

Original Communication

Hair Calcium Concentration is Associated with Calcium Intake and Bone Mineral Density

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Abstract: Calcium concentration in hair, representing intracellular calcium levels, is associated with systemic diseases such as coronary artery disease. To date, there are no previous studies which investigate the regulation of hair calcium levels. The aim of the study is to investigate whether hair calcium concentration is related to calcium intake and calcium content in bone – bone mineral density (BMD). An observational research study was conducted with 55 women over the age of 20 who visited a university hospital in Suwon, Korea. The average age of the women was 51.45. Depending on the concentration of hair calcium, participants were divided into quartiles to compare calcium intake and BMD. There was no difference in demographic, anthropometric, and biochemical characteristics between the highest quartile of hair calcium concentration and the rest of the quartiles. However, the highest quartile ingested significantly less calcium compared to the rest of the quartiles ($p < 0.05$). The highest quartile of hair calcium concentration also showed significantly lower BMD and T-score in the L1–4 vertebrae compared to the rest of the quartiles ($p < 0.05$). These results show that high hair calcium concentration was associated with low calcium intake and low BMD.

Key words: hair, calcium, dietary calcium, bone density, minerals

Introduction

Calcium is the most abundant mineral in vertebrates and stores 99 % of the element in bone. The remaining 1 % of calcium plays a crucial role in the maintenance and regulation of metabolic and physiologic homeostasis in the body via intracellular signal transduction, neuromuscular function, and structural stability of the skeleton [1]. Recently, many studies have found that calcium concentration measured in hair is useful to demonstrate the contribution of calcium to metabolic

dysfunctions or systemic diseases. Because serum calcium level is strictly regulated to maintain homeostasis, even in pathologic conditions caused by significant changes in calcium content, it is very difficult to detect changes using serum calcium. On the contrary, hair mineral analysis can measure the intracellular contents of calcium [2, 3], directly linked to metabolic imbalances. For instance, hypertensive obese patients with insulin resistance have a significantly higher hair calcium level compared to healthy controls [4]. Hair calcium level is inversely correlated with aortic calci-

fication [5] and high hair calcium level is associated with low mortality in coronary heart disease [6]. Prior to investigating the underlying mechanism by which hair calcium concentration is correlated with disease, it is important to elucidate whether changes in calcium supply, transport, and metabolism affect the concentration of calcium in the hair.

Calcium is presumed to be influenced by oral intake, transport among various calcium pools (especially bone), and renal excretion [7]. Calcium metabolism is also regulated by interactions among calciotropic hormones, such as parathyroid hormone (PTH), vitamin D, and calcitonin [8]. However, in almost all previous studies, the investigators didn't consider these factors. Furthermore the relationship between these regulatory factors and hair calcium concentration has not been confirmed. In a small study ($n = 8$), increased calcium intake in osteoporosis patients resulted in reduced hair calcium levels [9]. However, another study indicated that calcium intake was not associated with hair calcium concentration [10]. The hair calcium level may also be affected by calcium efflux from bone and influx into bone (Figure 1). Patients with hyperparathyroidism, which increases calcium efflux from bone, showed higher hair calcium concentrations compared to the reference value; after parathyroidectomy, the patients hair calcium levels fell to the reference level [9], which suggests that the calcium reservoir in bone is associated with calcium level in hair.

Under physiological conditions, calcium intake and calcium efflux from bone are important factors that determine hair calcium level. To date, no research has elucidated the influence of calcium intake and calcium reserves in bone on hair calcium concentration. The aim of this study was to investigate whether calcium intake and bone mineral density (BMD) are related to calcium concentration in hair.

Materials and Methods

Subjects

The study included 63 women, over 20 years of age, who underwent hair tissue mineral analysis, bone mineral densitometry, and calcium intake measurement by the 24-hour recall method at Ajou University's health promotion center from January 2007 to December 2009. The study was approved by the Institutional Review Board of Ajou University Hospital.

The exclusion criteria were any disease which influences calcium metabolism, such as small intestine resection, chronic renal failure, hyperparathyroidism, hypercalciuria, hyperthyroidism, and hypothyroidism. Individuals who were taking medications that can affect bone mineral density such as oral contraceptives or hormonal replacement therapy, any type of calcium or vitamin D supplements during the last 6 months, diuretics, steroid hormones, thyroid-related medications, and anti-convulsive agents were also excluded. The final data consisting of 55 women analyzed.

