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A western-style diet reduces bone mass and biomechanical bone strength to a greater extent in male compared with female rats during development

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Evidence from epidemiological and animal-feeding trials suggests that a western-style diet that is high in fat, and low in Ca, vitamin D and folic acid may result in low bone mass and poor bone quality: this leads to an increased risk of fragility fracture. The overall objective of the present study was to determine the effect of feeding a western-style diet (low in Ca (0·4 g/kg diet, Ca:P ratio 1:10), cholecalciferol (3 μg/kg diet), folic acid (0·23 mg/kg diet) and fibre (20 g/kg diet), and high in fat (200 g/kg diet)) for 17 weeks on bone mineral content (BMC) and the biomechanical bone strength of rat femurs. A secondary objective was to determine whether femurs from male and female rats (seven to eight rats per group) respond differently to the western-style diet. Male and female rats weighing 150–180 g were fed a western-style diet or a control diet for 17 weeks. At the end of the feeding trial, femur BMC was measured by ashing, and biomechanical properties were determined by three-point bending. Femur BMC and the majority of biomechanical properties measured were lower ($P<0·05$) among male and female rats fed a western-style diet compared with a control diet, despite similar weight gain and final body weight within genders. However, the western-style diet had a greater negative effect on femur BMC and biomechanical strength properties among male rats compared with females. This may be because male rats experienced greater overall body growth, as assessed by weight gain, than female rats, and suggests that the nutrient composition of the western-style diet did not support the development of strong femurs.

Western-style diet: Biomechanical bone strength: Bone mass: Rats

Osteoporosis is a debilitating disease that arises from a loss of bone mass and compromised integrity of bone structure: it leads to an increased risk of fragility fractures in both women and men. In turn, fragility fractures result in significant morbidity and even death (Melton *et al.* 1992; Atkinson & Ward, 2001; Binkley & Krueger, 2002; Cummings & Melton, 2002). While genetic factors contribute to an individual's risk of developing osteoporosis and subsequent fragility fractures (Peacock *et al.* 2002), modifiable factors such as nutrition can also influence bone health, either positively or negatively (Dawson-Hughes, 2001; Brown & Josse, 2002).

Interestingly, the rates of osteoporosis as assessed by bone mass can be similar among populations, although hip-fracture rates can be quite different (Ho *et al.* 1993, 1999; Bacon *et al.* 1996). For example, despite the fact that femur neck bone mineral density (BMD) of women in Hong Kong and North America is similarly low, hip-fracture rates are lower among women in Hong Kong (Ho *et al.* 1993, 1999). When rates of hip fracture in men and women were compared among nine countries, the rates were highest in North America (Canada, USA) and European countries (Finland, Sweden, Switzerland and Scotland) compared with Hong Kong, Venezuela and Chile (Bacon *et al.* 1996). Another study has also shown that the incidence of hip fractures is lower among Asians compared with several non-Asian countries (Schwartz *et al.* 1999). The lower fracture rates may be due to differences in the geometry of the hip (shorter femoral neck) that may be protective against fractures during falls, to a lower incidence of falls or to differences in diet (Koh, 2002). It is possible that the western diet itself may contribute to the higher rates of fragility fracture as the western diet, in general, is high in fat and low in Ca, vitamin D and folic acid, when compared with dietary recommendations (Cavadini *et al.* 2000; Gray-Donald *et al.* 2000; Starkey *et al.* 2001). However, fortification of specific foods with folic acid has improved folic acid intakes among North Americans (Choumenkovitch *et al.* 2002; Ray *et al.* 2002). The eating patterns of children and adolescents living in North America and Europe indicate that they do not meet the current dietary recommended intakes for Ca and vitamin D and, furthermore, consume higher levels of fat than recommended (Food and Nutrition Board, Institute of Medicine, 1997; Munoz *et al.* 1997; Cavadini *et al.* 2000; Starkey *et al.* 2001; Alexy *et al.* 2002). Moreover, adult men and women living in North America and Europe, in general, also have lower dietary intakes of Ca

Abbreviations: BMC, bone mineral content; BMD, bone mineral density.

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and vitamin D and higher dietary intakes of fat than recommended (Millen *et al.* 1996; Food and Nutrition Board, Institute of Medicine, 1997; Borody *et al.* 1998; Becker 1999; Volatier & Verger, 1999; Gray-Donald *et al.* 2000; Hannon *et al.* 2001; Starkey *et al.* 2001). Together, a diet high in fat and low in Ca and vitamin D may compromise bone health.

