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High fat diet enriched with saturated, but not monounsaturated fatty acids adversely affects femur, and both diets increase calcium absorption in older female mice

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Abstract

Diet induced obesity has been shown to reduce bone mineral density (BMD) and Ca absorption. However, previous experiments have not examined the effect of high fat diet (HFD) in the absence of obesity or addressed the type of dietary fatty acids. The primary objective of this study was to determine the effects of different types of high fat feeding, without obesity, on fractional calcium absorption (FCA) and bone health. It was hypothesized that dietary fat would increase FCA and reduce BMD. Mature 8-month-old female C57BL/6J mice were fed one of three diets: a HFD (45% fat) enriched either with monounsaturated fatty acids (MUFAs) or with saturated fatty acids (SFAs), and a normal fat diet (NFD; 10% fat). Food consumption was controlled to achieve a similar body weight gain in all groups. After 8wk, total body bone mineral content and BMD as well as femur total and cortical volumetric BMD were lower in SFA compared with NFD groups ($P < 0.05$). In contrast, femoral trabecular bone was not affected by the SFAs, whereas MUFAs increased trabecular volume fraction and thickness. The rise over time in FCA was greater in mice fed HFD than NFD and final FCA was higher with HFD ($P < 0.05$). Intestinal calbindin-D_{9k} gene and hepatic cytochrome P450 2r1 protein levels were higher with the MUFA than the NFD diet ($P < 0.05$). In conclusion, HFDs elevated FCA overtime; however, an adverse effect of HFD on bone was only observed in the SFA group, while MUFAs show neutral or beneficial effects.

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Keywords

Bone; calcium absorption; fatty acids; high-fat diet; mice

1. Introduction

Total body Ca balance is determined by intestinal Ca absorption and urinary Ca excretion. Since plasma Ca concentration fluctuates constantly as a result of environmental and physiological impacts [1], maintaining Ca balance is important to ensure optimal metabolic function and structural integrity of bone [1, 2]. Two major pathways of luminal Ca entering the circulation are paracellular and transcellular movement [1, 3]. In particular, in the transcellular movement of Ca, crucial transporters including the apical epithelium protein channel transient receptor potential cation channel subfamily V member 5 and member 6 (TRPV6/TRPV5), intracellular calbindin-D9k (CalbD9k)/ CalbD28k, and the basolateral plasma membrane Ca ATPase act together and transport Ca into the blood [1]. The transcellular movement of Ca is principally regulated by the calciotropic hormone 1,25-dihydroxycholecalciferol, the bioactive metabolite of vitamin D that directly enhances Ca absorption in the small intestine and reabsorption in the kidney when serum Ca concentration is low. Other factors such as dietary fat also affect intestinal Ca absorption.

Dietary fat can stimulate or inhibit intestinal Ca absorption depending upon the type and amount of fat intake. High fat diets (HFD) may reduce Ca absorption by forming insoluble Ca soaps [4]. A moderately high fat ad libitum feeding also results in changes of the duodenal oxidation state that lowers Ca absorption in mice [5]. In addition, studies suggest that HFD that are typically rich in saturated fatty acids (SFAs), negatively affect bone mineral density (BMD) during growth in rat studies [6]. This is supported by observational studies in adult humans showing that a high fat intake is negatively associated with BMD [7, 8]. Conversely, we and others found that dietary fat intake was positively related to Ca absorption in both human and rodent studies [9–11]. Since obesity is associated with compromised bone quality both in clinical trials [12, 13] and rodent studies [14], it is possible that excessive fat in the diet is a contributing factor. Observational studies examining the effects on bone, however, suggest that dietary monounsaturated fatty acids (MUFAs) act differently than other fatty acids [15, 16]. The effect of MUFAs on Ca absorption has not been examined previously but others have suggested that the type of fatty acids differentially influences intestinal Ca absorption [17]. Fat intake or type may also affect liver function and influence the hydroxylation of vitamin D to 25-hydroxyvitamin D through the enzyme Cyp2r1.

In this study, we hypothesized that excess dietary fat, in the absence of diet-induced obesity, would increase fractional Ca absorption (FCA) in an older and estrogen insufficient mouse model. The primary objective was to examine whether high fat feeding affects Ca metabolism and its active transporters in the small intestine and bone in this older female model. In a secondary objective, we hypothesized that the type of dietary fatty acid (MUFAs or SFAs enriched) would differentially affect Ca metabolism and bone mass, mineral density, and quality.