Methods

Hair mineral analysis

The Centers for Disease Control (CDC) defines the protocol for analysis of calcium content in hair samples as follows: hair samples (80 mg) are collected from four different points on the occipital scalp [11]. The proximal portion of the hair samples (3–4 cm from skin) are cut with stainless steel sampling scissors. The participants are asked not to chemically process (dye, perm, straighten, or frost) their hair for at least 2 weeks prior to sample collection. The hair also has to be free of all gels, oils, and hair creams before sample collection. The collected hair was pre-washed (using a non-ionic detergent). Samples were stored in pre-cleaned plastic bags that were rigorously tested.

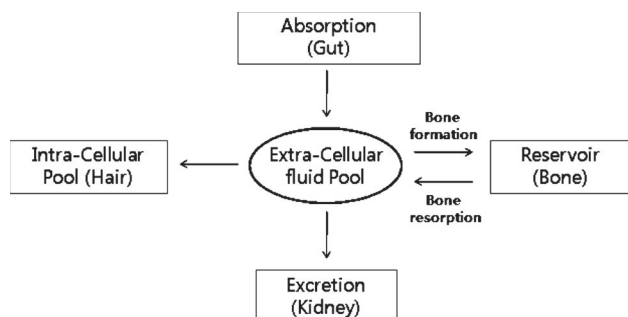


Figure 1: Hypothetic diagram of calcium transport and metabolism that influences hair calcium concentration.

Calcium measurements were performed using a microwave temperature-controlled digestion technique and Perkin-Elmer Mass Spectrometer in a licensed and certified clinical laboratory. The laboratory is regularly inspected by the Clinical Laboratory Division of the Department of Health and Human Services (Trace Elements Inc., Addison, TX, USA). The concentrations of calcium are reported in units of mg% (mg/100 g of hair with dry weight).

Calcium intake

Calcium intake was measured by the 24-hour recall method. A nutritionist interviewed each participant and recorded the type and amount of food ingested. Each mineral in the daily diet was catalogued and evaluated by the dietetic computer program Can-Pro ver. 3.0 (Computer Aided Nutritional Analysis Program, The Korean Nutrition Society). Calcium was divided into vegetable calcium and animal calcium. Calcium level of hair was reported as mg.

Bone mineral density

Bone mineral density, as measured by dual-energy x-ray absorptiometry (DEXA, Lunar Expert-XL, USA), provides excellent precision and accuracy in the measurement of total bone calcium [8]. Bone mineral density reflects the mobilization of calcium from bone, especially after menopause [12]. Bone mineral content (BMC, g), area (cm²), and T-score were measured at the 1st, 2nd, 3rd, and 4th lumbar spine (L1–4), femoral neck, and total femur. Bone mineral density (BMD, g/cm²) was calculated as the BMC (g) divided by area (cm²).

Measurements

All participants underwent a full physical examination. Anthropometric measurements were obtained with individuals wearing light clothing and no shoes. Height was measured to the nearest 0.1 cm and weight was measured to the nearest 0.1 kg. Body mass index (BMI) was calculated by dividing the weight (kg) by height squared (m²). The basal metabolic rate was measured by estimating body composition with Inbody 720 (Biospace Inc., Seoul, Korea) after an overnight fast.

Blood samples were collected after an overnight fast. Fasting glucose, total cholesterol, high-sensitivity C-reactive protein (hs-CRP), calcium, phosphorus,

blood urea nitrogen (BUN), creatinine, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were measured in each blood sample.

Statistical analysis

Data were analyzed using SPSS software (version 16.0, SPSS Inc., Chicago, IL). Variables are shown as means with standard deviation. Values of hair calcium concentration, calcium intake, and BMD were normally distributed, as confirmed by the one-sample Kolmogorov-Smirnov test. According to the hair calcium concentration, participants were divided into four quartiles using the SPSS statistics software. The four quartiles were defined as follows: Q1 (n = 14, 19–69 mg%), Q2 (n = 13, 70–105 mg%), Q3 (n = 14, 111–176 mg%), and Q4 (n = 14, 186–365 mg%). The groups were compared using ANOVA. The Student's *t*-test was used to determine significant differences among independent variables between the fourth quartile (Q4) and the rest of the quartiles (Q1–3). A *p* value < 0.05 was considered statistically significant.

Results

The basic characteristics of the study subjects are presented in Table I. The average hair calcium concentration was 136.07 mg% (Reference range: 22–97 mg%). There was no significant difference in demographic, anthropometric, and biochemical characteristics among each quartile (data not shown).