Using rodent models, the effects of low vitamin D and/or Ca have been shown to be detrimental to bone mass (Thomas *et al.* 1991; Bielaczyc *et al.* 1997; Geng *et al.* 2000; Creedon & Cashman, 2001; Geng & Wright, 2001; Kaastad *et al.* 2001). Other authors have reported that high-fat diets have negative effects on bone metabolism (Salem *et al.* 1992; Zernicke *et al.* 1995; Wohl *et al.* 1998). A high-fat diet, particularly a diet rich in saturated fat, resulted in a lower bone mass and lower biomechanical bone strength in roosters (Wohl *et al.* 1998). A high-fat, high-sucrose diet had a similar effect in growing (Salem *et al.* 1992) and aged (Zernicke *et al.* 1995) rats. Diets low in folic acid may also lead to abnormalities in bone metabolism. Moderate to high levels of homocysteine, which may result from inadequate levels of folic acid, are associated with low BMD and impairments in connective tissue (Krumdieck & Prince, 2000). Despite the fact that these dietary insults may occur together and not in isolation in everyday life, the effects of combining these particular dietary risk factors (high fat, low Ca, low vitamin D, low folic acid) on bone metabolism have not been studied.

To our knowledge, no studies have reported the effect of this western-style diet (i.e. the combined effect of reduced Ca, vitamin D and folic acid and increased fat) on bone mass and biomechanical bone strength in rats. The overall objective of the present study was to determine the effect of feeding a western-style diet for 17 weeks on bone mineral content (BMC) and biomechanical bone strength of femurs from male and female rats. A secondary objective was to determine whether there are any differences among male and female rats with regard to the effects of a western-style diet on BMC and biomechanical bone strength.

Materials and methods

Animals and diets

Male and female rats (Fisher 344; Charles River Co., St-Laurent, Que., Canada) weighing 150–180 g were randomized to a control diet (diet no. 01112801; Research Diets Inc., New Brunswick, NJ, USA) or the western-style diet (diet no. 16378B; Research Diets Inc.) for 17 weeks (Table 1). The composition of the western-style diet was developed by Newmark *et al.* (2001) and was shown to induce colon tumours in rodents. Major differences in nutrient composition between the western-style diet and the control diet are shown in Table 2. Other dietary components were adjusted to provide similar levels in the two diets on an energy-density basis (Newmark *et al.* 2001).

Rats had free access to food and distilled water during the study period. Fresh food was provided every 2–3 d. Food intake was measured for 1 week (weeks 14–15). Body weight was measured once every 2 weeks. At necropsy, femurs were excised and stored at −70°C until analyses were performed. The Animal Ethics Committee at the University of Toronto approved the protocol and the guidelines established by the Canadian Council on Animal Care (1984) were followed.

Table 1. Diet composition of control diet and western-style diet

Ingredient	Control diet (g/kg)	Western-style diet (g/kg)
Casein	200	240
Maize oil	50	200
Maize starch	500	75
Sucrose	0	336
Maltodextrin 10	150	75
Cellulose	50	20
Mineral mix*†	35	21
Monosodium phosphate	0·00	7·98
Monopotassium phosphate	0·00	7·91
Calcium carbonate	0·00	0·88
Vitamin mix‡§	10	12
Vitamin cholecalciferol (2·5 mg)	0·0000	0·0012
Folic acid	0·00000	0·00023
L-Cystine	0·0	3·6
DL-Methionine	3	0
Choline bitartrate	2·0	1·2

* Mineral mix for control diet (per kg mineral mix): calcium phosphate dibasic 500·00 g, magnesium oxide 24·00 g, potassium citrate 220·00 g, potassium sulfate 52·00 g, sodium chloride 74·00 g, chromium potassium sulfate 0·55 g, cupric carbonate 0·30 g, potassium iodate 0·01 g, ferric citrate 6·00 g, manganous carbonate 3·50 g, sodium selenite 0·01 g, zinc carbonate 1·60 g, sucrose 118·03 g.

† Mineral mix for western-style diet (per 0·5 kg mineral mix): magnesium oxide 24·00 g, potassium citrate 220·00 g, potassium sulfate 52·00 g, sodium chloride 74·00 g, chromium potassium sulfate 0·55 g, cupric carbonate 0·30 g, potassium iodate 0·01 g, ferric citrate 6·00 g, manganous carbonate 3·50 g, sodium selenite 0·01 g, zinc carbonate 1·60 g, sucrose 118·03 g.

