprivation or excess. The adverse effects of high-fat diets on bone metabolism in previously conducted rodent and human studies are likely the consequence of the changes in caloric intake causing obesity and insulin resistance or weight loss (22, 23).

An explanation for our results remains speculative. Eucaloric high-fat, low-carbohydrate feeding decreases insulin concentrations. The insulin receptor is also expressed by osteoblasts. Upon binding of insulin to its receptor in osteoblasts, expression of osteoprotegerin is reduced (32). Osteoprotegerin normally hinders osteoclast differentiation; therefore, decreased insulin concentrations will result in reduced bone resorption by the osteoclast, in line with the decreased CTx concentrations we observed. Carbohydrate deprivation also significantly decreased IGF-1 concentrations. Osteoclasts express IGF-1 receptors (33). In vitro, IGF-1 induces
receptor activator of NFκB ligand (RANKL) synthesis and subsequently osteoclastogenesis (33). This matches the decreased IGF-1 concentrations and concurrent decreased CTx concentrations we observed in our subjects on a high-fat, low-carbohydrate diet. Products of lipid oxidation have been found to contribute to the preferential differentiation of bone marrow progenitor cells away from the osteogenic lineage and the promotion of osteoclastic differentiation (9, 10, 34-38). This is contrary to the decreased CTx concentrations on the high-fat, low-carbohydrate diet we observed, which would indicate reduced osteoclast activity. Therefore, it is likely that other factors are involved.

We did not observe a significant effect of dietary carbohydrate/fat ratios on P1NP concentrations. This absence of difference should be interpreted with care and a larger sample size and longer study period are required to prove that dietary fat and/or carbohydrate content indeed has no effect on bone formation. The remodeling cycle of bone takes approximately three months (39), whereas our intervention only lasted 11 days. The results from this study therefore do not inform us on the long-term effects of dietary composition on bone metabolism. Since we only investigated bone turnover markers, we do not know whether there is also an effect on bone quality and bone mass.

The clinical relevance of the results of this study need to be considered. Our data indicate that short-term changes in dietary fat and carbohydrate content affect bone resorption, leading to either protection from bone loss or adverse effects on bone. In addition to well-known dietary factors involved in bone metabolism, like calcium and vitamin D, carbohydrate and fat could possibly serve as additional modifiable dietary factors to maintain bone health.

In conclusion, an eucaloric high-fat, low-carbohydrate diet decreased bone resorption, while a low-fat, high-carbohydrate intake had the opposite effect. There was no significant effect of dietary carbohydrate/fat ratio on bone formation.
References