2. Methods and materials

2.1 Animals and diets

Eight-month-old female retired breeder C57BL/6J mice (n=29) weighing approximately 27g each were purchased from Jackson Laboratory. After arrival, mice had free access to a purified diet (slightly modified AIN93M formula) and tap water and were housed in groups of three or four in breeding cages in an environmentally controlled room (19–26°C; relative humidity 40–70%; 12 h light/dark cycle). All procedures were approved by the Rutgers University Institutional Animal Care and Use Committee. After one-week stabilization, weight-matched mice were randomly divided into three groups and provided with different diets for 8wk (**Table 1**). The normal fat diet (NFD) contained 15% of calories from protein, 75% of calories from carbohydrate, and 10% of calories from fat. The 45% HFD was either enriched with MUFAs (15% protein, 39% carbohydrate, and 46% fat), or SFAs (19% protein, 37% carbohydrate, and 44% fat). All diets contained similar amount of dietary Ca, and the actual measured values were 0.6%, 0.7%, and 0.7% of Ca in the NFD, MUFA, and SFA groups, respectively. Mice were pair-weighted fed during the feeding intervention, and the food intake of the HFD groups was controlled twice a week to maintain similar weight gain as the NFD control group [18]. One mouse from the SFA group was removed due to tooth abscess and weight loss. Food intake of a 7d period was measured twice during the study at the beginning (d 1-7) and the end (d 50-56) of the study. After 8wk, food was removed for approximately 16h before the mice were asphyxiated with CO₂. Blood samples were collected using cardiac puncture, and mice were killed by exsanguination. Liver, kidney, and uterus were quickly removed, weighed, and frozen in liquid N₂. The small intestine was removed and flushed with saline, and the mucosa was obtained by scraping on ice. The mucosa was immediately frozen in dry ice.

2.2 Calcium absorption

Priori to any feeding intervention, eighteen mice (n=6 per dietary group) were randomly selected to assess Ca absorption radioisotope method. Intestinal Ca absorption and metabolism was measured in the same mice during the beginning (wk0) and the end (wk8) of the study using slightly modified method as previously described [19]. Briefly, mice were individually kept in metabolic cages. After a 4d adaptation period (d 1-4), mice received an intramuscular injection of 2 MBq (0.054 mCi) of ⁴⁵Ca to trace the endogenous Ca secretion in the small intestine. Calcium balance was determined over a 5d period (d 5-9). Throughout the 5 days, urine samples were collected and the pH was adjusted to <2 with HCl to avoid Ca precipitation; fecal samples were collected and ashed at 600°C for 18h and dissolved in HCl (3 mol/L). Fecal and urinary ⁴⁵Ca were determined by liquid scintillation counting. Fecal and urinary total Ca concentration (Ca_{total}) was determined using atomic absorption spectrometry.

2.3 Calculations

Calcium balance, FCA, and endogenous fecal Ca were calculated using the following equations [19]:

$$\begin{aligned}
 \text{Ca consumption (mmol/d)} &= \text{mean food consumption (g/d)} \\
 &\times \text{food Ca (mmol/g)} - \text{Ca balance (mmol/d)} \\
 &= \text{Ca consumption (mmol/d)} \\
 &- (\text{fecal Ca}_{\text{total}} + \text{urine Ca}_{\text{total}}) \text{ (mmol/d)} - \text{Endogenous fecal Ca (mmol/d)} \\
 &= \text{urine Ca}_{\text{total}} \text{ (mmol/d)} \\
 &\times \left(\text{fecal } ^{45}\text{Ca (mmol/d)} / \text{urine } ^{45}\text{Ca (mmol/d)} \right)
 \end{aligned}$$

$$\begin{aligned}
 \text{Unabsorbed dietary Ca (mmol/d)} &= \text{fecal Ca}_{\text{total}} \text{ (mmol/d)} \\
 &- \text{endogenous fecal Ca (mmol/d)} - FCA \\
 &= (\text{Ca consumption (g/d)} - \text{unabsorbed dietary Ca (g/d)}) \\
 &/ \text{Ca consumption (g/d)} - \text{Intestinal Ca secretion (mmol/d)} \\
 &= \text{endogenous fecal Ca (mmol/d)} / (1 - FCA)
 \end{aligned}$$