‡ Vitamin mix for control diet (per 10 g vitamin mix): retinyl palmitate 2·2 mg, cholecalciferol 25 μg, tocopheryl acetate 34 mg, menadione sodium bisulfite (625 mg menadione/g) 0·5 mg, biotin (10 mg/g) 0·2 mg, cyancocobalamin (1 mg/g) 10 μg, folic acid 2·0 mg, nicotinic acid 30·0 mg, calcium pantothenate 16·0 mg, pyridoxine-HCl 7·0 mg, riboflavin 6·0 mg, thiamine-HCl 6·0 mg, sucrose 9·7800 g.

§ Vitamin mix for western-style diet (per 10 g vitamin mix): retinyl palmitate 2·2 mg, tocopheryl acetate 34 mg, menadione sodium bisulfite (625 mg menadione/g) 0·50 mg, biotin (10 mg/g) 0·2 mg, cyancocobalamin (1 mg/g) 10 μg, nicotinic acid 30·0 mg, calcium pantothenate 16·0 mg, pyridoxine-HCl 7·0 mg, riboflavin 6·0 mg, thiamine-HCl 6·0 mg, sucrose 9·790 g.

Femur dimensions

Femurs were cleaned of soft tissue and stored at −70°C until biomechanical strength testing was performed.

Table 2. Major differences in nutrient composition between the control and western-style diet*

Nutrients	Control diet	Western-style diet
Ca g/kg	5·2	0·4
P g/kg	4·0	4·0
Ca:P ratio	1·0:0·8	1·0:10·0
Cholecalciferol (μg/kg)	25	3
Folic acid (mg/kg)	2·00	0·23
Fibre (g/kg)	50	20
Fat (maize oil) (g/kg)	50	200
Protein (g/kg)	200	240
Carbohydrate (g/kg)	660	500

* For details of diets, see Table 1.

Femur length was measured using digital calipers. Because the bone shafts were asymmetrical, width at the mid-point of the diaphysis was measured in two directions: width A in the medio-lateral direction and width B in the anterior-posterior direction.

Femur bone mineral content

The BMC of femurs was determined by ash technique. Femurs were placed in acid-washed crucibles and placed in a muffle furnace at 550°C for 72 h.

Femur biomechanical bone strength testing

Femur biomechanical strength was measured by three-point bending using an electromechanical materials testing system (model 4442, Universal Testing System; Instron Corp., Canton, MA, USA) using specialized software (Series IX Automated Materials Tester, version 8.15.00) as described by Ward *et al.* (2001*a*,*b*). Femurs that had been cleaned of soft tissue were removed from the −70°C freezer and rehydrated in physiological saline (9 g NaCl/l) for 4 h at room temperature. All femurs were tested 2 weeks post-excision. Immediately prior to testing, the hydrated femur weights were determined. The posterior side of the femurs was placed on two supports that were separated by a distance of 15 mm and bent until fracture by lowering the crosshead positioned at the mid-shaft at a constant speed of 6 mm/min (Ward *et al.* 2001*a*,*b*). Visual inspection ensured that all femurs were bent in the same direction.

Load–deformation curves were generated and several biomechanical characteristics were determined from these curves: (1) yield load (a measure of the elastic limit of a bone); (2) resilience (the energy absorbed by the bone until the yield point is reached); (3) peak load (a measure of the maximum force that the bone withstood before fracture); (4) toughness (a measure of the work energy that was required to fracture the bone); (5) ultimate stiffness (a measure of the extrinsic rigidity of the femur). To account for differences in the size of individual femurs, biomechanical properties that take into account bone size were calculated. The second moment of inertia was calculated as follows:

$$I = (\pi/64) \times (b_2 b_1^3 - a_2 a_1^3),$$

where b_2 is the outer diameter of the bone shaft perpendicular to the load direction, b_1 is the outer diameter of the bone shaft in the load direction, a_2 is the inner diameter perpendicular to the load direction and a_1 is the inner diameter in the load direction (Ejersted *et al.* 1993). Young's modulus, which represents the intrinsic stiffness of the intact bone, was calculated according to the following equation:

$$E = (L^3 ST)/(48\ I),$$

where E is Young's modulus, L is the distance between the base supports, ST is the value of ultimate stiffness generated by the computer software and I is the second moment of inertia (equation shown earlier).

