Original Article

Effects of a high intake of unsaturated and saturated oils on intestinal transference of calcium and calcium mobilization from bone in an ovariectomized rat model of osteoporosis

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Intestinal transference of calcium and rate of bone turnover were evaluated in ovariectomized rats fed for 15 days with a high amount (30%) of lipid enriched with monounsaturated (groundnut oil), polyunsaturated (sunflower oil) and saturated (coconut oil) fatty acids. The results were compared with those for sham-operated control and ovariectomized groups fed a normal diet (7% groundnut oil). Irrespective of the saturation and unsaturation characteristics, all lipids (edible oils) used in our study considerably decreased the rate of *in situ* intestinal transference of calcium. Likewise, the activities of intestinal mucosal enzymes, alkaline phosphatase (AP) and calcium ATPase (Ca^{2+}-ATPase) were decreased significantly in all the segments of the small intestine in a descending gradient. Significant changes in bone turnover and bone calcium (Ca) mobilization were confirmed in these animals by marked alterations in plasma AP activity, urinary calcium and phosphate excretion and calcium to creatinine (Ca:creatinine) ratio. Lipid supplementation (30%) in such ovariectomized rats using groundnut oil (monounsaturated), sunflower oil (polyunsaturated) or coconut oil (saturated) for 15 days further enhanced all of the above observed parameters. These results suggest that the intake of high amounts of lipids with different unsaturation and saturation characteristics may be an important factor in determining bone loss in ovariectomized rats.

Key words: ovariectomy, high lipid diet, oils, saturated, unsaturated, intestinal, epithelium, calcium, bone turnover, osteoporosis.

Introduction

Fat is an important dietary component which affects both growth and health. It is widely accepted that a high level of fat in the diet is detrimental to health, while recommendations for lower dietary fat levels and changes in the pattern of fatty acid consumption are regularly made.[1,2] In recent years there has been an increased focus on replacing some of the individual saturated fat intake with polyunsaturated fat.[3]

It has been demonstrated that fatty acid composition of intestinal brush border, basolateral and microsomal membranes can be altered by dietary variation of saturated and polyunsaturated fat.[4–8] Further, it has been suggested that lipid composition and functional activities of the biological membranes can be modified by diets varying in fatty acid composition.[9–11] If it is true that 'membranes do what their lipids tell them', then the lipid components of biological membranes are the main determinants of their physicochemical properties. Indeed, polyunsaturated fatty acids have been reported as playing an important role in regulating the physicochemical properties of the intestinal epithelial membrane.[12] Evidence has suggested that transport of nutrients from the lumen to the interior of the gastrointestinal epithelium is regulated by physicochemical properties of the brush border membrane. Similarly, the exit of absorbed nutrients from the enterocyte to the circulatory system is governed by basolateral membrane properties.[13–15]

Many important cell functions such as transport processes are affected by modulation of the activities of the membrane enzymes through changing of fatty acyl unsaturation. Such unsaturation has been reported to be induced in the membranes of cells when subjected to dietary fat supplementation. A significant proportion of this unsaturated fatty acyl moiety in mammalian cell membranes is required to maintain a 'fluid' state for the proper activity of a number of membrane proteins, such as $(Na^+ + K^+)$-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase. Moreover, Ca^{2+} transference efficiency is also affected by high lipid diets in infants[16] and in rats.[17–19]

Thus, we investigated how saturation and unsaturation characters of the different edible oils influence the various physiological phenomena; namely, intestinal transference of Ca^{2+} and the activities of relevant enzymes, alkaline phosphatase (AP) and Ca^{2+}-ATPase, and the relation of this to

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bone turnover in an ovariectomized rat model of osteoporosis.

Materials and methods

Animals and diets

Female Wistar rats weighing 120–150 g were used for this study. They were housed in an environmentally controlled animal laboratory after being divided into five groups consisting of five rats (randomly selected) in each group: (A) sham-operated control; (B) bilaterally ovariectomized; (C) ovariectomized maintained on a sunflower oil (high polyunsaturated) enriched diet; (D) ovariectomized maintained on a groundnut oil (high monounsaturated) enriched diet; (E) ovariectomized maintained on a coconut oil (high saturated) enriched diet.

