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

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감사의 글

먼저 연구에 대한 착상에서부터 결론을 내기까지 지도와 조언을 아끼지 않으셨던 김범택 지도교수님께 감사의 인사를 드립니다. 바쁘신 와중에도 함께 의논해 주시고 더 나은 방향을 제시해 주신 덕분에 논문을 완성할 수 있었습니다. 논문 심사를 해주신 지도위원 박준은, 박일중 교수님께도 감사드립니다. 또한 새로운 시각으로 바라보고 고민할 수 있는 기회를 주신 이득주 교수님과, 느슨해짐을 반성하고 항상 노력할 수 있도록 도와주신 김광민 교수님, 머리만이 아닌 가슴으로 느끼고 발전할 수 있게 도와주신 박새별 교수님께 감사드립니다. 연구의 약점에 대해 조언해 주시고 열심히 할 수 있게 힘을 주신 주남석 교수님과 영문 교정에 시간을 내어 주신 조두연 교수님께 감사의 말씀을 전하고 싶습니다.

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부족한 머느리를 항상 사랑으로 이해해 주시고 아껴주시는 시부모님과 그리 대단하지 않은 딸이지만 항상 믿고 지지해 주시는 부모님, 저를 언제나 신뢰하고 따라주는 동생들에게 감사드립니다. 마지막으로 힘들고 지칠 때 마다 옆에서 용기를 주고 포기하지 않도록 이끌어준 사랑하는 남편 임신규씨와 논문을 쓰는 동안 뱃속에서 잘 자라고 건강하게 태어난 아들 임수빈에게 감사의 인사를 전합니다.

- ABSTRACT -

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

Background : Calcium concentration in hair representing intracellular calcium level, is associated with systemic diseases such as coronary artery disease. However there is no previous study to show how hair calcium level is regulated. The aim of the study is to investigate whether hair calcium concentration is related to calcium intake and calcium contents in bone - bone mineral density(BMD).

Methods : Observational research was conducted with 63 women aged above 30 year old who visited a university hospital in Suwon, Korea. Depending on concentration of hair calcium, participants were divided into quartiles to compare their calcium intake and BMD.

Results : 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 than the rest of the quartiles.($p < 0.05$) The highest quartile of hair calcium concentration also demonstrated significantly lower BMD and T-score at L1-4 vertebrae than the rest.($p < 0.05$)

Conclusion : High hair calcium concentration is associated with low calcium intake and low BMD.

Key words : Hair, Calcium, Dietary Calcium, Bone mineral density

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I. INTRODUCTION

Hair mineral test is a measuring tool to demonstrate intracellular contents of minerals and toxic molecules.(Klevay et al, 1987; Harkins and Susten, 2003) Calcium the most abundant mineral in body, is stored 99% in bone - the biggest calcium reservoir in body, while spared 1% of calcium plays a crucial role on maintaining and regulating metabolic and physiologic homeostasis in body, such as intracellular signal transduction, neuroendocrine function and musculoskeletal stability.(Wang et al, 2006)

The changes in hair calcium concentration is associated with abnormal metabolic function and systemic diseases in body. For instance, obese hypertensive patients with insulin resistance, had significantly higher hair calcium level than healthy controls.(Suliburska et al, 2011) Hair calcium level showed inversed correlation with aortic calcification.(Bacso et al, 1986) High hair calcium level was also associated with low mortality due to coronary heart disease.(MacPherson and Bacsó, 2000) However, it has not revealed actually how hair calcium concentration is connected with those diseases. Prior to investigating those relationship, it is important to figure out which physiologic conditions that can change calcium metabolism in body, may influence calcium concentration in hair.

The contents of hair calcium is presumed to be under the influence of food intake, metabolism and renal excretion of calcium.(National Osteoporosis Foundation, 2010) The relationship between calcium intake and hair calcium concentration has not confirmed yet. In very small sized study(n=8), increased calcium intake in osteoporosis patients reduced hair calcium level.(Miekeley et

al, 2001) But in other study, calcium intake is not associated with hair calcium concentration.(Song et al, 2007)

The contents of hair calcium may also be affected by calcium efflux and influx originated from resorption and formation in bone.(fig. 1.) Patients with hyperparathyroidism in which condition calcium efflux from the bone increases, demonstrated higher hair calcium concentration than reference value, and after parathyroidectomy hair calcium fell down to reference level(Miekeley et al, 2001), which suggests the critical role of calcium reservoir in bone in regulating hair calcium level.