2.4 Bone densitometry

Total body BMD and bone mineral content (BMC) were measured using dual-energy X-ray absorptiometry (DXA) (GE-Lunar PIXImus mouse densitometer; software version 2.10.41). After full body measurement (headless), individual bones such as femur, distal femur (20% from the femoral end), tibia, humerus, radius, and lumbar spine (L1-5) were dissected, wrapped with phosphate buffered saline soaked gauze, and stored at -20°C . The BMD and BMC at anatomical bone sites were evaluated by placing excised bone on a Delrin block in the PIXImus as described previously [20]. The CV values of three repeated BMD scans of total tibia and total femur BMD were 2.2% and 1.4%, respectively. Femoral length from the trochanter to the medial condyle and tibia length from the center of the condyles to the medial malleolus were measured (Manostat Vernier micrometric caliper, 15-100-500).

2.5 In vitro micro-computed tomography

Left femurs were scanned using the microcomputerized tomography system (μCT 35, Scanco Medical AG, Brüttisellen, Switzerland) to analyze the femoral geometric parameters in a limited number of samples, since some were lost or damaged due to a severe hurricane. A scout view of the entire femur was first performed to measure the length of the femur from the upper extremity to the lower extremity. Then the distal end of the femur corresponding to a 0-1.2 mm region above the highest point of growth plate was scanned at $6\mu\text{m}$ isotropic voxel size, 55 keV energy, 145 μA intensity, 300 ms integration time, and 1000 projections, using a 0.5 mm Al filter and a standard, manufacturer-provided beam-hardening correction algorithm, resulting in a total scan time of about 20 min to acquire a total of 220 μCT slices per 180 degree of scan. All images were first smoothed by a Gaussian filter (sigma=1.2, support=2.0) and then threshold corresponding to 30% of the maximum available range of image gray scale values. The images of the secondary spongiosa regions 0.3-0.9 mm above the highest point of growth plate were contoured for trabecular analysis. For cortical area, we scanned and analyzed the 0.3-mm region centered at the mid-point of each femur (diaphysis) at $6\mu\text{m}$ isotropic voxel size. Geometric trabecular

volumetric bone mineral density (vBMD), bone volume fraction (BV/TV), trabecular thickness (Tb.Th), trabecular separation (Tb.Sp), trabecular number (Tb.N), structure model index (SMI), cortical bone area (Ct.Ar), cortical bone thickness (Ct.Th) and polar moment of inertia (J) were calculated by 3D standard microstructural analysis provided by manufacturer [21].

2.6 Real-time PCR

Total RNA from intestinal mucosa and kidney was extracted according to the TRIzol protocol (Ambion, Life technologies). The RNA concentrations were quantified using a Nano-drop (ND-1000, V3.2.1, NanoDrop Tech, Inc, Wilmington, DE), and the quality of RNA was accessed by 1.5% agarose gel electrophoresis with ethidium bromide staining. Reverse transcription and real-time PCR were performed using MX3000P (Stratagene, La Jolla, CA). Primers (*Trpv6*, *CalbD9k*, and *Trpv5*) were designed using Roche primer design (Integrated DNA Technologies, Coralville, IA). Gene expression of intestinal and renal Ca transporters was calculated relative to the reference gene elongation factor 1 α gene (EF1 α) as previously described [22].

2.7 Liver fat

Liver fat was extracted according to Folch's method as previously described [23]. Briefly, Liver tissues were homogenized in 1xPBS, and hepatic total lipid was extracted with 2:1 (v:v) chloroform-methanol and 50mM KCl. Following centrifugation, the lower lipid-containing layer was isolated and dried under N₂. The percentage fat was calculated by dividing the isolated fat weight by the weight of the tissue sample.

2.8 Western blotting

The liver samples were homogenized with a Tissue-Tearor (Biospec Products, Bartlesville, OK), and the protein concentration of homogenates was determined using the Bradford Protein Assay (Bio-Rad) with BSA as the standard. Hepatic cytochrome P450 2r1 (Cyp2r1) protein level was quantified using the slightly modified procedure as previously described [24]. Briefly, total protein (30 μ g) was separated by electrophoresis on gradient (4-12%) SDS-PAGE gels (Novex, Thermo Scientific) followed by electro transfer to nitrocellulose membranes (Bio-Rad). After blocking, membranes were probed with rabbit Cyp2r1 primary anti-mouse antibodies (Abcam, Cambridge, UK) and anti-rabbit secondary antibodies (GE Healthcare). Bound antibodies were detected using the ECL western blotting detection reagent (GE Healthcare) with X-OMAT LS film. Films were scanned and digitized signals were quantified using the gel analysis software (UN-SCAN-IT gel, version 6.1). Used membranes were stripped with stripping buffer (Thermo Scientific) and re-probed with mouse β -actin primary antibodies to correct for unequal loading and transfer.

