

## Trace lipid from whey-mineral complex enhances calcium availability in young ovariectomized rats

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(Received 1 March 1994 – Revised 7 June 1994 – Accepted 24 June 1994)

The effects of the trace lipids (L1 and L2) extracted from two kinds of whey-mineral complex on bone metabolism were studied in young ovariectomized (OVX) rats. The concentrations of oestradiol in the L1 and L2 oils were 305 and 1313 pg/ml respectively, while the concentrations of progesterone were 34.3 and 36.9 ng/ml respectively. Trace amounts of  $1\alpha,25$ -dihydroxycholecalciferol ( $1\alpha,25$ - $(OH)_2D$ ) were detected: 56 pg/ml in the L1 oil and 132 pg/ml in the L2 oil. The OVX rats were orally given 0.4 ml each test oil twice weekly. Other animals were given with only a vehicle (control) or an oil mixture with similar levels of oestradiol and progesterone to those in L2 oil (PE oil) for comparison. The Ca absorption rate, bone density of the humerus and femoral Ca content in the rats given L2 were significantly higher than those in the control rats ( $P < 0.05$ ). Serum calcitonin and  $1\alpha,25$ - $(OH)_2D$  from rats given L2 were both significantly higher than those from the control rats, while serum progesterone in the L2 group was slightly lower. It is suggested that the bone metabolism of rats given L2 reflected the compound effect of  $1,25$ - $(OH)_2D$  and oestradiol in the L2 oil. In contrast, the relatively low level of these hormones might have been associated with the mediocre bone characteristics of the L1 group. It is suggested from these results that the trace level of hormones could be one of the reasons why whey-mineral complex showed good Ca availability.

**Bone: Calcium: Hormones: Rat: Whey-mineral complex**

Whey-mineral complex (WMC) prepared from acidic milk whey has been reported as a mineral supplement due to its good mineral availability (Igarashi *et al.* 1990; Tsuchita *et al.* 1992). We have previously implicated the influence of components other than the minerals in WMC on bone metabolism by comparing two WMC with different compositions (Tsuchita *et al.* 1993). Preliminary analyses indicated that the lipid extracted from WMC contained trace amounts of progesterone and oestradiol. Although sex steroid and corticosteroid hormones are related to bone metabolism (Nordin *et al.* 1981; Roe *et al.* 1988), the hormones naturally present in milk have practically no effect when taken orally. For example, the level of oestrogen in milk and milk products is too low for biological activity (Hoffmann, 1977). However, the composition of the lipid extracted from WMC was different from that of milk fat, showing less triacylglycerol, but larger amounts of cholesterol or compound lipids, so that the overall effect of the lipid on bone metabolism was not clear. The present study was conducted to determine whether the trace lipid from WMC would influence bone metabolism in young ovariectomized rats and to evaluate the contribution of a very low dose of oestradiol and progesterone.

Table 1. *Chemical and hormone composition of two whey-mineral complexes (WMC)*

	WMC1	WMC2
Major components (g/kg)		
Moisture*	59	90
Protein†	23.0	104.6
Fat‡	2	2
Lactose§	260.0	3.3
Calcium	182	264
Phosphorus¶	84	146
Magnesium	3.3	4.5
Hormones**		
Oestradiol (pg/g)	5.3	33.6
Progesterone (ng/g)	0.88	0.94
1 $\alpha$ ,25-dihydroxycholecalciferol (pg/g)	1.0	3.4

\* Weight loss after heating at 98° for 5 h.

† Kjeldahl N  $\times$  6.38.

‡ Röse-Gottlieb method.

§ Enzymic method using a measuring kit (Boehringer Mannheim Yamanouchi Co., Japan).

|| Atomic absorption spectrophotometry.

¶ Spectrophotometric method (Allen, 1940).

\*\* Hormones were determined in the lipid extracted from the WMC by the method described below. Assays were carried out using diagnostic measuring kits: oestradiol, progesterone, Diagnostic Products Co., CA, USA; 1 $\alpha$ ,25-dihydroxycholecalciferol, Amersham Japan Co., Tokyo, Japan.