Under physiologic condition, calcium intake and calcium output from the bone can be two major factors regulating hair calcium level. So far, no researches have illustrated influences of calcium intake and calcium reservoir in bone on calcium concentration in hair. The aim of the study is to investigate whether calcium intake and calcium level - bone mineral density(BMD). is related to hair calcium concentration.

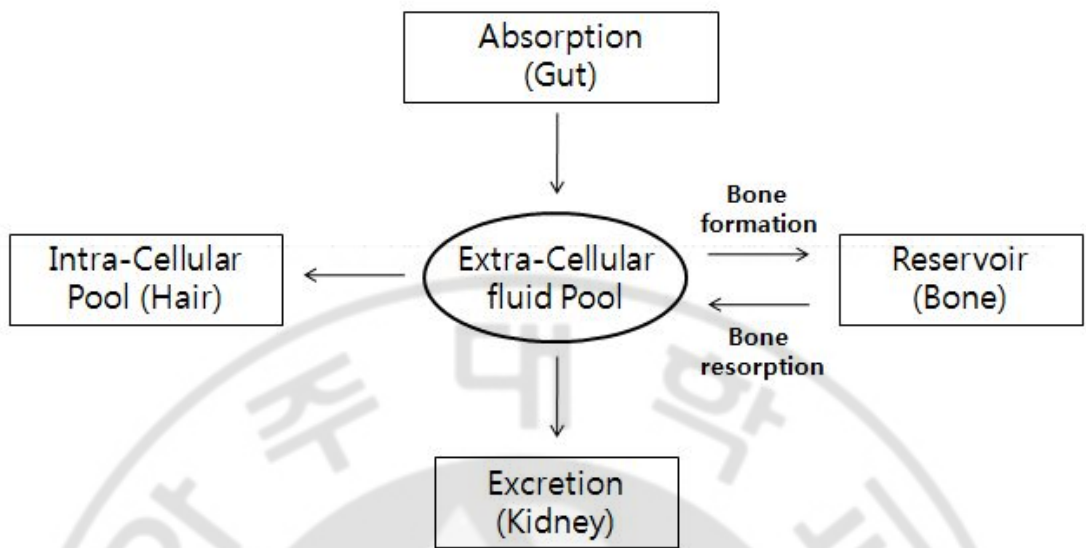


Fig. 1. Hypothetic diagram of calcium transport and metabolism that influences hair calcium concentration.

II. MATERIALS AND METHODS

A. Subjects

Sixty-three women aged above 30 years who did hair tissue mineral analysis, bone mineral densitometry and calcium intake measurement by 24-hour recalls method in Ajou university health promotion center from January 2007 to December 2009, were included for the study.

The exclusion criteria were individuals with diseases that influence calcium metabolism such as small intestine resection status, chronic renal failure, hyperparathyroidism, hypercalciuria, hyperthyroidism, and hypothyroidism. Individuals who was taking any medications that can affect bone mineral density, such as oral contraceptives or hormonal replacement therapy, any type of calcium supplements, steroid hormones, thyroid related medications and anti-convulsion agents, were also excluded. Finally data from fifty-five women were analyzed.

B. Methods

1. Hair Mineral Analysis

Hair samples (80 mg) were collected from four different points of occipital scalp. The samples were cut proximal portion of hair (3-4 cm from skin) with stainless steel sampling scissors. The participants were asked not to chemically process their hair for at least 2 weeks, such as dying, perming, straightening, or frosting. The hair also had to be free of all gels, oils, and hair creams before sample collection.

Measurements were performed using a microwave temperature -

controlled digestion technique and Perkin-Elmer Mass Spectrometer in a licensed and certified clinical laboratory that undergoes regular inspections with the Clinical Laboratory Division of the Department of Health and Human Services (Trace Elements Inc., Addison, TX, USA). The spectrophotometer of minerals were reported as unit of mg% (mg/100g of hair).