Under light ether anaesthesia, bilateral (dorsolateral) ovariectomies were performed in the groups B, C, D and E and animals of group A were subjected to sham-operation. The animals of all groups were provided with a control diet[20] composed of 71% carbohydrates (equal parts of arrowroot starch and sucrose), 18% protein (casein), 7% fat (groundnut oil) and 4% salt mixture,[21] while vitamins were supplied according to Chatterjee *et al*.[22] The animals were maintained on this diet for 1 week after the operation. After 1 week, the animals of group C, D and E were supplied with a high-lipid diet[23] for 15 days, while groups A and B were provided with a normal diet. The composition of the high-lipid diet included 48% carbohydrates (equal parts of arrowroot starch and sucrose) and 30% fat (sunflower oil for group C, groundnut oil for group D and coconut oil for group E). The other ingredients of the diet and the supply of vitamins were the same as described above.[20–22]

The percentage composition of fatty acids[23] of the normal and high-lipid diets supplied to the various groups of animals in the present investigation are shown in Table 1. The animals of all the groups were maintained on a 12 h light/dark schedule with free access to water supply. During the period of diet supplement, group A was pair-fed with experimental groups B, C, D and E so as to overcome the impact of any altered food intake in the experimental groups.

Preparation of intestinal loops

After the experimental period was over, the body weights of all animals were recorded. They were fasted for 16 h and then anaesthetized with urethane (1.7 mg/g body weight). The preparation of intestinal loops for the study of Ca^{2+} transference *in situ* was made following the method of Levine *et al*.[24] The abdomen of each animal was opened through a midline incision and duodenal, jejunal and ileal segments were located. Two ligatures, one proximal and the other distal, were applied tightly on each loop, measuring approximately 8 cm in all of the duodenal, jejunal, and ileal segments. Loops were selected so that each contained 8–10 blood vessels and care was taken that no major blood vessel was occluded by the ligature.

Measurement of intestinal calcium transference

For the measurement of intestinal Ca^{2+} transference, 1 mL of Tris-HCl buffer solution containing 0.2 mmol $CaCl_2$ was injected into the lumen of each loop with a syringe. Tris-HCl buffer solution was prepared following the method of Singh *et al*.[25] The intestinal loops were placed in their usual positions and the abdomen was closed. After1 h, animals were sacrificed, the preselected loops were removed and the fluid from each loop was collected separately. The lumens were also washed several times with distilled water. The collected fluid was then made up to a definite volume with distilled water. A fraction of this fluid was used for the estimation of calcium using the method described by Adeniyi *et al*. using a double-beam spectrophotometer (Shimadzu, 160 A; Shimadzu Corporation, Kyoto, Japan).[26] The difference between the amount of Ca^{2+} introduced and the amount of it left unabsorbed was used as an estimate of the amount of Ca^{2+} absorbed. The intestinal part constituting the loop was dried on a watch glass in an electric oven at 90°C to attain a constant weight, which was recorded as the weight of the dried loop.

Preparation of enzyme extracts

After sacrificing the animal and opening the abdomen, the whole of the small intestine was quickly removed. The portion comprising the duodenum, jejunum and ileum was separated and chilled in ice. Intestinal mucosa was collected as described by Maenz and Cheeseman,[27] and the scrapings were homogenized according to the method of Koyama *et al*.[28] Mucosal scrapings were homogenized with five volumes of Tris-HCl buffer (pH 7.4).

Estimation of enzyme activities

The activity of AP was estimated using the *p*-nitrophenyl phosphate method of Maenz and Cheeseman.[27] The protein content of the homogenate used for the study was determined

Table 1. Percentage composition of fatty acids of the normal and high lipid diets supplied to the various groups of animals in the present investigation

Groups	Percentage composition of fatty acids							
	C4–12	C14	C4–16	C18	C16:1	Oleic acid C18:1	Essential linoleic acid C18:2	Fatty acids and other polyunsaturated acids
Group A*	–	trace	9	3	trace	65	17	trace
Group B*	–	trace	9	3	trace	65	17	trace
Group C*	–	–	–	–	trace	14	73	1
Group D*	–	trace	9	3	trace	65	17	trace
Group E*	63	18	9	2	–	8	1.6	–