There was no difference in demographic, anthropometric, and biochemical characteristics between the highest quartile of hair calcium concentration and the rest of the quartiles (Table II). Total calcium intake for the highest quartile was significantly less compared to quartiles 1, 2, and 3. Intake of animal calcium and vegetable calcium showed similar trends to total calcium intake and there were no significant differences. There was no significant difference in the intake of protein, lipid, and carbohydrate between the highest quartile of hair calcium concentration and the rest of the quartiles.

The highest quartile of hair calcium concentration had significantly lower BMD in the L1–4 vertebrae compared to quartiles 1–3. The BMD of the femoral neck and total femur of the highest quartile were not significantly different compared to Q1, Q2, and Q3. The highest quartile of hair calcium concentration had

Table I: Basic characteristics of the research subjects (N = 55).

Basic characteristics		Min	Max	Mean	SD	
Age (years)		32	76	51.45	±	9.58
Height (cm)		140.9	178.1	157.40	±	6.19
Weight (kg)		41.5	109.8	60.60	±	11.27
BMI (kg/m2)		17	34.9	24.37	±	3.49
SBP (mmHg)		82	163	121.89	±	17.10
DBP (mmHg)		51	98	75.62	±	10.75
Fasting glucose (mg/dL)		70	172	98.45	±	18.78
Total cholesterol (mg/dL)		122	251	192.40	±	31.70
hs-CRP (mg/dL)		0	3.36	0.14	±	0.45
Calcium (mg/dL)		8.3	10.1	9.07	±	0.40
Phosphorus (mg/dL)		2.2	9.1	3.99	±	1.67
BUN (mg/dL)		7.2	32.6	13.53	±	4.27
Creatinine (mg/dL)		0.5	1.6	0.79	±	0.16
ALP (U/L)		32	143	61.69	±	19.90
AST(GOT) (U/L)		13	39	21.55	±	5.76
ALT(GPT) (U/L)		6	83	21.53	±	12.47
Hair calcium (mg%)		19	365	136.07	±	86.82
BMR (kcal)		1005.8	1883.5	1179.53	±	147.48
Nutritional intake						
Total calorie (kcal)		757.00	2673.00	1805.60	±	387.18
Total calcium (mg)		98.80	1496.52	815.05	±	284.55
Animal calcium (mg)		48.40	882.22	366.83	±	209.32
Vegetable calcium (mg)		50.40	942.45	448.22	±	151.59
Total protein (g)		15.22	123.54	79.06	±	20.49
Total lipid (g)		11.12	120.91	48.12	±	20.74
Total carbohydrate (g)		144.56	386.87	268.12	±	57.98
Phosphate (mg)		159.70	1812.68	1218.00	±	305.90
Sodium (mg)		199.00	8972.10	6013.12	±	1738.74
Bone mineral density measurement						
L1-4 vertebrae	BMD (g/cm2)	0.813	1.472	1.125	±	0.164
	BMC (g)	28.41	85.60	59.81	±	12.38
	T score	−2.8	3	0.0	±	1.3
Femur neck	BMD (g/cm2)	0.670	1.190	0.896	±	0.128
	BMC (g)	3.02	5.42	4.10	±	0.62
	T score	−2.3	2.5	−0.1	±	1.1
Femur total	BMD (g/cm2)	0.720	1.243	0.950	±	0.119
	BMC (g)	21.23	38.10	28.05	±	3.92
	T score	−2.1	2.6	0.0	±	1.0

Data was presented with mean ± SD (standard deviation).

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, hs-CRP; high-sensitivity C-reactive protein, BUN; blood urea nitrogen, ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, BMR; basal metabolic rate, BMD; bone mineral density, BMC; bone mineral contents.

Table II: Comparisons of characteristics between the highest quartile of hair calcium concentration (Q4) and the rest of the quartiles (Q1–3).