2. Calcium Intake

Calcium intake measured by 24-hour recalls method. The type and amount of food was recorded by direct interview with a nutritionist. Each mineral in daily diet was processed and evaluated using 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 units were mg.

3. Bone Mineral Density

BMD was measured bone mineral content (BMC, g), area (cm²) and T-score at the 1st, 2nd, 3rd and 4th lumbar spine (L1-4), femoral neck and total femur by using dual-energy X-ray absorptiometry (DEXA, Lunar Expert-XL, USA). Bone mineral density (BMD, g/cm²) was calculated by dividing area (cm²) by BMC (g).

4. Measurements

All participants underwent a full physical examination. Anthropometrical measurements of individuals wearing light clothing and no shoes were conducted. 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 weight (kg) by height squared (m^2).

The waist circumference was measured to the nearest 0.1 cm in midway between the lower rib margin and the anterior superior iliac spine with the subject standing with their feet placed together. The basal metabolic rate was measured by estimating body composition with Inbody 720 (Biospace Inc., Seoul, Korea).

Blood samples were collected after an overnight fast. Measurements of fasting glucose, insulin, total cholesterol, triglycerides, high-density lipoproteins (HDL), low-density lipoproteins (LDL), 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 performed in each blood sample.

C. Statistical Analysis

Data were analyzed using SPSS (version 16.0) software. Depending on concentration of hair calcium, participants were divided into quartiles. Each group, from the first quartile to the fourth quartile, was defined as Q1, Q2, Q3 and Q4. T - test was used to establish the significance of group differences for independent variables between the fourth quartile and the rest of the quartiles. A p value <0.05 was considered statistically significant.

III. Results

A. Basic Characteristics of the Research Subjects

The average age of women included in this study was 51.45. Their hair calcium concentrations was 136.07 mg% and their serum calcium concentration was 9.07 mg/dl. The average intake of total calcium, animal calcium and vegetable calcium is 815.05 mg/day, 366.83 mg/day, 448.22 mg/day. The average BMD and T-score of L1-4 vertebrae was 1.125 g/cm², -0.0, respectively. (Table 1.)

We compared the highest quartile of hair calcium concentration and the rest of the quartiles. 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 2.)

B. The Relationship between Calcium Intake and Hair Calcium Concentration

The highest quartile took significantly less total calcium than the rest of the quartiles. (686.51 mg vs. 858.94 mg, $p < 0.049$) Intakes of animal calcium and vegetable calcium showed similar trend to total calcium intakes. There was no difference in intakes of protein, lipid and carbohydrate between the highest quartile of hair calcium concentration and the rest of the quartiles. (Table 2, Fig. 2.)

C. The Relationship between Bone Mineral Density and Hair Calcium Concentration

The highest quartile of hair calcium concentration demonstrated significantly lower BMD of L1-4 vertebrae than the rest of the quartiles. (1.049 g/cm^2 vs. 1.150 g/cm^2 , $p < 0.044$) BMDs at femoral neck and total femur, of the highest quartile were not different from those of the rest. The highest quartile of hair calcium concentration demonstrated significantly lower T-score of L1-4 vertebrae than the rest of the quartiles. (-0.8 vs. 0.2 , $p < 0.020$) BMCs showed similar trend to BMDs and T-scores. (Table 2, Fig. 3.)

Table 1. Basic characteristics of the research subjects (N=55).

Basic characteristics	Mean	SE
Age (years)	51.45 ±	1.29
Height (cm)	157.40 ±	0.84
Weight (kg)	60.60 ±	1.52
Waist Circumference (cm)	83.53 ±	1.18
BMI (kg/m ²)	24.37 ±	0.47
SBP (mmHg)	121.89 ±	2.31
DBP (mmHg)	75.62 ±	1.45
Fasting glucose (mg/dl)	98.45 ±	2.53
Insulin (μIU/ml)	8.25 ±	0.64
Total cholesterol (mg/dl)	192.40 ±	4.27
Triglyceride (mg/dl)	118.45 ±	10.42
HDL-cholesterol (mg/dl)	55.76 ±	1.98
LDL-cholesterol (mg/dl)	112.95 ±	3.49
hsCRP (mg/dl)	0.14 ±	0.06
Calcium (mg/dl)	9.07 ±	0.06
In. Phosphorus (mg/dl)	3.99 ±	0.23
BUN (mg/dl)	13.53 ±	0.58
Creatinine (mg/dl)	0.79 ±	0.02
ALP (U/L)	61.69 ±	2.68
AST(GOT) (U/L)	21.55 ±	0.78
ALT(GPT) (U/L)	21.53 ±	1.68
Hair calcium (mg%)	136.07 ±	11.71
BMR (kcal)	1179.53 ±	20.07
Nutritional information		
Total calory (kcal)	1805.60 ±	52.21
Total calcium (mg)	815.05 ±	38.37
Animal calcium (mg)	366.83 ±	28.23
Vegetable calcium (mg)	448.22 ±	20.44
Total protein (g)	79.06 ±	2.76
Total lipid (g)	48.12 ±	2.80
Total carbohydrate (g)	268.12 ±	7.82
Phosphate (mg)	1218.00 ±	41.25