Statistical analyses

Within each gender, Student's *t* test was performed to detect statistically significant differences among treatments for data that followed a normal distribution. Data that did not have a normal distribution (resilience, food intakes) were tested using the Mann–Whitney rank sum test. Differences were considered significant if $P<0{\cdot}05$. To determine the contribution of BMC to the various biomechanical strength properties, linear regression analyses were performed for each gender, with BMC as the independent variable and each of the biomechanical strength properties as dependent variables in separate analyses. All statistical analyses were performed using SigmaStat software (version 2.0; Jandel Scientific, San Rafael, CA, USA). Results are presented as the mean values and standard deviations.

Results

Food intake and weight gain

By design, the western-style diet contained more fat, and less Ca, vitamin D, folic acid and fibre, than the control diet (Table 1). Thus, both male and female rats fed the western-style diet had higher ($P<0{\cdot}001$) intakes of fat and lower ($P<0{\cdot}001$) intakes of Ca, vitamin D, folic acid and fibre than rats fed the control diet. The mean daily food intake of male rats fed the western-style diet was significantly less ($P<0{\cdot}002$) than that of the control group (western-style diet 15 (SD 1) g/d, control diet 20 (SD 1) g/d). Although the energy content of the western-style diet was higher ($P<0{\cdot}001$) than the control diet, energy intakes were also less among male rats fed the western-style diet. Similarly, female rats fed the western-style diet consumed less food (western-style diet 8 (SD 1) g/d, control diet 11 (SD 1) g/d; $P<0{\cdot}001$) and their energy intakes were lower ($P<0{\cdot}001$) than female rats fed the control diet.

Both male and female rats fed the control or western-style diet gained weight over the study period. Final body weights were not significantly different among either male (western-style diet 347 (SD 12) g, control diet 343 (SD 16) g) or female (western-style diet 183 (SD 5) g, control diet 183 (SD 8) g) rats. Moreover, total weight gain over the study period did not differ among male (western-style diet 175 (SD 15) g, control diet 171 (SD 14) g) or female (western-style diet 36 (SD 8) g, control diet 37 (SD 9) g) rats, indicating that differences in mean daily food intakes and dietary interventions did not alter body-weight gain.

Femur dimensions and bone mineral content

Femurs from male rats fed the western-style diet weighed less ($P<0{\cdot}001$) and had a lower BMC ($P<0{\cdot}001$) than control rats (Fig. 1). In contrast, the BMC ($P<0{\cdot}001$), but not the weight ($P=0{\cdot}095$), of femurs from female rats differed among treatment groups (Fig. 1). Femur length or width (width A or width B) were not different among either male or female rats fed the western-style diet compared with their respective controls (results not shown).