Group A, normal diet containing 7% groundnut oil; Group B, normal diet containing 7% groundnut oil; Group C, high lipid diet containing 30% sunflower oil; Group D, high lipid diet containing 30%groundnut oil; Group E, high lipid diet containing 30% coconut oil. *In addition, animals of all groups received an extra 0.1% fatty acid only from arrowroot.

essentially following the method described by Lowry *et al*.[29] The activity of Ca^{2+}-ATPase was studied according to the method of Rorive and Kleinzeller.[30] Phosphate liberated during enzyme activity was estimated by the method of Lowry and Lopez.[31]

Estimation of urinary calcium, phosphate and creatinine

Urine was collected for 24 h (8 a.m. to 8 a.m.) according to the standard laboratory procedure,[32] as described elsewhere by Chanda *et al*.[33] Care was taken so that no urine was lost through evaporation. Total volume was measured. Ca^{2+}, phosphate and creatinine content of urine were estimated according to the methods described, respectively, by Adeniyi *et al*.,[26] Lowry and Lopez[31] and Nath.[34]

Estimation of plasma calcium and alkaline phosphatase

Blood was collected directly from the heart under urethane anaesthesia (1.7 mg/g body weight). Heparin was used as an anticoagulant. Plasma Ca^{2+} was estimated using the method of Adeniyi *et al*.[26] For estimation of plasma AP activity, the method described by Maenz and Cheeseman[27] was essentially followed.

Data

Data were expressed as mean ± SE. Statistical significance was determined using the one way analysis of variance (ANOVA) unless otherwise mentioned for Student's *t*-test. $P < 0.05$ was considered to be significant.

Results

Mucosal calcium transference profiles

The mucosal calcium transference profiles in the different intestinal segments of ovariectomized rats supplemented with different kind of edible oils are shown in Table 2. In order to ascertain whether calcium transference in the sham-operated control and hypogonadal (ovariectomized) states have any significant difference, we analysed the results of Student's *t*-tests of Group A and Group B and found significant ($P < 0.05$) differences. Note further that in the hypogonadal state, when high lipid was supplemented in the diet either with sunflower, groundnut or coconut oil, a greater decrease in mucosal transference of calcium in all segments was observed compared with the hypogonadal (ovariectomized) state alone.

Mucosal alkaline phosphatase activity profiles

The mucosal alkaline phosphatase activity profiles in the different intestinal segments of ovariectomized rats supplemented with different kind of edible oils are shown in Table 3. Similar to calcium transference results, alkaline phosphatase activity also showed significant ($P < 0.05$) differences in the sham-operated control and hypogonadal (ovariectomized) states. High lipid diet supplemented groups were found to be more prone to greater decreases in alkaline phosphatase activity in all segments of the hypogonadal rats.

Mucosal calcium ATPase activity profiles

The mucosal calcium ATPase activity profiles in the different intestinal segments of ovariectomized rats supplemented with different kinds of edible oils are shown in Table 4. Similar to mucosal calcium transference and alkaline phosphatase activity, mucosal calcium ATPase activity was also reduced in all high lipid diet supplemented groups and in all segments of the intestine, compared with the sham-operated control and hypogonadal (ovariectomized) state groups. A significant ($P < 0.05$, Student's *t*-test) difference of calcium

Table 2. Mucosal transference of Ca^{2+} in duodenal, jejunal and ileal segments of sham-control (Group A), ovariectomized (Group B), ovariectomized + sunflower oil fed (Group C), ovariectomized + groundnut oil fed (Group D) and ovariectomized + coconut oil fed (Group E) rats

Segments of small intestine	Group A	Group B	Group C	Group D	Group E	Significant level			Decrease (%)			
						Pa	Pb	Pc	A*vs*B	B*vs*C	B*vs*D	B*vs*E
Duodenum	7.65 ± 0.37	6.64 ± 0.29	6.15 ± 0.05	5.90 ± 0.11	5.77 ± 0.20	<0.001	<0.001	<0.001	13.2	7.4	11.14	13.10
Jejunum	7.27 ± 0.20	6.44 ± 0.15	6.12 ± 0.08	5.85 ± 0.10	5.73 ± 0.19	<0.001	<0.001	<0.001	11.4	5.0	9.16	11.02
Ileum	6.44 ± 0.28	5.52 ± 0.32	4.85 ± 0.15	4.70 ± 0.20	4.64 ± 0.33	<0.001	<0.001	<0.001	14.3	12.1	14.85	15.94