Sodium (mg)		6013.12	±	234.45
BMD measurement				
L1-4 vertebrae	BMD (g/cm ²)	1.125	±	0.022
	BMC (g)	59.81	±	1.67
	T score	0.0	±	0.2
Femur neck	BMD (g/cm ²)	0.896	±	0.017
	BMC (g)	4.10	±	0.08
	T score	-0.1	±	0.1
Femur total	BMD (g/cm ²)	0.950	±	0.016
	BMC (g)	28.05	±	0.53
	T score	0.0	±	0.1

Data was presented with Mean ± SE (Standard Error)

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL: high-density lipoproteins, LDL: low-density lipoproteins, hs-CRP: high-sensitivity C-reactive protein, BUN: blood urea nitrogen, ALP: alkaline phosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BMR: basal metabolic rate

Table 2. 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 value*
	Mean	SE	Mean	SE	
Age (years)	50.63	± 1.46	53.86	± 2.71	0.281
Height (cm)	157.61	± 0.82	156.79	± 2.30	0.676
Weight (kg)	60.70	± 1.73	60.29	± 3.29	0.907
Waist Circumference (cm)	83.76	± 1.33	82.86	± 2.57	0.743
BMI (kg/m ²)	24.36	± 0.55	24.40	± 0.93	0.972
Systolic BP (mmHg)	121.63	± 2.76	122.64	± 1.11	0.851
Diastolic BP (mmHg)	76.00	± 1.80	74.5	± 4.27	0.656
Fasting glucose (mg/dl)	99.73	± 2.90	94.71	± 5.22	0.393
Insulin (μIU/ml)	8.77	± 0.74	6.6	± 1.12	0.147
Total cholesterol (mg/dl)	196.51	± 5.02	180.36	± 7.48	0.100
Triglyceride (mg/dl)	113.20	± 9.99	133.86	± 29.11	0.393
HDL-cholesterol (mg/dl)	57.20	± 2.31	51.57	± 3.74	0.219
LDL-cholesterol (mg/dl)	116.68	± 4.13	102.01	± 5.71	0.067
HsCRP (mg/dl)	0.17	± 0.08	0.06	± 0.02	0.423
Calcium (mg/dl)	9.11	± 0.06	8.95	± 0.11	0.234
In. Phosphorus (mg/dl)	3.83	± 0.25	4.46	± 0.54	0.245
BUN (mg/dl)	13.37	± 0.55	13.99	± 5.22	0.642
Creatinine (mg/dl)	0.78	± 0.02	0.84	± 0.07	0.229
ALP (U/L)	61.41	± 2.73	62.5	± 7.08	0.862
AST(GOT) (U/L)	21.49	± 0.87	21.71	± 1.75	0.900
ALT(GPT) (U/L)	22.46	± 2.11	18.79	± 2.30	0.345
Hair calcium (mg%)	94.10	± 7.09	259	± 14.99	<.001
BMR (kcal)	1179.96	± 20.96	1178.29	± 50.67	0.971
Nutritional information					
Total calory (kcal)	1865.44	± 58.07	1630.36	± 104.52	0.049
Total calcium (mg)	858.94	± 42.03	686.51	± 80.02	0.049
Animal calcium (mg)	398.14	± 32.71	275.12	± 49.89	0.057
Vegetable calcium (mg)	460.80	± 22.39	411.39	± 46.47	0.297
Total protein (g)	82.19	± 2.96	69.89	± 6.10	0.052