	Q1–3 (N = 41)				Q4 (N = 14)				P	
	min	max	Mean	SD	min	max	Mean	SD	value*	
Age (years)	32	70	50.63	± 9.36	41	76	53.86	± 10.15	0.281	
Height (cm)	147.4	177.5	157.61	± 5.25	140.9	178.1	156.79	± 8.61	0.676	
Weight (kg)	41.5	109.8	60.70	± 11.05	47.0	88.7	60.29	± 12.29	0.907	
BMI (kg/m2)	17.0	34.9	24.36	± 3.54	19.4	33.1	24.40	± 3.47	0.972	
Systolic BP (mmHg)	82	163	121.63	± 17.65	103	147	122.64	± 15.98	0.851	
Diastolic BP (mmHg)	51	98	76.00	± 11.53	61	85	74.50	± 8.32	0.656	
Fasting glucose (mg/dL)	75	172	99.73	± 18.59	70	155	94.71	± 19.55	0.393	
Total cholesterol (mg/dL)	122	251	196.51	± 32.15	131	224	180.36	± 28.00	0.100	
hs-CRP (mg/dL)	0.00	3.36	0.17	± 0.52	0.00	0.22	0.06	± 0.07	0.423	
Calcium (mg/dL)	8.4	10.1	9.11	± 0.40	8.3	9.4	8.95	± 0.38	0.234	
Phosphorus (mg/dL)	2.2	9.1	3.83	± 1.57	2.9	8.9	4.46	± 1.95	0.245	
BUN (mg/dL)	0.5	1.1	13.37	± 0.12	0.6	1.6	13.99	± 0.24	0.642	
Creatinine (mg/dL)	7.2	21.6	0.78	± 3.51	8.4	32.6	0.84	± 6.13	0.229	
ALP (U/L)	32	102	61.41	± 17.50	34	143	62.50	± 26.48	0.862	
AST(GOT) (U/L)	14	39	21.49	± 5.55	13	34	21.71	± 6.56	0.900	
ALT(GPT) (U/L)	6	83	22.46	± 13.50	9	37	18.79	± 8.59	0.345	
Hair calcium (mg%)	19	176	94.10	± 45.37	186	365	259	± 56.08	<.001	
BMR (kcal)	1031.0	1883.5	1179.96	± 132.57	1005.8	1770.3	1178.29	± 189.60	0.971	
Nutritional intake										
Total calorie (kcal)	1211.38	2673.15	1865.44	± 371.81	757.40	2067.61	1630.36	± 391.08	0.049	
Total calcium (mg)	410.65	1496.52	858.94	± 269.09	98.80	1315.25	686.51	± 299.42	0.049	
Animal calcium (mg)	101.77	942.45	398.14	± 209.48	50.40	804.10	275.12	± 186.66	0.057	
Vegetable calcium (mg)	174.85	882.22	460.80	± 143.39	48.40	822.90	411.39	± 173.89	0.297	
Total protein (g)	41.85	123.54	82.19	± 18.93	15.22	100.65	69.89	± 22.81	0.052	
Total lipid (g)	18.92	120.91	50.68	± 21.57	11.12	63.20	40.63	± 16.59	0.119	
Total carbohydrate (g)	164.09	386.87	273.90	± 54.48	144.56	373.76	251.2	± 66.47	0.209	
Phosphate (mg)	710.00	1812.68	1274.00	± 272.17	159.70	1465.53	1054.1	± 349.17	0.019	
Sodium (mg)	2620.50	8972.10	6137.28	± 1539.23	199.00	8395.63	5649.49	± 2253.21	0.370	

Table II: Continued.

		Q1-3 (N = 41)			Q4 (N = 14)			P		
		min	max	Mean	SD	min	max	Mean	SD	value*
Bone mineral density measurement										
L1-4 vertebrae	BMD (g/cm2)	0.869	1.472	1.150	± 0.157	0.813	1.312	1.049	± 0.166	0.044
	BMC (g)	43.31	85.60	61.71	± 11.68	28.41	74.76	54.24	± 13.09	0.050
	T score	-2.3	3.0	0.2	± 1.3	-2.80	1.1	-0.8	± 1.4	0.020
Femur neck	BMD (g/cm2)	0.715	1.190	0.911	± 0.124	0.666	1.107	0.849	± 0.133	0.119
	BMC (g)	3.10	5.42	4.16	± 0.61	3.02	5.37	3.95	± 0.64	0.274
	T score	-1.6	2.5	-0.1	± 1.1	-2.30	1.7	-0.3	± 1.1	0.477
Femur total	BMD (g/cm2)	0.725	1.243	0.964	± 0.120	0.720	1.071	0.909	± 0.111	0.136
	BMC (g)	22.46	38.10	28.42	± 4.01	21.23	33.15	26.97	± 3.53	0.237
	T score	-1.7	2.6	0.1	± 1.0	-2.10	1.7	-0.4	± 1.1	0.158

Data was presented with Mean ± SD (standard deviation).