2.9 Estrogen status

At the end of the study, blood samples were drawn by cardiac puncture. Serum estradiol concentration was determined using a mouse ELISA assay kit (Calbiotech, Cat# ES 180S-100, Spring Valley, CA).

2.10 Statistical Analyses

Data are presented as means $\pm$ SD and figures are means $\pm$ SEM. One-way ANOVA was used to evaluate the statistical difference across dietary groups for experimental measurements. When the F ratio was significant, Tukey's HSD post-hoc analysis was conducted. A P value < 0.05 was considered significant, and a P value < 0.1 was considered a non-significant trend. The effect of dietary fat on Ca metabolism was evaluated using two-way ANOVA with dietary treatment and time as the independent factors. Repeated measure ANOVA was used to compare the weekly body weights among groups. Pearson correlation coefficient was used to assess the relation between liver fat and hepatic Cyp2r1 protein levels. A power analysis for FCA and BMD was done using a previous study examining energy restricted rodents vs. controls with identical methods (^{45}Ca and DXA). It was found that with α set at 0.05 and 80% power, 6 or fewer mice are necessary for statistical significance [19, 24]. In addition, 4 mice are adequate (80% power with $\alpha = 0.05$) for BV/TV [19]. Grubb's test to identify outliers was determined before statistical analysis. All analyses were conducted using the SAS statistical package (SAS Institute, Cary, NC, USA; v9.3).

3 Results

3.1 Caloric intake and body weight

The daily caloric intake per mouse as calculated from the 7-day food intake at the end of the study was 12 ± 2 , 7 ± 1 , and 8 ± 1 kcal/d, in the NFD, MUFA, and SFA groups, respectively ($P < 0.05$). There was a modest gain in weekly body weights throughout the study ($P = 0.32$, **Figure 1**). Delta weight change from weeks 0 to 8 did not differ among groups, with value equals to 1.3 ± 1.9 , 1.2 ± 2.2 , and 1.4 ± 2.3 g/8wk in the NFD, MUFA, and SFA groups, respectively ($P = 0.96$).

3.2 Calcium metabolism

Dietary intake of Ca was given at recommended levels (0.5% Ca) in all mice, and was 18 ± 3 , 14 ± 1 , and 16 ± 1 mg/day in the NFD, MUFA, and SFA groups, respectively ($P < 0.05$). There was a trend for SFAs and MUFAs to have higher final FCA values than the NFD diet ($P = 0.06$), and the FCA rise over time (from week 0 to 8) did not differ between groups ($P = 0.12$; **Table 2**). However, when analyzing the combined HFD groups (SFA and MUFA) compared with the NFD, final FCA was significantly higher ($P < 0.05$), and the rise over time in FCA between the initial and final weeks was greater in the HFD than in the NFD group ($P < 0.05$; **Supplemental Table 1**).

The high fat feeding tended to decrease fecal Ca excretion over the 8 weeks of food intervention when compared with the NFD group ($P = 0.06$, **Table 2**), but the greater rise in urinary Ca excretion and Ca balance in the HFD group compared with NFD was not statistically significant. None of the dietary treatments had a significant effect on final (wk8) Ca balance, endogenous fecal Ca loss, intestinal Ca secretion, or urinary Ca excretion.

3.3 Serum estradiol and uterine weight

Dietary fat did not change the final serum estradiol concentration, which was 6.6 ± 3.2 , 6.1 ± 3.3 , and 9.6 ± 3.9 pg/mL in the NFD, MUFA, and SFA groups, respectively ($P = 0.42$). In addition, functioning as an indicator of the estrogenic effect, the uterine weight was measured. There were no significant differences in uterine weight among groups, with the value equals to 87 ± 32 , 80 ± 36 , and 77 ± 25 mg in the NFD, MUFA, and SFA groups, respectively ($P = 0.61$).

3.4 Calcium transporter mRNA expression

Located at the apical epithelium, protein channel *Trpv6* transfers dietary Ca into the enterocytes cross the epithelial membrane. In this study, intestinal *Trpv6* mRNA abundance was similar among dietary groups (**Figure 2**) ($P = 0.49$). However, mRNA abundance of the intracellular Ca transporter *CalbD9k* in the small intestine was up regulated in the MUFA group when compared with the NFD group ($P < 0.0001$). Moreover, in the kidney, high fat feeding down regulated the mRNA abundance of the apical Ca channel *Trpv5* when compared with the NFD group ($P < 0.0001$) (**Figure 2**).