Total lipid (g)		50.68	±	3.37	40.63	±	4.43	0.119
Total carbohydrate (g)		273.90	±	8.51	251.2	±	17.76	0.209
Phosphate (mg)		1274.00	±	42.51	1054.1	±	93.32	0.019
Sodium (mg)		6137.28	±	240.39	5649.49	±	602.2	0.370
BMD measurement								
L1-4 vertebrae	BMD (g/cm ²)	1.150	±	0.025	1.049	±	0.044	0.044
	BMC (g)	61.71	±	1.82	54.24	±	3.50	0.050
	T score	0.2	±	0.2	-0.8	±	0.4	0.020
Femur neck	BMD (g/cm ²)	0.911	±	0.019	0.849	±	0.035	0.119
	BMC (g)	4.16	±	0.09	3.95	±	0.17	0.274
	T score	-0.1	±	0.2	-0.3	±	0.3	0.477
Femur total	BMD (g/cm ²)	0.964	±	0.019	0.909	±	0.030	0.136
	BMC (g)	28.42	±	0.63	26.97	±	0.94	0.237
	T score	0.1	±	0.2	-0.4	±	0.3	0.158

Data was presented with Mean ± SE (Standard Error)

BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, HDL: high-density lipoproteins, LDL: low-density lipoproteins, hs-CRP: high-sensitivity C-reactive protein, BUN: blood urea nitrogen, ALP: alkaline phosphatase, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BMR: basal metabolic rate

*P value from independent t-test comparing a difference between Q4 and Q1-3.

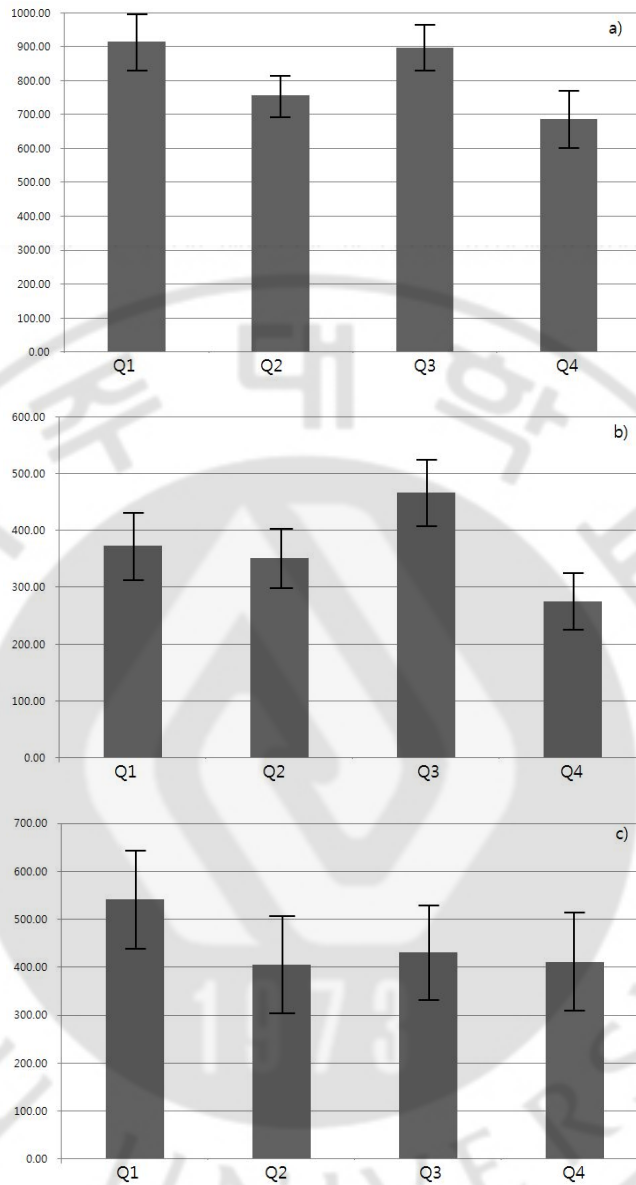


Fig. 2. Calcium intake according to quartiles of hair calcium concentration (mg). a) total calcium, b) animal calcium, c) vegetable calcium.