Values are expressed as mean ± SE ($n = 5$). Ca^{2+} is expressed in mmol/g dry weight/h. Pa, Significance based on F value and one-way analysis of variance groups A, B, C, D and E. Pb, Significance based on F value and one-way analysis of variance groups B, C, D and E. Pc, Significance based on F value and one-way analysis of variance groups C, D and E.

Table 3. Alkaline phosphatase activity of intestinal mucosal extracts in duodenal, jejunal and ileal segments of sham-control (Group A), ovariectomized (Group B), ovariectomized + sunflower oil fed (Group C), ovariectomized + groundnut oil fed (Group D) and ovariectomized + coconut oil fed (Group E) rats

Segments of small intestine	Group A	Group B	Group C	Group D	Group E	Significant level			Decrease (%)			
						Pa	Pb	Pc	A*vs*B	B*vs*C	B*vs*D	B*vs*E
Duodenum	237.4 ± 12.9	167.3 ± 10.8	161.0 ± 4.6	158.9 ± 5.3	146.0 ± 2.7	<0.001	<0.001	<0.001	29.5	3.8	5.0	12.8
Jejunum	151.1 ± 7.5	116.4 ± 4.2	107.0 ± 2.4	104.6 ± 1.4	100.0 ± 6.5	<0.001	<0.001	<0.001	23.0	8.1	10.1	14.1
Ileum	85.5 ± 2.7	65.3 ± 2.2	55.0 ± 4.8	52.7 ± 4.5	51.0 ± 6.5	<0.001	<0.001	<0.001	23.6	15.8	19.3	21.9

Values are expressed as mean ± S.E. ($n = 5$). Alkaline phosphatase activity is expressed as *p*-nitrophenol liberated in µmol/g protein /min at 37°C.
Pa, significance based on F value and one-way analysis of variance groups A, B, C, D and E. Pb, Significance based on F value and one-way analysis of variance groups B, C, D and E. Pc, Significance based on F value and one-way analysis of variance groups C, D and E.

ATPase activity was also observed between sham-operated control and hypogonadal state groups.

Plasma calcium and alkaline phosphatase activity profiles

The plasma calcium and alkaline phosphatase activity profiles of ovariectomized rats supplemented with different kind of edible oils are shown in Table 5. Compared with the sham-operated controls, ovariectomized animals showed a significant ($P < 0.05$, Student's *t*-test) increase in alkaline phosphatase activity. Similar increases in plasma AP activity were also noted when ovariectomized animals were supplemented with different kind of edible oils. Plasma calcium level, however, did not show any significant alteration under the conditions of our study.

Urinary calcium and phosphate excretion profiles and calcium to creatinine ratio

The urinary calcium and phosphate excretion profiles and calcium to creatinine ratio of ovariectomized rats supplemented with different kind of edible oils are shown in Table 6. Compared with sham-operated controls, ovariectomized animals showed a significant ($P < 0.05$, Student's *t*-test) increase in all three of the parameters. The increases in all parameters were further pronounced when ovariectomized animals were supplemented with different kind of edible oils, of which coconut oil was found to be most effective in producing these changes.

Discussion

This study demonstrates that maintenance of bilaterally ovariectomized rats for 2 weeks on a diet enriched with either polyunsaturated (sunflower), monounsaturated (groundnut) or saturated (coconut) oil produced a variable degree of reduction in the intestinal transference of Ca^{2+} as compared with bilaterally ovariectomized rats fed a normal diet only (Table 2). These results suggest that, under the conditions of our study, the addition of high amounts of lipids in the diet possibly had a negative influence on the intestinal transference of Ca^{2+}, which is quite consistent with earlier reports that Ca^{2+} transference efficiency is affected by high-lipid diet in rats[17–19] and in infants.[16]

Table 4. Calcium ATPase activity of intestinal mucosal extracts in duodenal, jejunal and ileal segments of sham-control (Group A), ovariectomized (Group B), ovariectomized + sunflower oil fed (Group C), ovariectomized + groundnut oil fed (Group D) and ovariectomized + coconut oil fed (Group E) rats

