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Original Research

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/6 J 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 ($P < .05$). 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 ($P < .05$). Intestinal calbindin-D_{9k} gene and hepatic

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 1α 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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cytochrome P450 2r1 protein levels were higher with the MUFA than the NFD diet ($P < .05$). 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.

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/6 J mice (n = 29) weighing approximately 27 g 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%

Table 1 – Dietary composition and energy content of experimental diets [1]

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

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