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The Effect of Alcohol Consumption on Korean Young Women's Bone Health: Cross-Sectional data from 2008-2011 Korea National Health and Nutrition Examination Survey

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ABSTRACT

Objectives: To assess the effect of alcohol on healthy Korean young women bone by AUDIT scores and drinking patterns; frequency, amount, binge drinking

Design: Cross-sectional study composed of three parts; health interview, health examination, nutrition survey

Setting: 2008-2011 Korea National Health and Nutrition Examination Survey

Participants: Of the 18,828 subjects who measured bone mineral density (BMD), n=1,183 healthy women aged 19 to 30 years old were selected.

Primary and secondary outcome measures: Mean BMD T-scores of total femur (TF), femur neck (FN) and lumbar (LB) by drinking patterns and AUDIT scores, and the odds of having low BMD (T-score <-1: either osteopenia or osteoporosis) at three sites by AUDIT scores

Results: After adjustment, the lower BMD were found at three sites in those who drank more and had higher AUDIT scores. These associations were significant by AUDIT scores at the TF (P=0.001) and FN (p=0.002) and by drinking frequency at FN (p=0.040). The adjusted odds ratio of having low BMD increased significantly, particularly at FN, in those who had higher AUDIT scores such as 16-17 harmful drinking (OR 5.01; 95%CI 1.33 to 18.95) and 20-40 alcohol dependence (OR 7.47; 95%CI 2.07 to 26.99), compared with young women who scored 0-7 low risk drinking or abstinence. No beneficial effect of moderate drinking was observed at all sites and the association between alcohol and bone health was most evident in FN while no significance was found in LB.

Conclusions: It is crucial to promote the awareness of alcohol harm on Korean young women's bone health. At the same time, since alcohol's effect on bone is complex with cumulative effects of various factors over the years and there is an absence of studies with young women in their twenties, more studies are needed with more precise and appropriate design to confirm our findings.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The first study to investigate the effect of alcohol on young female bone health at the total femur, femur neck and lumbar spine, using a nationwide sample data representative of Korean young women in their twenties
- Only healthy Korean young women, free of diseases known to influence bone metabolism, were considered in the study
- The study is limited by its cross-sectional nature
- Drinking variables based on the past year's experience were limited in their ability to fully reflect the effect of alcohol on bones by the extent and duration of alcohol exposure

INTRODUCTION

Osteoporosis is a skeletal disorder characterized by the reduction of bone density and quality, leading to weakness of the skeleton and increased risk of fractures, especially of the wrist, spine and hip.[1-2] Osteoporotic fractures are an important cause of mortality and morbidity and a considerable financial burden on economies.[1] With the trend toward aging populations, osteoporosis is a major public health concern in many countries, including Korea.

In Korea, the proportion of people over 65 years of age was 7.2% in 2000 and is expected to reach 32.3% by 2040.[3] According to the recent 5-year (2007-2011) Korean osteoporosis patients statistics released in 2013 by the Health Insurance Review Agency (HIRA), Korea, 93.7% of the patients were 50 years or older and the growing rate of the number of the total patients was 44.3% with an annual growth rate of 9.7%. There was a particularly substantial increase in elderly patients over 70 years old with 75.2% increase during the same period with associated medical care costs of about 72 billion won (about 7.1million dollars) in 2011, an increase of 35% from 2007 with 7.9% annual growth.[4]

Even though osteoporosis is considered an age-related disease, it is also affected by many other factors such as weight, dietary factors, family history of osteoporosis, menopausal status, exercise, smoking and drinking. Heavy drinking, particularly, is known to have detrimental effects on bone density while the effect of light or moderate drinking on bones remains mixed: It can be beneficial for postmenopausal women but no benefit was found for premenopausal women.[5-7] Many human and animal studies indicate that alcohol consumption interrupts bone growth and replacement of bone tissue, causing increased bone fragility and susceptibility to fractures.[8-13] Influencing directly or indirectly on bone metabolism, alcohol consumption during adolescence and young adulthood, before the mid 30's, prevents the attainment of optimal Peak Bone Mass(PBM) which is a major contributor

to the development of strong and healthy bones in later years.[14-15]

The Annual Korea National Health and Nutrition Examination Survey (KNHANES), however, suggests that high alcohol consumption among Korean young women in their twenties was outstanding in every drinking indicator: Of female drinkers, high risk drinking (on average more than 5 glasses per occasion more than twice per week) and weekly binge drinking (on average more than 5 glasses at a sitting with more than once per week) rates in this young female group are 10.6% and 17.4%, higher than the 8%, 14.8% of all women aged over 19 years old respectively. The rates of their yearly and monthly drinking were also higher at 86.5% and 57.7%, respectively, than any other female age groups.[16] Besides, alcohol use in this life stage, particularly the formation of unhealthy drinking habits, may have deleterious effects on health in later years.

Most research on the effects of alcohol on bone health has focused on middle-aged females over the age of 40 or postmenopausal women, when the excessive bone loss is the key concern rather than adequate PBM attainment. There are few studies on the association of alcohol use with the bone health of Korean women younger than 35 years of age, even though optimal bone growth and development typically occur in this life stage. The aim of this study, therefore, is to assess the effect of alcohol use on Korean young female adults bone health by drinking patterns, using national based data from the Korea National Health and Nutrition Examination Survey.