Segments of small intestine	Group A	Group B	Group C	Group D	Group E	Significant level			Decrease (%)			
						Pa	Pb	Pc	A*vs*B	B*vs*C	B*vs*D	B*vs*E
Duodenum	16.3 ± 0.66	13.8 ± 0.32	12.9 ± 0.32	11.2 ± 0.63	9.5 ± 0.75	<0.001	<0.001	<0.001	15.3	6.52	18.84	31.16
Jejunum	11.4 ± 0.74	8.8 ± 0.25	8.3 ± 0.09	8.0 ± 0.27	7.1 ± 0.33	<0.001	<0.001	<0.001	22.8	5.68	9.09	19.32
Ileum	7.7 ± 0.33	6.1 ± 0.33	5.5 ± 0.08	5.3 ± 0.16	4.7 ± 0.09	<0.001	<0.001	<0.001	20.8	9.84	13.11	22.95

Values are expressed as mean ± SE ($n = 5$). Calcium ATPase activity is expressed as phosphate liberated in mmol/g protein/min at 37°C. Pa, Significance based on F value and one-way analysis of variance groups A, B, C, D and E. Pb, Significance based on F value and one-way analysis of variance groups B, C, D and E. Pc, Significance based on F value and one-way analysis of variance groups C, D and E.

Table 5. Plasma biochemistry of sham-control (Group A), ovariectomized (Group B), ovariectomized + sunflower oil fed (Group C), ovariectomized + groundnut oil fed (Group D) and ovariectomized + coconut oil fed (Group E) rats

Parameters	Group A	Group B	Group C	Group D	Group E	Significant level			Decrease (%)			
						Pa	Pb	Pc	A*vs*B	B*vs*C	B*vs*D	B*vs*E
Ca^{2+} (mg/dL)	7.12 ± 0.11	7.20 ± 0.35	7.34 ± 0.10	7.38 ± 0.18	7.58 ± 0.10	NS	NS	NS	1.12	2.0	2.50	5.30
A P (U/L)	109.0 ± 5.12	136.0 ± 5.66	156.0 ± 4.55	162.0 ± 4.53	169.0 ± 6.54	<0.001	<0.001	<0.001	24.8	14.7	19.1	24.3

Values are expressed as mean ± SE ($n = 5$). NS denotes not significant. Pa, Significance based on F value and one-way analysis of variance groups A, B, C, D and E. Pb, Significance based on F value and one-way analysis of variance groups B, C, D and E. Pc, Significance based on F value and one-way analysis of variance groups C, D and E. AP, alkaline phosphatase.

Table 6. Urine biochemistry of sham-control (Group A), ovariectomized (Group B), ovariectomized + sunflower oil fed (Group C), ovariectomized + groundnut oil fed (Group D) and ovariectomized + coconut oil fed (Group E) rats

Parameters	Group A	Group B	Group C	Group D	Group E	Significant level			Decrease (%)			
						Pa	Pb	Pc	A*vs*B	B*vs*C	B*vs*D	B*vs*E
Phosphate (mg)	35.6 ± 2.4	45.5 ± 2.2	50.5 ± 1.2	56.9 ± 2.7	59.5 ± 2.9	<0.001	<0.001	<0.001	27.80	10.99	24.90	30.80
Ca^{2+} (mg)	3.27 ± 0.08	9.61 ± 0.55	10.8 ± 0.14	11.2 ± 0.28	11.92 ± 0.20	<0.001	<0.001	<0.001	194.00	11.97	17.00	24.04
Ca:Cr ratio (mg:mg)	1.70 ± 0.13	2.43 ± 0.07	2.68 ± 0.10	2.74 ± 0.07	2.82 ± 0.07	<0.001	<0.001	<0.001	42.90	10.30	12.80	16.00

Values are expressed as mean ± SE ($n = 5$). Urinary calcium (Ca) and creatinine (Cr) excretion is expressed in mg/24 h urine. Urinary phosphate excretion is expressed in mg/dL. Pa, Significance based on F value and one-way analysis of variance groups A, B, C, D and E. Pb, Significance based on F value and one-way analysis of variance groups B, C, D and E. Pc, Significance based on F value and one-way analysis of variance groups C, D and E.