3.5 Bone densitometry and geometry

Total body and femur BMD was lower in SFA mice than in NFD mice ($P < 0.05$) (**Table 3**). Total body BMC was also lower in the SFA group than in the NFD group ($P < 0.05$), but no statistically significant effects of HFD on BMC or BMD were found at distal femur, tibia, humerus, radius, and lumbar spine (**Table 3**). Tibia lengths were similar in all groups with value equals to 17.5 ± 0.9 , 16.2 ± 0.7 , and 17.8 ± 0.6 mm in the NFD, MUFA, and SFA groups, respectively ($P = 0.75$). In addition, femur length did not significantly differ among groups, with length equals to 16.2 ± 0.7 , 16.5 ± 0.6 , 16.2 ± 0.7 mm in the NFD, MUFA, and SFA groups, respectively ($P = 0.71$).

Femoral geometric parameters were measured in a limited number of samples (**Table 4**). Trabecular BV/TV (%) was higher in the MUFA group than the NFD group ($P < 0.05$). Additionally, Tb.Th were higher in the MUFA group when compared with the NFD group ($P < 0.05$). There was a lower SMI with MUFA intake compared with the other diet groups ($P < 0.05$). No significant differences among diet groups were found for trabecular vBMD ($P = 0.23$), Tb.N ($P = 0.51$), or Tb.Sp. ($P = 0.61$). In the cortical region, the cortical vBMD was less in the SFA group when compared with the NFD group ($P < 0.05$). High fat feeding tended to be associated with a higher cortical porosity ($P < 0.07$), when compared the HFD group with the NFD group. Other cortical parameters including Ct.Ar ($P = 0.45$), Ct.Th ($P = 0.85$), and *J* ($P = 0.97$) were similar among diet groups.

3.7 Hepatic fat and Cyp2r1 protein level

The liver fat in this weight-controlled study did not significantly differ among groups, with 36 ± 14 , 31 ± 26 , and $32 \pm 13\%$ in the NFD, MUFA, and SFA groups, respectively ($P = 0.77$). Protein levels of 25-hydroxylase Cyp2r1 that plays an important role in the hepatic synthesis of 25-hydroxycholecalciferol (25(OH)D) was found to be higher in the MUFA

than NFD group ($P < 0.05$) (**Figure 3**). Additionally, there is an insignificant inverse correlation between liver fat and hepatic Cyp2r1 protein levels ($r = -0.145$; $P = 0.48$).

4. Discussion

There is evidence that dietary fat affects bone metabolism, but previous studies have only examined a high fat intake when there were also excess caloric intake and greater body weight [7, 26]. In the current study, our findings indicate that controlled HFD feeding in mature mice increases intestinal FCA, whereas only the MUFA enriched diet increases the expression of intestinal CalbD_{9k} mRNA and hepatic Cyp2r1 protein. The lower areal BMD, cortical BMC and vBMD in the femur with the SFA-enriched diet, compared with a neutral or positive effect of the MUFA-enriched diet suggests that bone is differentially affected by the type of dietary fatty acids. Hence, these findings support our primary hypothesis that high fat feeding without excess caloric intake increases Ca absorption in older female mice. Additionally, compared with dietary MUFAs, high SFAs intake adversely affected total body and femoral cortical bone parameters, but showed no differential role on Ca metabolism.

Different types of dietary fatty acids have differential effects on osteoblastic activity [27–30]. In addition, there is a negative effect of HFD-induced obesity on the bones of young rodents [31–32]. Our findings indicate that there is also an adverse effect of HFD on mature bones even in the absence of obesity, but this only occurs in the presence of a high SFAs (not MUFAs) intake. In a cross-sectional study using the NHANES III dataset, dietary SFAs was associated with reduced femoral neck BMD in humans [7]. SFAs may adversely affect bones through several mechanisms. Rodent studies indicate that SFAs increase bone resorption by elevating the expression of inflammatory cytokines and the receptor activator of NF- κ B ligand [33]. SFA mediates osteoclastogenesis and may decrease osteoclast apoptosis [29]. Additionally, SFAs interfere with osteoblastogenesis and bone formation [34].