## MATERIALS AND METHODS

### *Preparation of lipids*

Two types of WMC (WMC1 and WMC2) prepared by separate methods were used as lipid sources in the extractions (Tsuchita *et al.* 1993). Their chemical compositions and hormone contents are shown in Table 1. Lipid was extracted from the material with diethyl ether for 6 h using Soxhlet apparatus. The treated material was dried and then mixed with a fivefold volume of a chloroform-methanol (2:1, v/v) mixture in a Waring blender. The chloroform-methanol extraction was conducted three times. After dehydrating by adding anhydrous Na<sub>2</sub>SO<sub>4</sub>, the solvents were removed from the diethyl ether extract and the chloroform-methanol extract by vacuum rotary evaporation. The diethyl ether extract and chloroform-methanol extract were combined and mixed (L1 from WMC1 and L2 from WMC2). Using thin-layer chromatography, the lipid extracted from these WMC was shown to contain larger amounts of cholesterol, its esters and phospholipid, and less triacylglycerol compared with the lipid composition of butter oil (results not shown). To administer the lipids to eight rats, L1 and L2 were newly obtained from 1520 g WMC1 and 1040 g WMC2. Assuming that eight rats each consume 20 g diet daily for 28 d, 1520 g WMC1 and 1040 g WMC2 represent ten times the amounts consumed as a 6 g/kg Ca diet when WMC1 or WMC2 is added as the sole source of Ca. The recovery of L1 was 1.86 g, and of L2, 1.29 g. L1 or L2 was dissolved in 0.2 ml ethanol and then mixed with 25.0 ml soyabean oil as a vehicle (L1 oil or L2 oil). The concentrations of oestradiol in the L1 and L2 oils were 305 and 1313 pg/ml respectively, while the concentrations of progesterone were 34.3 and 36.9 ng/ml respectively. A trace amount of 1 $\alpha$ ,25-dihydroxycholecalciferol (1 $\alpha$ ,25-(OH)<sub>2</sub>D) was detected: 56 pg/ml in the L1 oil and 132 pg/ml in the L2 oil. An oil mixture including standard progesterone and 17 $\beta$ -oestradiol (Sigma Chemical Co., MO, USA) was prepared for comparison (PE oil: progesterone, 40.0 ng/ml; 17 $\beta$ -oestradiol, 1427 pg/ml).

### *Animals and diets*

Forty 3-week-old Sprague–Dawley female rats (Japan SLC, Shizuoka, Japan) were housed in individual aluminium cages in a temperature-controlled ( $23 \pm 2^\circ$ ) room with  $50 \pm 5\%$  humidity and a 12 h light–dark cycle, and fed on a stock diet (CE-7, Clea Japan, Tokyo, Japan). On the fifth day of feeding, thirty-two rats were ovariectomized (OVX) under diethyl ester anaesthesia. The remaining animals were sham-operated (SHAM). At 1 week after the operation the rats were fed on a low-Ca diet (Ca, 0.1 g/kg diet; P, 3 g/kg diet). After 30 d feeding the OVX rats were separated into four groups of eight animals each according to a similar mean body weight of 208 g. Four groups of OVX rats and one group of SHAM rats were then fed *ad lib.* on a Ca-supplemented diet (Ca, 3 g/kg diet; P, 3 g/kg diet) and deionized water for 28 d. The low-Ca diet and the Ca-supplemented diet were prepared according to the AIN-76A purified diet composition with slight modification for minerals (American Institute of Nutrition, 1977, 1980). During the feeding period, three groups of OVX rats were orally given 0.4 ml of one of the test oils (L1 oil, L2 oil, or PE oil) twice weekly. One group of OVX rats and the SHAM rats were given only the vehicle. Urine samples were collected during days 2–4, 9–11, 16–18, and 23–25 of the experimental period to determine hydroxyproline and cyclic AMP concentrations. Faecal samples were also collected during days 23–25 for a mineral balance study. Body weight and feed intake were recorded three times weekly. After 28 d the animals were fasted overnight, anaesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg body weight), and blood samples were taken from the aorta ventralis to determine biochemical indices. Both femurs, humeri, and ulnae were excised, and the surrounding flesh was removed.