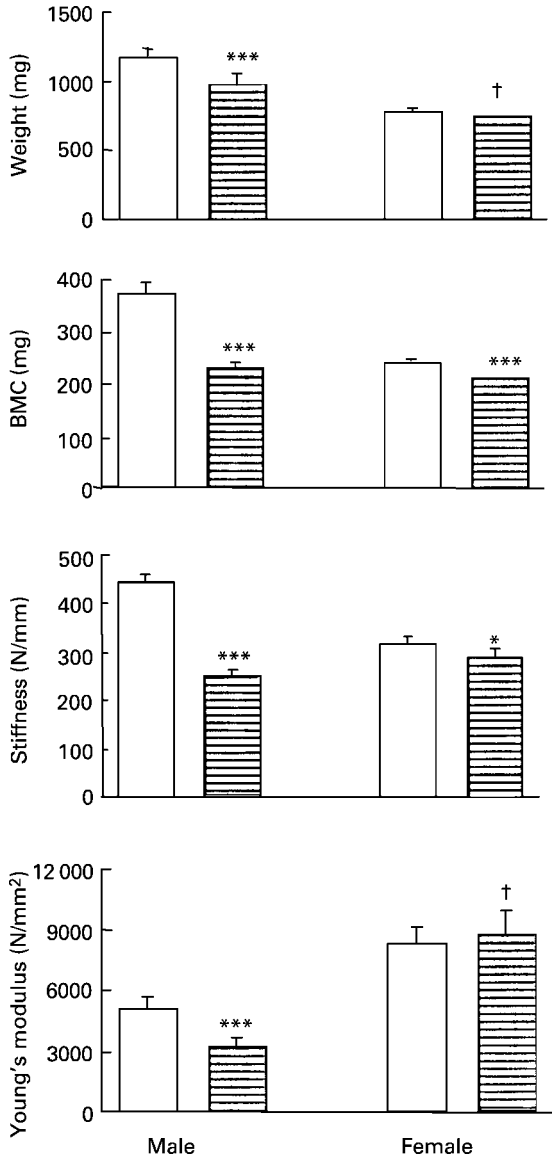

Fig. 1. Femur weight, bone mineral content (BMC), stiffness and Young's modulus among male and female rats (seven to eight rats per group) fed a control (□) or western-style (▤) diet. For details of diets and procedures, see Tables 1 and 2 and p. 590. Mean values were significantly different within gender group: *$P<0{\cdot}05$, ***$P<0{\cdot}001$. Mean values were not significantly different within gender group: †$P>0{\cdot}05$.

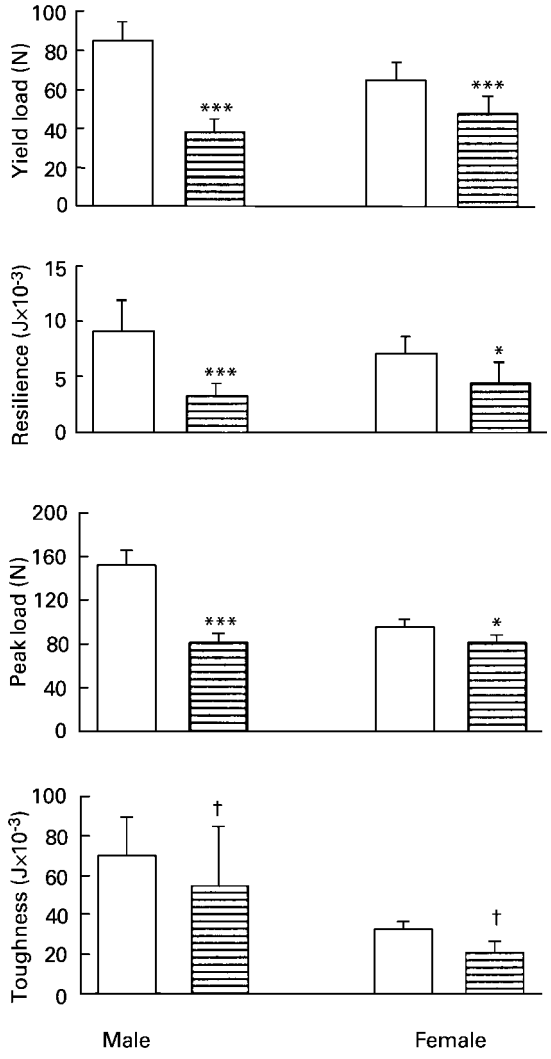

Fig. 2. Femur yield load, resilience, peak load and toughness among male and female rats fed a control (□) or western-style (▤) diet. For details of diets and procedures, see Tables 1 and 2 and p. 590. Mean values were significantly different within gender group: *$P<0{\cdot}05$, ***$P<0{\cdot}001$. Mean values were not significantly different within gender group: †$P>0{\cdot}05$.

Femur biomechanical properties

The yield load and the resilience of femurs were lower ($P<0{\cdot}001$ and $P<0{\cdot}001$ respectively) among male rats receiving the western-style diet compared with controls (Fig. 2). Similarly, the peak load (Fig. 2) was lower ($P<0{\cdot}001$) among male rats fed the western-style diet, while femur toughness was not different among male rats (Fig. 2). Femur stiffness (Fig. 1) was lower ($P<0{\cdot}001$) among male rats fed the western diet compared with their respective control groups. Young's modulus, which takes into consideration size differences in the femurs at the mid-point, was lower ($P<0{\cdot}001$) among male rats fed the western-style diet (Fig. 1).

Similarly to the male rats, the yield load (Fig. 2), resilience (Fig. 2) and peak load (Fig. 2) of femurs from female rats were lower ($P<0{\cdot}001$, $P<0{\cdot}05$ and $P<0{\cdot}05$ respectively) among rats fed the western-style diet compared with the control diet, while toughness was not different among females (Fig. 2). Femur stiffness was lower ($P<0{\cdot}05$) among female rats fed the western-style diet compared with their respective control groups (Fig. 1). In contrast to the male rats, Young's modulus was not different among female rats (Fig. 1).