BMI; body mass index, SBP; systolic blood pressure, DBP; diastolic blood pressure, hs-CRP; high-sensitivity C-reactive protein, BUN; blood urea nitrogen, ALP; alkaline phosphatase, AST; aspartate aminotransferase, ALT; alanine aminotransferase, BMR; basal metabolic rate, BMD; bone mineral density, BMC; bone mineral contents.

*p value from independent T-test comparing a difference between Q4 and Q1-3

a significantly lower T-score in the L1–4 vertebrae compared to Q1, Q2, and Q3. BMC showed a similar trend to BMD and T-score.

Discussion

The highest quartile of hair calcium concentration in our study showed less calcium intake and lower BMD in the lumbar vertebrae compared to lower hair calcium concentration quartiles. These data suggest that the amount of calcium intake and amount of calcium released from bone can influence hair calcium levels.

In contrast to our study, an observational study reported that there was no significant association between calcium intake and hair calcium levels in premenopausal women [10]. However, the study included subjects who took calcium supplements, which can confound the relationship between calcium intake and hair calcium concentration. In a small clinical study of eight osteoporosis patients, taking calcium supplements for six months, hair calcium concentrations were shown to decrease [9], which suggests that calcium intake decreases calcium concentration in hair, which is consistent with our findings. A low calcium intake augments intracellular calcium concentrations [13]. Hair calcium concentration decreases as serum PTH level decreases after six months of calcium supplements [9], and serum PTH level is inversely correlated with dietary calcium intake [14]. Taken together, these findings support the results of our current study elucidating the underlying mechanism that low calcium intake induces hypersecretion of PTH, which in turn accelerates bone loss and augments intracellular calcium concentration in hair [13, 15].

An observational study with premenopausal women did not demonstrate a correlation between BMD and hair calcium level [9]. It is difficult to identify the influence of the main calcium reservoir, bone, on hair calcium levels in premenopausal women because calcium mobilization is not as active prior to menopause, as compared to after menopause when calcium efflux is increased due to the dramatic acceleration of bone resorption in the absence of estrogen [16]. In contrast to the study with premenopausal women, patients with diseases that increase bone resorption and decrease bone mineral density, such as hyperparathyroidism, hyperthyroidism, and osteomalacia, had a higher concentration of hair calcium compared to the reference range [9]. As our study revealed, serum calcium concentration does not reflect these pathologic conditions whereas hair calcium does, because serum calcium is

meticulously regulated for metabolic and physiologic homeostasis in the body [8]. Our observation infers that the change in calcium efflux due to bone loss can be more easily detected by hair calcium concentration than by serum calcium level.

Bone resorption is more apparent in the lumbar vertebrae compared to the proximal femur because the vertebral bone consists of mostly trabecular bone, which is metabolically more responsive to estrogen deficiency compared to cortical bone in the proximal femur after menopause [17]. This explains the reason for the BMD differences between groups observed only in the vertebrae and not in the femur in our study.

The hair can provide quality information regarding intracellular calcium [18], allowing a better understanding of the underlying pathophysiology of many systemic illnesses and calcium mobilization in the body. Calcium homeostasis in the body is largely regulated through integrated hormonal systems [10], which includes estrogen and two major calcium-regulating hormones: PTH [19] and $1,25(\text{OH})_2 \text{D}$ [20]. To obtain a more precise illustration of calcium metabolism in the body, monitoring changes in calcium-regulating endocrine factors and lifestyle information are required. The current study described a portion of calcium metabolism and mobilization of calcium in the body under physiological conditions and revealed the relationship between hair calcium level, calcium intake, and BMD. Hair calcium concentration is influenced by dietary calcium intake and calcium efflux from calcium reserves in the bone, implying the connection between two observations that hair calcium is related to vascular calcification and that osteoporosis is associated with vascular calcification.

There are several limitations to our study. We used 24-hour recall to assess calcium intake. Among retrospective dietary assessment tools such as the 24-hour recall, food frequency questionnaire (FFQ), and diet history interview [21], the 24-hour recall provides for more precise amounts of foods than other tools [22]. To improve accuracy, all subjects in our study were interviewed by a professional nutritionist. Moreover, in our research, the participants were asked to refrain from chemically processing their hair for at least 2 weeks. Because scalp hair grows at an average rate of 1 cm per month [23] and we took 3 cm of hair from each subject, we cannot completely exclude external exposure influencing the results.

Higher calcium level in hair is associated with less calcium intake and lower BMD, suggesting that lower calcium intake and increased calcium efflux from bone can augment intracellular calcium concentration, which is linked to metabolic imbalances.

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