### *Analytical methods*

The bone densities for the humerus and ulna were obtained by microphotodensitometry of roentgenographic images as previously reported (Tsuchita *et al.* 1993). The femurs and the faecal samples were dried, weighed, and ashed overnight in a muffle furnace at  $600^\circ$ . The ashed samples were then dissolved in 1 M-HNO<sub>3</sub> for Ca analysis by atomic absorption spectrophotometry (Shimadzu AA-640-13, Shimadzu Corporation, Kyoto, Japan). P was determined by a spectrophotometric method (Allen, 1940), and urinary Ca content was determined directly as already described. Plasma Ca, inorganic P (Pi) and alkaline phosphatase (EC 3.1.3.1) activity were determined using an autoanalyzer (Hitachi 736, Hitachi Ltd., Tokyo, Japan), and urinary hydroxyproline was estimated colorimetrically (Bergman & Loxley, 1970). Cyclic AMP was measured by a competitive binding assay, using a cyclic AMP<sup>[3H]</sup> assay system (Amersham Japan Co., Tokyo, Japan). Serum and urinary creatinine was determined colorimetrically using a measuring kit (Wako Pure Chemical Ind., Osaka, Japan). Oestradiol and progesterone in the serum and extracted lipids were measured by a radioimmunoassay, using diagnostic assay kits (Diagnostic Products Corporation, CA, USA). Serum immunoreactive rat calcitonin was evaluated by a diagnostic measuring kit (Mitsubishi Petrochemical Co., Tokyo, Japan), based on human calcitonin, which recognizes human calcitonin 1-32 and has previously been shown to detect rat calcitonin (Roos *et al.* 1978).  $1\alpha,25\text{-(OH)}_2\text{D}$  was measured by a competitive radio-binding assay, following a non-HPLC sample purification procedure, using a commercial assay system (Amersham Japan Co.).

### *Statistical analysis*

All data were analysed by a one-way analysis of variance (ANOVA). When significant *F* ratios were found, individual means were compared by the Bonferroni test ( $P < 0.05$ ).

**Table 2. Mineral balance and percentage tubular reabsorption of phosphorus (% TRP) in ovariectomized (OVX) and sham-operated (SHAM) rats receiving lipid extracts from whey-mineral complexes, or soya-bean oil (control) twice weekly by oral administration\***  
(Mean values with their standard errors for eight rats per dietary group)

Treatment ...	OVX													
	L1			L2			PE			Control			SHAM Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<b>Ca</b>														
Absorption (mg/d)	26.80 <sup>(a)</sup>	2.44	36.07 <sup>(b)</sup>	2.53	30.57 <sup>(ab)</sup>	1.20	27.85 <sup>(a)</sup>	1.35	30.29	1.35	30.29	1.35	30.29	3.06
Absorption rate (%)	59.63 <sup>(a)</sup>	3.00	77.00 <sup>(b)</sup>	0.96	63.85 <sup>(a)</sup>	1.45	61.30 <sup>(a)</sup>	1.45	65.04 <sup>a</sup>	1.45	65.04 <sup>a</sup>	1.45	65.04 <sup>a</sup>	3.80
Urinary excretion (mg/mg creatinine)	3.45 <sup>a</sup>	0.23	2.54 <sup>a</sup>	0.31	2.67 <sup>a</sup>	0.77	1.93 <sup>a</sup>	0.33	6.42 <sup>b</sup>	0.33	6.42 <sup>b</sup>	0.33	6.42 <sup>b</sup>	0.83
<b>P</b>														
Absorption (mg/d)	53.76	3.95	54.49	3.89	55.94	2.64	52.47	2.02	53.41	2.02	53.41	2.02	53.41	4.06
Absorption rate (%)	88.90	1.91	90.37	0.76	88.39	0.96	89.32	0.96	87.41	0.96	87.41	0.96	87.41	1.66
Urinary excretion (mg/mg creatinine)	244.2	7.0	190.5	8.9	227.8	19.7	197.1	17.3	231.3	17.3	231.3	17.3	231.3	12.4
% TRP <sup>†</sup>	54.42 <sup>(a)</sup>	2.06	68.17 <sup>(b)</sup>	2.97	64.13 <sup>(ab)</sup>	3.41	67.20 <sup>(ab)</sup>	3.43	65.46 <sup>b</sup>	3.43	65.46 <sup>b</sup>	3.43	65.46 <sup>b</sup>	2.80