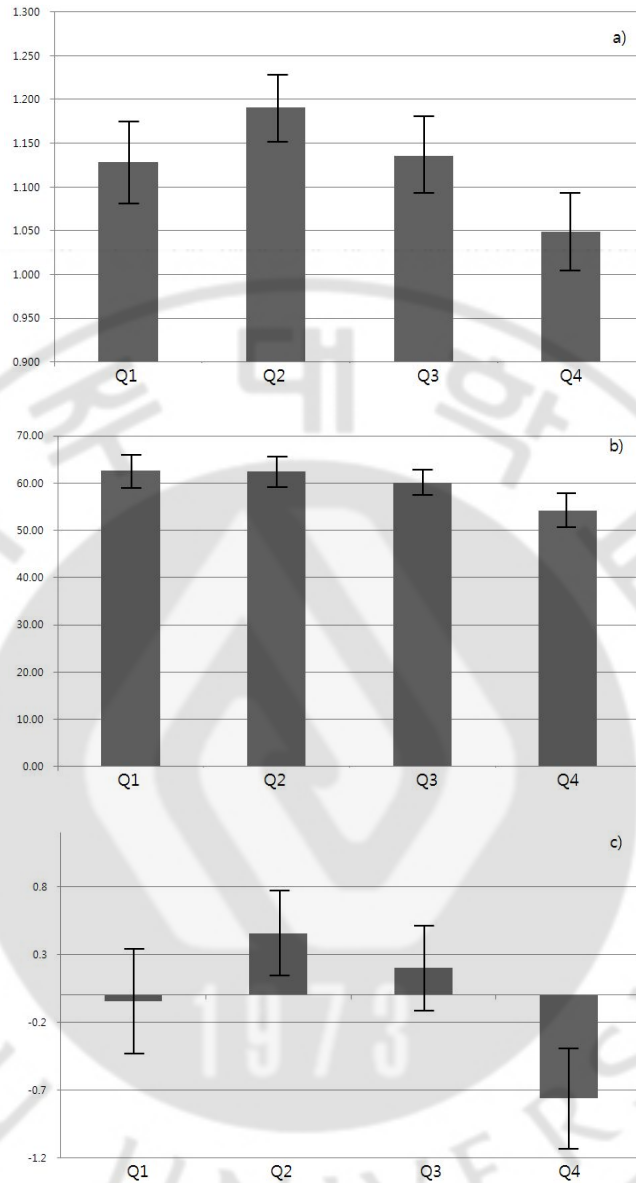


Fig. 3. Lumbar vertebrae BMD measurement according to quartiles of hair calcium concentration. a) BMD (g/cm²), b) BMC (g), c) T score.

IV. DISCUSSION

The highest quartile group of hair calcium concentration in the study demonstrated less calcium intake and lower BMD at lumbar vertebrae than the other groups, suggesting calcium intake and calcium reservoir in bone influences hair calcium level.

An observational study showed no significant association between hair calcium concentration and calcium intake in pre-menopausal women.(Song et al, 2007) In that study, subjects who took calcium supplements were included, which can blur the relationship between hair calcium concentration and calcium intake. In a small sized clinical research, hair calcium concentration in eight osteoporosis patients, decreased after taking calcium supplement for six months(Miekeley et al, 2001), showing calcium intake can change calcium concentration in hair, which is in consistence with our study. There are some evidences supporting calcium intake influences hair calcium concentration through serum parathyroid hormone (PTH). Hair calcium concentration changes according to serum PTH level.(Miekeley et al, 2001) Dietary calcium intake is inversely correlated with the PTH concentration.(Kinyamu et al, 1998) These findings make the inference possible that low calcium intake induces PTH hypersecretion which led to elevated hair calcium level.