There are no known mechanisms suggesting that MUFAs would have a negative effect on bone quality, and our findings indicate that BMD and cortical variables were similar in MUFA-enriched and NFD groups. Furthermore, we found that some femoral trabecular variables were higher in the mice fed the MUFAs-enriched diet, although the absolute values were low in all groups. The overall low values in these mice are likely attributed to their older age and history of repetitive pregnancies and lactations (retired breeders) [35]. It is possible that high dietary MUFAs have a protective effect on trabecular bone in general, but it is not clear if this would also occur under other conditions, such as younger, virgin or ad-libitum fed. Nevertheless, our findings are consistent with cross sectional reports in humans showing a positive effect of MUFA intake on bone [15, 36] and a reduction in fracture risk in an elderly population [16].

Intestinal Ca absorption varies with several factors including vitamin D concentration, estrogen status, and dietary nutrients [25]. In particular, fat consumption has been shown to have inhibit or have null effects on Ca absorption [4, 5, 37]. In contrast, we found that FCA tended to be higher with HFD feeding. The trend for higher Ca absorption in the HFD groups is consistent with findings that dietary fat is a significant positive predictor of FCA in clinical trials [9, 10, 38]. However, the underlying mechanisms of how dietary fat affects Ca

absorption remain unclear. A high fat intake increases circulating estrogen and vitamin D concentrations that would indirectly increase Ca absorption [39, 40]. However, we did not find that estrogen status differed between groups. It is possible that the higher FCA was due to a hyper-permeability of the intestinal membrane, which would increase passive leakage of dietary Ca [41]. In fact, SFA overconsumption, and not obesity, has been shown to reduce the integrity of tight junctions and increase intestinal permeability [11]. We suggest that a high fat feeding affects both active and passive transport to raise intestinal Ca absorption and these findings occur in the absence of excess energy intake.

An indirect estimation of intestinal transcellular movement of Ca can be achieved by measuring the expression of Ca absorption-related genes such as *Trpv6*, *CalbD_{9k}*, and plasma membrane Ca ATPase. These Ca transporters transfer dietary Ca across the enterocytes from the intestinal lumen to the blood circulation [1, 3]. The fat-induced greater *CalbD_{9k}* gene expression in this study is consistent with findings that short chain fatty acids elevated *CalbD_{9k}* expression *in vitro* [42]. Nevertheless, it is unclear whether a higher FCA or transcellular Ca transport was activated by high fat diet since only *CalbD_{9k}* mRNA and not *Trpv6* was elevated. The down regulation of renal *Trpv5* gene expression by high fat intake may suggest an attenuated transcellular Ca movement and reabsorption by the kidney [43]. However, urinary Ca excretion did not significantly differ among diet groups in this study. Also, SFA and MUFA diets induce a higher intestinal Ca absorption, suggesting that this is not contributing to differences in bone.

Hepatic Cyp2r1 is the major enzyme responsible for the first step of vitamin D hydroxylation to 25(OH)D [44, 45]. Because fatty liver is associated with low serum 25(OH)D concentration [46, 47], we measured hepatic fat and found that liver fat did not differ among diet groups. However, there was an up-regulation of Cyp2r1 protein expression in the MUFA group despite an absence of fatty liver. This finding may offer a mechanism to explain the higher serum 25(OH)D found in elderly people consuming a MUFA-rich diet compared with diets rich in polyunsaturated fat or low in fat diets [48]. Others have used intestinal Caco-2 cells to show that oleic acid compared with other fatty acids produces greater cholecalciferol basolateral efflux [49]. It is possible that a greater intestinal vitamin D absorption or conversion in the liver contributes to a higher vitamin D status with MUFA. Since the rise in Cyp2r1 was not significantly higher in the SFA enriched diet, differential vitamin D metabolism due to differences in dietary fatty acid type may be influencing bone.

One limitation of this study is that the protein intake was higher in the SFA than other diet groups (19% vs. 15%). Nevertheless, because the protein intake was sufficient in all groups and within the normal range of intake (15-22%) [50, 51], we would expect no differential effect on bone. For example, a previous study found that even at 40% (vs. 17%) protein intake had no effect on bone parameters in older mice [52]. Similarly, the vitamin/mineral content differed slightly between groups since intake was lower in the HFD compared to the NFD group. However, in all groups there was sufficient intake of micronutrients, and therefore it is not expected to differentially affect bone or Ca metabolism. Specifically, all mice were consuming approximately 2000 IU/kg vitamin D and 0.6-0.7% Ca that are both above the threshold intake to influence BMD and BMC in mice [53]. Serum 25(OH)D concentration was not measured in this study. It would be interesting to know if a higher

hepatic Cyp2r1 protein in the MUFA group is positively associated with a higher serum 25(OH)D and if this affects Ca absorption or bone. In the same way, the proposed changes in Ca transporter function, based on mRNA abundance, and would need additional measurements of protein levels and activity to confirm these changes. Also, it is possible that a longer term study [13] would have resulted in greater differences in bone between treatment groups.