L1, lipid extract from whey-mineral complex 1; L2, lipid extract from whey-mineral complex 2; PE, oil mixture containing progesterone and oestradiol.

<sup>a</sup> Mean values within a row with unlike superscript letters were significantly different,  $P < 0.05$  (Bonferroni test). Superscript letters in parentheses show the comparison within the groups of OVX rats.

\* For details of treatments and procedures, see pp. 300-301.

†  $(1 - (\text{urinary P} \times \text{serum creatinine}) / (\text{serum P} \times \text{urinary creatinine})) \times 100$ .

**Table 3. Body weight, femur mineral content, and bone density of the humerus and ulna in ovariectomized (OVX) and sham-operated (SHAM) rats receiving lipid extracts from whey-mineral complexes, or soya-bean oil (control) twice weekly by oral administration\***  
(Mean values with their standard errors for eight rats per dietary group)

Treatment ...	OVX													
	L1			L2			PE			Control			SHAM Control	
	Mean	SE		Mean	SE		Mean	SE		Mean	SE		Mean	SE
Body weight (g)	290.6*	3.7		289.9*	4.2		292.3*	4.9		288.9*	6.5		233.8 <sup>b</sup>	5.4
Femur														
Dry wt (mg)	420.4 <sup>ab</sup>	3.8		448.3*	6.8		445.0*	8.2		435.5 <sup>ab</sup>	9.1		399.6 <sup>b</sup>	12.4
Calcium (mg)	76.92 <sup>abc(ab)</sup>	2.06		79.53 <sup>a(a)</sup>	1.00		77.63 <sup>ab(a)</sup>	1.22		71.63 <sup>bc(b)</sup>	1.42		69.57 <sup>c</sup>	2.95
Phosphorus (mg)	30.63	0.92		32.72	0.79		33.58	0.64		32.85	0.74		33.28	1.89
Magnesium (mg)	15.49	0.41		15.91	0.39		16.19	0.38		16.04	0.29		15.79	0.68
Calcium:phosphorus	2.51 <sup>(a)</sup>	0.02		2.42 <sup>ab(ab)</sup>	0.05		2.31 <sup>abc(ab)</sup>	0.01		2.16 <sup>bc(c)</sup>	0.08		2.11 <sup>c</sup>	0.06
Bone density														
Humerus	55.86 <sup>(a)</sup>	1.97		66.00 <sup>b(b)</sup>	2.22		60.25 <sup>ab(ab)</sup>	2.10		57.29 <sup>a(a)</sup>	1.41		56.25 <sup>a</sup>	1.75
Ulna	51.38 <sup>(a)</sup>	0.75		57.38 <sup>b(b)</sup>	1.18		54.88 <sup>ab(ab)</sup>	0.99		53.25 <sup>ab(a)</sup>	0.94		51.75 <sup>a</sup>	1.82

L1, lipid extract from whey-mineral complex 1; L2, lipid extract from whey-mineral complex 2; PE, oil mixture containing progesterone and oestradiol.

<sup>a, b, c</sup> Mean values within a row with unlike superscript letters were significantly different,  $P < 0.05$  (Bonferroni test). Superscript letters in parentheses show the comparison within the groups of OVX rats.