An observational study with premenopausal women showed no correlation between hair calcium level and BMD.(Song et al, 2007) It is difficult to see the influence of main calcium reservoir - bone on hair calcium level in young women for calcium mobilization in premenopausal time is not as active as in postmenopausal period when calcium efflux due to bone

resorption increases drastically.(Nordin et al, 1981) The patients with diseases that increase bone resorption and decrease bone mineral density such as hyperparathyroidism, hyperthyroidism, and osteomalacia, demonstrated higher concentration of hair calcium than reference range.(Miekeley et al, 2001) As our study showed, serum calcium concentration is not affected at all by these pathologic conditions for serum calcium is meticulously regulated for metabolic and physiologic homeostasis in body.(Peacock, 2010) Any conditions which can pour calcium load in extracellular fluid calcium pool such as postmenopausal bone loss raises hair calcium concentration rather than elevated in serum calcium level. It is also possible that calcium imbalance in extracellular fluid caused by insufficient calcium intake activates bone mineral density change just after menopause is more apparent at lumbar vertebrae than proximal femur, because vertebral bone consists of trabecular bone which is more sensitive to estrogen deficiency after menopause than cortical bone while proximal femur is composed of cortical bone.(Riggs et al, 2008) It may be the reason, in our study femoral bone density was different between groups. Compensation mechanism to make up calcium deficit by mobilizing calcium from bone – the largest calcium reservoir in body though elevated PTH level.(National Osteoporosis Foundation, 2010)

Our research has a few limitations that we used 24-hour recalls to assess calcium intake and we measured BMD only as an indicator for calcium pool in bone. Among retrospective Dietary assessment tools such as multiple 24-hour recalls, food frequency questionnaires (FFQ) and diet history interviews(Arab et al, 2011), 24-hour recalls have recently become favored for it provides more precise amount of foods than other tools(Schatzkin et al, 2003). To improve accuracy, all subjects in our research, were interviewed by

professional nutritionist. Bone mineral content, as measured by dual x-ray absorptiometry, provides excellent precision and accuracy to measure total bony calcium.(Peacock, 2010) BMD reflects mobilization of calcium from bone especially after menopause.(Seeman, 2002) Current study is first research that illustrates calcium metabolism and mobilization in body, under physiological condition. The hair is one of cells in body, can provide quality information for intracellular contents paving a way to understand underlying pathophysiology of many systemic illnesses.

Our study demonstrated that lost balance of calcium metabolism such as insufficient calcium intake or lower BMD, affects hair calcium concentration independent of systemic diseases. Before interpreting hair calcium concentration in relevance with systemic disease, calcium balance and mobilization in body should be taken into consideration. Calcium homeostasis is largely regulated through an integrated hormonal system.(Peacock, 2010) It involves two major calcium-regulating hormones such as PTH(Potts and Gardella, 2007) and 1, 25(OH)₂D(Jurutka et al, 2001), as well as serum ionized calcium(Brown, 2007). To get more precise feature of calcium metabolism in body, further researches including calcium regulating endocrine factors like PTH and vitamin D are required.

V. CONCLUSION

Higher calcium level in hair is associated with less calcium intake and lower BMD at lumbar vertebrae. Hair calcium concentration can be under the influence of dietary calcium intake and calcium efflux from calcium reservoir in bone. Many previous studies investigating connections between hair calcium concentration and systemic diseases were not concerned about physiological conditions that influence calcium concentration in hair. Prior to investigating those relationships, it is important to take into account of physiological conditions such as calcium intake and BMD and their influences on calcium concentration in hair.