In conclusion, high compared to normal fat intake induced a higher Ca absorption and this did not differ due to the type of dietary fatty acids. In contrast, only the high fat feeding with SFA adversely affected total and femoral areal bone mineral density under conditions of sufficient Ca intake and in the absence of excess caloric intake.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

25(OH)D	25 hydroxycholecalciferol
BMC	bone mineral content
BMD	bone mineral density
BV	bone volume
BV/TV	bone volume fraction
Ca	calcium
CalbD_{9k}	calbindin-D _{9k}
Ct.Ar	cortical total cross-sectional bone area
Ct.Po	cortical porosity
Ct.Th	cortical cross-sectional thickness
Cyp2r1	cytochrome P450 2r1
EF1α	elongation factor 1 α gene
FCA	fractional calcium absorption
HFD	high fat diet
J	polar moment of inertia

NFD	normal fat diet
MUFA	monounsaturated fatty acid
SFA	saturated fatty acid
SMI	structure model index
Tb.N	trabecular number
Tb.Sp	trabecular separation
Tb.Th	trabecular thickness
Trpv5	transient receptor potential cation channel subfamily V member 5
Trpv6	transient receptor potential cation channel subfamily V member 6
TV	total volume
vBMD	volumetric bone mineral density

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FIGURE 1.

Weekly body weights in mice throughout the 8wk of feeding with NFD or HFD enriched with MUFAs or SFAs (NFD, $n=10$; MUFA, $n=10$; SFA, $n=8$). Values are means $\pm$ SEM. $P < 0.05$ by repeated measure ANOVA for the effect of diets on body weight over 8wk. MUFA, monounsaturated fatty acid; NFD, normal fat diet; SFA, saturated fatty acid.

FIGURE 2.

Relative mRNA abundance of intestinal Ca transporter *Trpv6* and *CalbD_{9k}* and renal *Trpv5* in mice after 8wk of feeding NFD or HFD enriched with MUFAs or SFAs (n=6 for each group). All values are means $\pm$ SEM. $P < 0.05$ by one-way ANOVA following Tukey's post-hoc test. *CalbD_{9k}*, calbindin-D_{9k}; MUFA, monounsaturated fatty acid; NFD, normal fat diet; SFA, saturated fatty acid; *Trpv5*, transient receptor potential cation channel subfamily V member 5; *Trpv6*, transient receptor potential cation channel subfamily V member 6.

FIGURE 3.

Hepatic vitamin D 25-hydroxylase Cyp2r1/ β -actin protein level in mice after 8wk of feeding with normal fat diet and high fat diets enriched with MUFAs or SFAs (NFD, n=10; MUFA, n=10; SFA, n=8). All values are means $\pm$ SEM. $P < 0.05$ by one-way ANOVA using Tukey's post-hoc test. Cyp2r1, cytochrome P450 2r1; MUFA, monounsaturated fatty acid; NFD, normal fat diet; SFA, saturated fatty acid.

Table 1Dietary composition and energy content of experimental diets¹

Ingredient	NFD	MUFA	SFA
g/kg diet			
Casein	146	183	242
L-cystine	3	4	3
Corn Starch	540	249	77
Maltodextrin	98	189	106
Sucrose	98	34	240
Cellulose	49	61	60
Soybean Oil	6	36	33
Coconut Oil	0	0	20
Lard	11	64	188
Olive Oil	26	153	0
Mineral Mix	10	12	14
Vitamin Mix V10001	10	12	12
Choline bitartrate	2	2	2
Calcium, mg/g diet	5.7	7.2	7.1
Energy, kcal/g diet	3.9	4.8	4.7
% Energy			
Carbohydrate	75	39	37
Protein	15	15	19
Fat	10	46	44
Saturated (%kcal of fat)	20	20	41
Monounsaturated (%kcal of fat)	60	60	41
Polyunsaturated (%kcal of fat)	20	20	18

¹ Prepared by Research Diets Inc., New Brunswick, NJ. MUFA, monounsaturated fatty acids; NFD, normal fat diet; SFA, saturated fatty acid.