\* For details of treatments and procedures, see pp. 300-301.

Table 4. Serum calcium, inorganic phosphorus (Pi), alkaline phosphatase (EC 3.1.3.1) and hormone concentrations in ovariectomized (OVX) and sham-operated (SHAM) rats receiving lipid extracts from whey-mineral complexes, or soya-bean oil (control) twice weekly by oral administration\*

(Mean values with their standard errors for eight rats per dietary group)

Treatment ...	OVX														
	L1			L2			PE			Control			SHAM Control		
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	
Calcium (mg/l)	54.1	0.9	53.5	1.3	56.0	0.8	54.0	1.2	52.5	1.7	52.5	1.7	52.5	1.7	
Pi (mg/l)	48.0	2.2	54.9	4.2	55.5	2.8	54.2	1.7	56.3	1.9	56.3	1.9	56.3	1.9	
Alkaline phosphatase (IU/l)	138.6	12.3	117.0	4.3	129.1	6.1	121.4	11.9	109.3	10.7	109.3	10.7	109.3	10.7	
Progesterone (ng/ml)	15.68 <sup>ab</sup>	2.30	12.04 <sup>a</sup>	2.65	15.90 <sup>ab</sup>	2.27	14.98 <sup>ab</sup>	1.90	26.86 <sup>b</sup>	5.07	26.86 <sup>b</sup>	5.07	26.86 <sup>b</sup>	5.07	
Calcitonin (pg/ml)	108.9 <sup>a(a)</sup>	10.7	177.1 <sup>b(b)</sup>	5.1	166.5 <sup>b(b)</sup>	11.8	126.9 <sup>c(a)</sup>	7.9	134.4 <sup>abc</sup>	17.7	134.4 <sup>abc</sup>	17.7	134.4 <sup>abc</sup>	17.7	
1 $\alpha$ ,25-(OH) <sub>2</sub> D (pg/ml)	29.34 <sup>ab(a)</sup>	2.65	44.08 <sup>a(a)</sup>	3.93	32.47 <sup>b(ab)</sup>	2.66	26.13 <sup>b(b)</sup>	3.45	37.73 <sup>ab</sup>	3.51	37.73 <sup>ab</sup>	3.51	37.73 <sup>ab</sup>	3.51	

L1, lipid extract from whey-mineral complex 1; L2, lipid extract from whey-mineral complex 2; PE, oil mixture containing progesterone and oestradiol; 1 $\alpha$ -25-(OH)<sub>2</sub>D, 1 $\alpha$ ,25-dihydroxycholecalciferol.

<sup>a,b,c</sup> Mean values within a row with unlike superscript letters were significantly different, *P* < 0.05 (Bonferroni test). Superscript letters in parentheses show the comparison within the groups of OVX rats.

\* For details of treatments and procedures, see pp. 300-301.