Our data further indicate that such inhibition of Ca^{2+} transference by addition of high amounts of lipids in the diet possibly depends upon the degree of unsaturation and saturation characteristics of the lipids (Table 2). Saturated fat (coconut oil) produced a more pronounced ($P < 0.001$) decrease in Ca^{2+} transference compared with mono-unsaturated (groundnut oil) or polyunsaturated fat (sunflower oil). It has been reported that dietary fat affects lipid and fatty acid composition of biological membranes, including the microvillus membrane.[5,7,8] It has also been reported that many important cell functions such as transport processes are affected by modulation of the activities of the membrane enzymes, namely Na^+-K^+-ATPase, Ca^{2+}-ATPase and Mg^{2+}-ATPase, through changing fatty acyl unsaturation.[9] An increase in such unsaturation has often been considered necessary for increased membrane fluidity.[7] It is therefore plausible that in our study the addition of high amounts of lipids in the diet with different degrees of saturation and unsaturation characters had an influence on membrane unsaturation, thus affecting membrane protein activity and resulting in the inhibition of Ca^{2+} transference.

Ca^{2+} transference in our study showed segmental variation and was seen to have a descending gradient from the duodenum to ileum irrespective of groups (Table 2). This corroborates well with the earlier observation of Wills.[35] Our results further suggest that ovarian hormones possibly have an influence on the intestinal transference of Ca^{2+} given that bilaterally ovariectomized rats, compared with sham-operated control, showed a significant decrease in the transference of Ca^{2+}.

Our studies with AP and Ca^{2+}-ATPase suggest that, irrespective of groups, both these enzymes showed segmental variation in their activities in the rat small intestine (Tables 3 and 4). This confirms the earlier observation of Toofanian and Teshfam.[36] Results further revealed that, compared with controls, activities of both of these enzymes were significantly inhibited in bilaterally ovariectomized rats as well as in rats of different groups in which high amount of lipids were added in the diet. These results suggest that the possible cause of reduction of Ca^{2+} transference in our study might be an inhibition of activities of these enzymes as both the enzymes have been reported to be involved in Ca^{2+} transference. Our results also indicate that AP and Ca^{2+}-ATPase may make a significant contribution to the regulation of intestinal transference of Ca^{2+}. Our results thus confirm the earlier proposal of Wasserman and Fullmer that AP and Ca^{2+}-ATPase make a significant contribution to the regulation of intestinal transference of Ca^{2+}.[37]

Compared with sham-operated controls, bilaterally ovariectomized rats showed an increased loss of urinary Ca and phosphate. This loss was further enhanced when these animals were fed with diets enriched with high amounts of lipids (Table 6). Compared with sham-operated controls, bilaterally ovariectomized rats did not have an altered plasma Ca level. This observation is in agreement with an earlier report by Lindsay *et al*.[38] The plasma Ca level also was not changed when ovariectomized animals were fed with diets containing different edible oils (Table 5).

Thus, with respect to Ca homeostasis our results suggest that the experimental conditions of the present study may be highly conducive for the development of a hypocalcemic condition. This suggestion has its own merit as an increased urinary loss of Ca, as occurred in our study, has always been associated with a simultaneous decrease in intestinal transference of Ca^{2+}. These factors are two of the most important for the development of hypocalcemia and secondary increase in parathormone secretion.[39,40]

Biochemical markers of bone turnover, namely plasma AP activity and urinary Ca to creatinine ratio, were found to be enhanced when ovariectomized animals were fed with high amounts of lipids in their diets. A rise in serum AP and the urinary Ca to creatinine ratio has been linked with collagen degradation, bone resorption and osteoporosis.[38,41–43] Thus, the positive influence of high lipid supplementation on bone turnover and bone loss, under the conditions of the present study, is apparent.

The observations made in the present investigation may have far reaching implications should bone loss and development of osteoporosis in a hypogonadal situation be assessed in light of dietary habits, such as the preference of using one kind of edible oil over the others. This is because many dietary components have an important biochemical and physiological impact for a healthy life, particularly in women of menopausal age.

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