Calcium metabolism in mice before and after 8wk of feeding with normal fat diet or with high fat diets enriched in MUFAs or SFAs[/]**Table 2**

	Baseline (wk0)				Final (wk8)				<i>P</i>	
	NFD	MUFA	SFA	NFD	MUFA	SFA	Diet	Time	Diet × Time	
Urinary Ca, $\mu\text{mol/d}$	1.80 ± 1.3	1.87 ± 1.0	1.48 ± 1.0	2.24 ± 1.5	3.36 ± 3.3	3.17 ± 1.7	0.64	0.08	0.67	
Fecal Ca, mmol/d	0.30 ± 0.06	0.27 ± 0.05	0.33 ± 0.05	0.31 ± 0.07	0.18 ± 0.05	0.24 ± 0.04	0.01	0.01	0.06	
Ca balance, mmol/d	0.10 ± 0.03	0.12 ± 0.03	0.09 ± 0.02	0.14 ± 0.05	0.16 ± 0.04	0.15 ± 0.03	0.25	0.001	0.61	
Endogenous fecal Ca, mmol/d	0.11 ± 0.05	0.08 ± 0.05	0.07 ± 0.05	0.13 ± 0.06	0.24 ± 0.03	0.19 ± 0.08	0.96	0.01	0.25	
Intestinal Ca secretion, mmol/d	0.11 ± 0.09	0.13 ± 0.11	0.10 ± 0.06	0.23 ± 0.13	0.39 ± 0.20	0.38 ± 0.22	0.23	0.001	0.45	
FCA, %	31.9 ± 14	33.6 ± 10	24.9 ± 8	38.5 ± 9.8	57.7 ± 11	56.7 ± 20	0.10	0.001	0.12	

[/] All values are means ± SD (NFD, n=6; MUFA, n=6; SFA, n=6); means in a row with different superscripts differ, *P* < 0.05 by two-way ANOVA. FCA, fractional calcium absorption; MUFA, monounsaturated fatty acid; NFD, normal fat diet; SFA, saturated fatty acid.

Table 3

Bone densitometry in mice after 8wk of feeding with normal or high fat diets enriched with MUFAs or SFAs¹,

	Diet			<i>P</i>
	NFD	MUFA	SFA	
BMC, g				
Total body	0.41 ± 0.04 ^a	0.39 ± 0.03 ^{ab}	0.37 ± 0.04 ^b	0.02
Whole femur	0.026 ± 0.002	0.025 ± 0.002	0.025 ± 0.001	0.09
Distal femur	0.007 ± 0.001	0.007 ± 0.001	0.006 ± 0.002	0.12
Tibia	0.024 ± 0.002	0.024 ± 0.004	0.023 ± 0.002	0.69
Humerus	0.013 ± 0.001	0.013 ± 0.001	0.012 ± 0.001	0.85
Radius	0.009 ± 0.001	0.009 ± 0.001	0.008 ± 0.001	0.58
Lumbar spine	0.047 ± 0.004	0.045 ± 0.004	0.045 ± 0.002	0.62
BMD, g/cm ²				
Total body	0.046 ± 0.003 ^a	0.046 ± 0.002 ^a	0.044 ± 0.001 ^b	0.02
Whole femur	0.047 ± 0.002 ^a	0.047 ± 0.004 ^a	0.044 ± 0.002 ^b	0.03
Distal femur	0.062 ± 0.006	0.061 ± 0.013	0.053 ± 0.001	0.20
Tibia	0.045 ± 0.002	0.046 ± 0.004	0.043 ± 0.002	0.12
Humerus	0.040 ± 0.001	0.040 ± 0.001	0.038 ± 0.001	0.24
Radius	0.029 ± 0.001	0.029 ± 0.001	0.028 ± 0.004	0.12
Lumbar spine	0.047 ± 0.002	0.046 ± 0.001	0.046 ± 0.001	0.39

¹ All values are means ± SD (NFD: n=10; MUFA, n=10; SFA, n=8). Means in a row with different superscripts differ, *P* < 0.05 (one-way ANOVA followed by Tukey's post hoc test). BMC, bone mineral content; BMD, bone mineral density; MUFA, monounsaturated fatty acid; NFD, normal fat diet; SFA, saturated fatty acid.