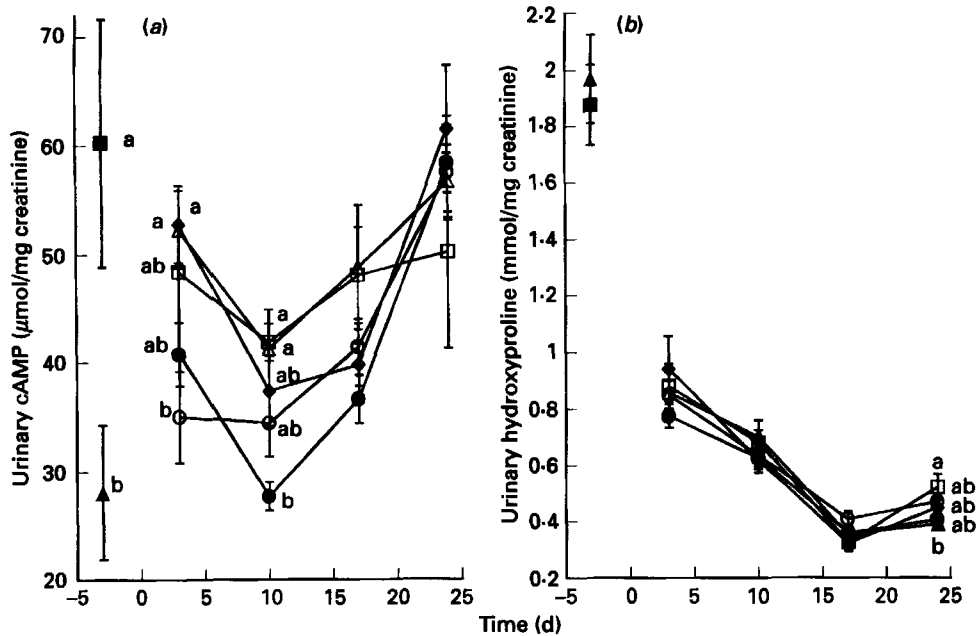


Fig. 1. (a) Urinary cyclic AMP excretion and (b) urinary hydroxyproline excretion by ovariectomized (OVX) rats receiving lipid extracts from whey-mineral complexes (L1,  $\circ$  and L2,  $\bullet$ ), an oil mixture containing progesterone and oestradiol (PE oil,  $\blacklozenge$ ) or soya-bean oil (control,  $\triangle$ ) twice weekly by oral administration. Sham-operated (SHAM) rats also received soya-bean oil ( $\square$ ). Values for the OVX rats ( $\blacktriangle$ ) and SHAM rats ( $\blacksquare$ ) were obtained at the end of a period on a low-Ca diet. All animals were fed on a Ca-supplemented diet from days 0 to 28. Values are means with their standard errors represented by vertical bars. Mean values for the same day not sharing a common superscript letter were significantly different,  $P < 0.05$  (Bonferroni test).

## RESULTS

### Mineral balance

The Ca absorption rate in rats given the L2 oil was significantly higher than in all the other groups (Table 2). The Ca absorption in rats given L2 oil was also high among the groups of OVX rats. The Ca absorption and the absorption rate of the L1 group were similar to those of the control (OVX) group. The highest urinary Ca excretion was observed in the SHAM group, while there was no significant difference in the values among the groups of OVX rats. There was no significant difference in quantitative values for P balance among the groups (Table 2). The percentage tubular reabsorption of P (% TRP) for the L1 group was significantly lower than that for the L2 and control groups.

### Bone mineral analysis and bone density

The final body weight of the SHAM group was significantly lower than that of the groups of OVX rats, as previously reported (Clark & Tartelin, 1982), but no significant difference was observed among the groups of OVX rats (Table 3). There was also no significant difference in the dry weight of the femur among the groups of OVX rats (Table 3). The Ca content in the femur of the L2 group was significantly higher than that of the control and SHAM groups, the value for the PE group also being high when compared with the other groups of OVX rats. All the groups had similar P and Mg contents in the femur, although the L1 group had a higher femoral Ca:P ratio than did the control and SHAM groups (Table 3). The rats given the L2 oil showed significantly higher bone density for the

humerus than all other groups except the PE group (Table 3). Likewise, the bone density of the ulna in the L2 group was higher than that of the control and L1 groups.

#### *Biochemical indices in serum and urine*

There were no significant differences in serum Ca, Pi and alkaline phosphatase activity among the five groups (Table 4). Serum progesterone in the rats given the L2 oil was significantly lower than that in the SHAM rats given the vehicle, although the difference was not significant when compared with the other groups of OVX rats. Serum oestradiol was only detected in a few samples ( $> 10$  pg/ml), and these values are not shown. Serum calcitonin in the L2 group was significantly higher than that in the L1 and control groups (Table 4). The value for the PE group was also higher than that for the L1 group when compared with the groups of OVX rats. Serum  $1\alpha,25\text{-(OH)}_2\text{D}$  in the rats given the L2 oil was significantly higher than that in the rats given the control oil, although no significant difference was observed among the other groups (Table 4). The animals given the L1 and L2 oils tended to give a lower urinary cyclic AMP excretion; the value for the L1 group was significantly lower during days 2–4, and that for the L2 group was lower than that for the control group during days 9–11 (Fig. 1). The change in urinary hydroxyproline excretion was similar among the groups of OVX rats throughout the experimental period, although the values were much higher at the end of the period of low-Ca-diet feeding (Fig. 1).