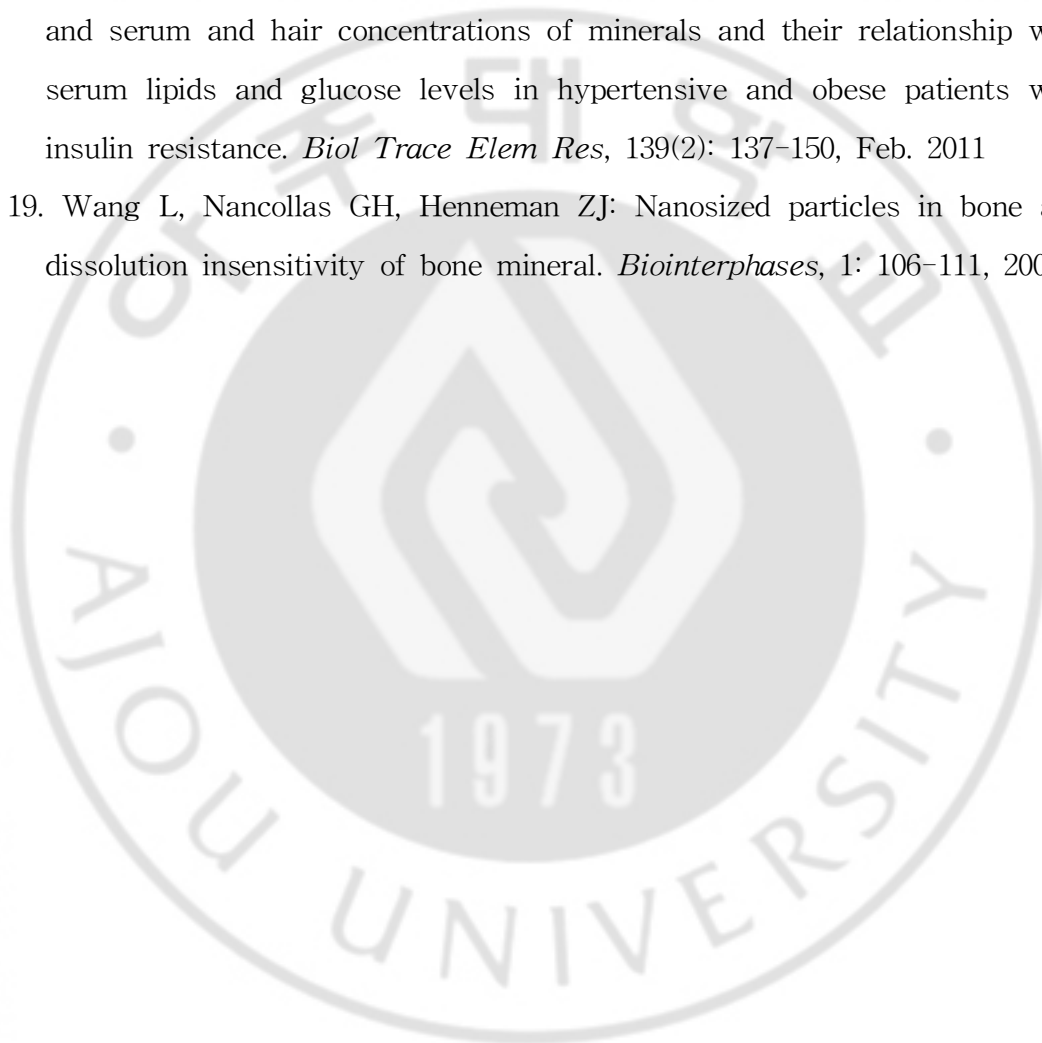
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모발 내 칼슘 농도와 연관된 칼슘 섭취와 골밀도

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연구배경 : 모발 내 칼슘 농도는 세포 내 칼슘 축적을 반영하고 관상동맥질환과 같은 전신 질환과 관련이 있다. 이전에 모발 내 칼슘을 조절하는 인자에 대한 밝힌 연구는 없었다. 이 연구의 목적은 모발 내 칼슘 농도와 연관된 칼슘 섭취량 및 골밀도를 이용한 뼈의 칼슘량에 대해 알아보는 것이다.

방법 : 아주대학교 건강 검진 센터를 내원한 30세 이상 여자 63명을 대상으로 하였다. 모발 내 칼슘 농도에 따라 사분위하고 농도가 가장 높은 군 (N=14)과 나머지 군 (N=41)으로 분류하여 칼슘 섭취량과 골밀도를 비교하였다.

결과 : 모발 내 칼슘 농도에 따라 분류한 두 군에서 인구 사회학적 특성은 통계적으로 유의한 차이를 보이지 않았으나 총 칼슘 섭취량은 모발 내 칼슘 농도가 가장 높은 4분위 군에서 통계적으로 유의하게 낮은 것으로 나타났다.($p<0.05$) 골밀도 검사에서는 제 1번에서 4번 요추부의 BMD, T값이 모발 내 칼슘 농도가 가장 높은 4분위 군에서 통계적으로 유의하게 낮았다.($p<0.05$)

결론 : 높은 모발 내 칼슘 농도는 칼슘 섭취 부족 및 낮은 골밀도와 관련이 있다.

핵심어 : 모발, 칼슘, 식이 칼슘, 골밀도