Contribution of bone mineral content to biomechanical strength properties

Among male rats, linear regression analysis demonstrated that BMC was significantly related to yield load ($P<0{\cdot}001$, r 0·965), resilience ($P<0{\cdot}001$, r 0·856), peak

load ($P<0{\cdot}001$, r 0·977), stiffness ($P<0{\cdot}001$, r 0·964) and Young's modulus ($P<0{\cdot}001$, r 0·852), but not toughness. The same analysis among female rats indicated that the BMC was significantly related to yield load ($P=0{\cdot}009$, r 0·714), peak load ($P<0{\cdot}001$, r 0·856) and stiffness ($P=0{\cdot}003$, r 0·783), but not resilience, toughness or Young's modulus.

Comparison of the effect of the western-style diet on male v. *female rats*

To determine if male or female rats were affected differently by the western-style diet compared with the control diet, femur outcomes were expressed as a percentage of the value of the rats fed control diet for each gender (Table 3). For all femur outcomes, male rats were more sensitive to the effects of the western-style diet than female rats when compared with their respective control group (Table 3).

Discussion

The present study is the first to report the effect of a western-style diet, characterized by a diet high in fat, and low in Ca, vitamin D, folic acid and fibre, on bone mass and biomechanical bone strength in a rodent model. Although both male and female rats fed the western-style diet had markedly lower femur BMC and the majority of the biomechanical properties measured were lower, the negative effects on bone metabolism were more evident among male compared with female rats. Male rats may have been more sensitive to this diet as they had a greater body-weight gain over the 17-week study period, although body-weight gain was similar among male rats fed the control or western-style diet. Male rats gained approximately five times more body weight than female rats over the study period. Similarly to male rats, body-weight gain did not differ among female rats fed control or western-style diet. Femur BMC was markedly lower, but body weights did not differ among groups, suggesting that either lean and/or fat mass were also altered with the western-style diet. Despite lower food intakes among male and female rats fed the western-style diet, body-weight gain was not different within genders and suggests that measurements of food intakes over a 1-week period were not indicative of food intakes throughout the entire feeding trial.

Table 3. Comparison of the effect of western-style diet on male *v.* female rats*

(Mean values)

	Male	Female
Femur outcome	% of control†	% of control†
Weight	83	95
BMC	61	88
Yield load	45	73
Resilience	36	62
Peak load	53	89
Toughness	81	104
Stiffness	56	91
Young's modulus	62	105

BMC, bone mineral content.
* For details of diets and procedures, see Tables 1 and 2 and p. 590.
† Results are expressed as a percentage of the values obtained for the respective control group within each gender.

The majority of the biomechanical properties that were measured were negatively altered by the western-style diet among both male and female rats: this suggests that both the matrix and mineral component of femurs were affected (Burr, 2002). However, the fact that the yield load and peak load among male and female rats were significantly correlated with BMC indicates that the reduction in mineral content of the femurs was largely responsible for the weakening of the femurs. The lower BMC of femurs observed among both male and female rats fed the western-style diet is in agreement with the lower yield point and the resilience measurements. Because the three-point bending test evaluates changes in bone biomechanics at the mid-point, a site composed of a substantial quantity of cortical bone, this indicates that cortical bone was affected by the western-style diet. This finding is similar to that of several other groups that have demonstrated that a high-fat, high-sucrose diet compromises the biomechanical properties of cortical as well as trabecular bone in developing female rats (Li *et al.* 1990; Salem *et al.* 1992).

The higher fat content of the western-style diet may have altered serum sex steroid levels. Feeding a high-cholesterol diet to developing male rats resulted in a reduction in serum testosterone (Tanaka *et al.* 2001). In turn, a lower level of testosterone could mediate a negative effect on bone metabolism.