## DISCUSSION

### *Calcium and phosphorus balance*

The present study shows the marked effect of the lipid extracted from WMC, especially of L2, on Ca absorption and bone mineral deposition. In contrast with this, no effect on Ca absorption was reportedly produced by supplementing with WMC2 in young male rats, although the bone density was high (Tsuchita *et al.* 1993). This discrepancy might have arisen from the different dose of the lipid; the total amount of L2 administered to a rat was more than ten times as much as the lipid from WMC2 in the diet. The effect of oestrogen administration on intestinal Ca absorption has been reported (Russell *et al.* 1986), and an increase in the serum level of  $1\alpha,25\text{-(OH)}_2\text{D}$  has been shown in normal rats (Baksi & Kenny, 1978) and OVX rats (Ash & Goldin, 1988). The serum  $1\alpha,25\text{-(OH)}_2\text{D}$  level in the rats given L2 was higher than that in the rats given standard  $17\beta$ -oestradiol and progesterone, whose concentrations were similar to those in L2. Therefore,  $1\alpha,25\text{-(OH)}_2\text{D}$  rather than oestradiol contained in L2 would have contributed to the high level of serum  $1\alpha,25\text{-(OH)}_2\text{D}$  that caused the high Ca absorption in those rats given L2 (Armbrecht *et al.* 1980). It is well known that parathyroid hormone (PTH) is one of the most important factors for stimulating  $1\alpha$ -hydroxylase (*EC* 1.14.13.13) activity, although the relatively low urinary cyclic AMP excretion from the rats given L1 and L2 does not support an increase in PTH.

The level of oestradiol given to the L2 group was 405 pg/rat per d or 3240 pg/rat in total during the experimental period, which is lower than the levels reported in the literature:  $5.0 \mu\text{g}/100$  g body weight (Russell *et al.* 1986); 1.0, 2.5, and  $5.0 \mu\text{g}/100$  g body weight (Wronski *et al.* 1988*b*). Oestrogen administration caused an increase in serum calcitonin in humans (Austin & Heath, 1981), and in rats (First *et al.* 1981). While the administration of oestradiol to the L2 and PE groups could have been associated with a higher level of serum calcitonin, the rats given L1 gave the lowest value among all the groups. This could partly have been due to the relatively low level of oestradiol in L1. However, whether an effect of progesterone on oestradiol or of another unknown factor in L1 was involved remains unclear.



The P retention in rats fed with WMC tended to be higher than in rats fed with  $\text{CaCO}_3$  (Tsuchita *et al.* 1993), but no significant difference in P balance was observed among the experimental groups. However, the P metabolism of those rats given L1 seems to have been affected; the % TRP and serum Pi in the L1 group were slightly lower than the levels in the other groups, although the differences were not significant.

#### *Bone mineralization*

Histological studies have shown that the initial rapid phase of bone loss in OVX rats is associated with an increased bone turnover (Wronski *et al.* 1986, 1988*a*). Oestrogen treatment prevents osteopenia and depresses the bone turnover in OVX rats (Wronski *et al.* 1988*b*) via the function of PTH or calcitonin (Gennari *et al.* 1987). In the present study, not only ovariectomy but also a low-Ca diet accelerated the bone loss in OVX rats (Kalu *et al.* 1989) as shown by the urinary hydroxyproline excretion (Fig. 1). However, any inhibitory effect of oestradiol in the test oils on bone turnover is obscure, no significant difference in urinary hydroxyproline being apparent among the OVX rats after feeding with a Ca-supplemented diet. On the other hand, the high serum calcitonin and low urinary cyclic AMP excretion in rats given L2 suggest the effect of oestrogen on bone metabolism. It has been shown that exogenous  $1,25\text{-(OH)}_2\text{D}$  increases bone mass in OVX rats as well as in intact rats (Lindgren & DeLuca, 1982). Therefore, the high bone density of the humerus and high femoral Ca content in those rats given L2 could reflect the effect of oestradiol and  $1,25\text{-(OH)}_2\text{D}$  on the bone, in addition to the high Ca absorption. The Ca content and Ca:P ratio of the femur in the L1 group were similar to the figures for the L2 and PE groups, while the bone density of the humerus was similar to that of the control group. The different bone characteristics between the L1 and L2 groups might have been caused by the levels of oestradiol and  $1,25\text{-(OH)}_2\text{D}$  in the test oils. The administration of progesterone prevents bone loss in OVX rats (Lindsay *et al.* 1978; Barbagallo *et al.* 1989; Barendolts *et al.* 1991), but the combination of oestrogen and progesterone has no effect on bone minerals (Lindsay *et al.* 1978; Barbagallo *et al.* 1989). Another report suggests that progesterone antagonizes the oestrogen-related inhibition of bone formation (Turner *et al.* 1989). Therefore, although the concentrations of progesterone in L1 and L2 were very small when compared with the dose used in these reported studies, the smaller oestradiol:progesterone ratio in L1 than in L2 might have affected bone formation.

#### *Hormones in milk and milk products*

An advance in measuring hormones using a radioimmunoassay technique enables comparison among the values reported for milk and milk products (Koldovsky & Thornburg, 1987). Processing milk causes a large part of the steroid hormones to move with the fat; progesterone and oestrogens are contained in butter to a much higher level than in milk or whey (Hoffmann, 1977; Wolford & Argoudelis, 1979). The level of naturally occurring hormones in dairy products reported in the literature is so small that no biological significance has been attributed to them (Hoffmann, 1977). In fact, there has been no study to show any effect of the hormones in milk on bone in animals or humans, probably because of their low level or of the obvious effect of Ca in milk. However, the present study shows the effect of oestradiol and progesterone in PE oil as well as in L2 oil on bone metabolism in OVX rats. The concentrations of oestradiol in the L1 and L2 oils (305 and 1313 pg/ml) were higher than that in butter (82.3 pg/g; Wolford & Argoudelis, 1979), whereas the concentrations of progesterone in the L1 and L2 oils (34.3 and 36.9 ng/ml) were lower than that in butter (132.9 ng/g, Ginther *et al.* 1976). The concentrations of  $1,25\text{-(OH)}_2\text{D}$  in the L1 and L2 oils (56 and 132 pg/ml) were comparable with that in milk (29 pg/ml; Takeuchi *et al.* 1988). Therefore, although the level of

hormones was very low, it is suggested that the bone metabolism in rats given L2 reflects the compound effect of 1,25-(OH)<sub>2</sub>D and oestradiol in L2. Other steroid hormones such as oestrone, oestriol, androgens and corticosteroids have been detected in milk and milk products (Hoffmann, 1977; Pope & Swinburne, 1980), and these have some effects on bone metabolism (Schot & Schuurs, 1990). The results of a preliminary analysis by a radioimmunoassay showed trace amounts of androstenedione and dehydroepiandrosterone, but no oestrone in the lipid extracted from WMC (results not shown). Further work to analyse these hormones or unknown components in the lipid and to evaluate the effect on bone metabolism in OVX rats would contribute to understanding of the good Ca availability from WMC.

Postmenopausal bone loss has been prevented by treatment with oestrogen and by Ca supplementation (Ettinger *et al.* 1987; Riis *et al.* 1987). In this regard, WMC with trace amounts of 1,25-(OH)<sub>2</sub>D and oestradiol could be a good mineral supplement for those who are at risk of osteoporosis.

The authors are grateful to Dr T. Inomata, Azabu University, for the bone density analyses and I. Sekiguchi for the mineral analyses.

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