Other investigators have reported that among rats older (24 weeks of age) than those in the present study, female rats are more sensitive to a low-Ca diet than male rats (Geng & Wright, 2001). Geng & Wright (2001) fed rats various levels of Ca in diets for 31 d (0·2, 5·0, 10·0 or 17·5 g Ca/kg) and measured changes in bone mass within the axial and appendicular skeleton. To assess the axial skeleton, six representative bones, all of which contain a higher proportion of trabecular rather than cortical bone, were selected for analyses (Geng & Wright, 2001), while bones selected for analysis of appendicular skeleton contained a higher level of cortical bone. Female rats fed 0·2 g Ca/kg diet, similar to the level of Ca in our western-style diet, experienced a greater loss in the axial skeleton compared with female rats fed 17·5 g Ca/kg diet. There were no significant differences in the axial skeleton or appendicular skeleton among female rats fed 0·2 or 5·0 g Ca/kg diet (which was similar to our control diet), although values were 10·4 and 8·0 % higher for axial and appendicular skeleton respectively among rats fed 5·0 g Ca/kg compared with rats fed 0·2 g Ca/kg. In contrast to the findings in the present study, male rats fed the 0·2 g Ca/kg diet did not experience the significant reduction in bone mass experienced by female rats. In addition, bone loss in the appendicular skeleton was less than that of the axial skeleton. Trabecular bone is more metabolically active; thus, it makes biological sense that bones with a greater quantity of trabecular bone were affected more by the low-Ca diet (Geng & Wright, 2001). The difference in the gender response between our present study and

other reports is likely to be due to the fact that we studied developing rats rather than older, adult rats. It appears that during a period of rapid growth (i.e. body-weight gain), male rats are more susceptible to nutritional insults. It is also possible that dietary components other than the low Ca, such as the high-fat content, as discussed earlier, or the low-vitamin D or low-folic acid content of the western-style diet affected bone loss.

The precise role of folic acid in bone metabolism, particularly in regard to low dietary levels, have not been clearly defined. However, patients with homocysteinuria develop osteoporosis and have impaired cross-linking of type 1 collagen that can ultimately result in weakened bones (Lubec *et al.* 1996). While it is not possible to conclude whether low dietary folic acid directly resulted in bone abnormalities due to impaired processing of collagen, our present finding that BMC was significantly related to yield load and peak load suggests that the reduction in mineral content of the femur contributed significantly to the weakening of femurs among rats fed the western-style diet.

In conclusion, a western-style diet previously shown to induce colon neoplasms (Newmark *et al.* 2001), colonic hyperproliferation (Richter *et al.* 1995; Risio *et al.* 1996, 2000; Newmark *et al.* 2001) and mammary ductal epithelia cell hyperproliferation and hyperplasia (Khan *et al.* 1994; Xue *et al.* 1996, 1999) also results in impaired bone metabolism including both reductions in BMC and weakened bone properties, suggesting an increased susceptibility to fracture. Moreover, findings from the present study demonstrate that this detrimental effect is greater among male rats compared with female rats. Because multiple dietary components were altered in the western-style diet compared with the control diet, future studies could investigate the contribution of each individual dietary insult to more fully understand the mechanisms by which the western-style diet exerted its effects. Assessment of other bones, differing in the content of trabecular and cortical bone, could also provide further insight into whether a western-style diet affects these two types of bone in a similar manner. Moreover, strength testing at the femur neck, a common site of osteoporotic fracture in man, would provide more insight into the risks of hip fracture due to a western-style diet in men and women: fractures of the femur shaft, measured in the present study, are rare in man.

From a public health perspective, the findings from the present study are important, since the prevalence of osteoporosis is increasing among both men and women due to the aging population (Cummings & Melton, 2002). Because bone health can be influenced by diet, it is essential to promote awareness regarding recommended intakes of nutrients important for bone, and nutrients that may impair bone metabolism, such as a low dietary Ca:P ratio as well as high dietary fat. Using animal models, diets with a low Ca:P ratio are known to stimulate secondary hyperparathyroidism and increase bone resorption (Pettifor *et al.* 1984; Anderson *et al.* 2001). However, it should be noted that rats are relatively hyperphosphataemic compared with human subjects (Horn *et al.* 2000) and thus a low Ca:P ratio in human subjects may result in a different response. While the effects of a low dietary Ca:P ratio on bone metabolism in human subjects await investigation, the dietary Ca:P ratio is low among North Americans due to a higher consumption of prepared foods, a lower consumption of dairy products such as milk and higher intakes of carbonated beverages (Anderson *et al.* 2001). Moreover, since high dietary fat is also associated with other diseases such as cardiovascular disease and possibly some cancers, it is particularly prudent to advocate the consumption of recommended levels of nutrients to protect against or reduce the risk of developing multiple chronic diseases.

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