METHODS

The KNHANES is a cross-sectional survey conducted by the Korea Centers for Disease Control and Prevention and the Korean Ministry of Health and Welfare since 1998.

The survey was made up of three parts: a health interview survey, a health examination survey, and a nutrition survey. KNHANES represents a nationwide study of non-institutionalized civilians and used a stratified and multistage probability sampling design with a rolling survey-sampling model. Using a structured questionnaire, trained interviewers conducted face to face interviews.

Subjects

We used KNHANES data collected between 2008 and 2011. A total of 37,753 people (80.7% of the total target population of 46,777), all of whom provided written consent, participated in the survey and, 18,828 of them had their bone mineral density (BMD) measured. Among them, only female respondents aged from 19 to 30 years old were included in the present analysis. Those diagnosed with hypertension (n=5), hyperlipidemia (n=7), cardiac infarction/angina (n=1), arthritis (n=22), osteoarthritis (n=14), rheumarthiritis (n=10), osteoporosis (n=5), tuberculosis (n=21), asthma (n=35), renal failure (n=2), diabetes (n=7), thyrosis (n=29), stomach cancer (n=2), liver cancer (n=2), breast cancer (n=2), cervical cancer (n=2), other cancers (n=3), hepatitis B (n=6), hepatitis C (n=2), and thyroid gland cancer (n=4) were excluded. Pregnant women (n=2) were also excluded. Finally, a total of 1,183 subjects were selected for analysis in the present study.

Variables

Bone status variables

T-scores of bone mineral density of the total femur (TF), femur neck (FN) and lumbar spine (LB) was used as a continuous variable or as a binary variables (T-score≥-1, T-score< -1), to determine bone health status and characteristics of the subjects by bone status. According to WHO's standard, T-scores of ≥-1 are considered normal, -2.5<T-score<-1, osteopenia, and T-score≤-2.5, osteoporosis but, in this study, we categorized them into 2

groups: The normal (T-score≥-1) and low BMD groups (T-score<-1, osteopenia or osteoporosis). In order to measure BMD at these 3 sites, whole body dual-energy X-ray absorptiometry (DXA) was performed with a QDR Discovery (formerly known as the QDR 4500A) fan beam densitometer (Hologic, Inc., Bedford, MA, USA) following procedures recommended by the manufacturer. The results of DXA were analyzed using the standard techniques of the Korean Society of Osteoporosis and Hologic Discovery software (version 13.1).

Drinking variables

Drinking variables were assessed through the questions about whether they had ever drunk alcohol in their lifetime or not, frequency of alcohol consumption and amounts of alcohol consumed per occasion in the past year. In this study, abstainers were defined as those who never drank in her lifetime or who drank less than one per month with 1-2 glasses per occasion in the past year. Those who had not drunk at all only in the past year were excluded as missing values since the reason they stopped drinking could have been due to health problems which can have confounding effect on our analysis if included in the study. The drinking frequency was divided into 3 groups: less than once per month, monthly (more than once per month), weekly& daily (more than twice per week). For drinking amount, the number of glasses people drank per occasion was categorized into less than 4, 5-6 glasses and more than 7 glasses. In this study, 1 glass contains roughly 8g of pure alcohol which can be found in 220ml of regular beer with about 4.5% alcohol and 50ml of distilled spirits (soju) with about 19% alcohol. The amount of alcohol was computed as {amount of drink (ml) x volume of alcohol (%) x density of ethanol at room-temperature (0.8)}/100. With 8g of pure alcohol per glass, less than 4 glasses were considered equal to less than 32g of pure alcohol. In the analysis, those who drank either less than once per month or less than 4 glasses were

regarded as moderate drinkers. If the number of glasses they drank at one sitting was more than 5, more than one per week, they were defined as binge drinkers. AUDIT (Alcohol Use Disorders Identification Test) scores were also considered. The subjects were grouped according to their AUDIT scores: Abstinence or low risk drinking (0-7 points), more than low risk drinking (8-15 points), harmful and hazardous drinking (16-19 points) and alcohol dependence (20-40 points).

Other variables

We considered age, height, weight, BMI, starting age of smoking and drinking, physical activity, nutritional intake, age of menarche, family history of osteoporosis, oral contraceptive and female hormone use as potential confounding factors. The KNHANES health examination measured height and body weight, and body mass index (BMI) was calculated from the measured weight and height measurements as weight/height^2 (kg/m^2). Information for age, starting age of smoking and drinking, physical activity, age of menarche, family history of osteoporosis, oral contraceptive and female hormone use was examined through the health interview survey. Two types of physical activities were considered: "intense physical activity" for those who practice intense physical activity for more than 20 minutes at a time and more than 3 days per week and "intermediate physical activity" for those who practice intermediate physical activity for more than 30 minutes at a time and more than 5 days per week. All data for nutritional intake was collected by using a 24-hour dietary recall. Part of the health examination survey included the collection of blood samples which were used for biochemical measurements.

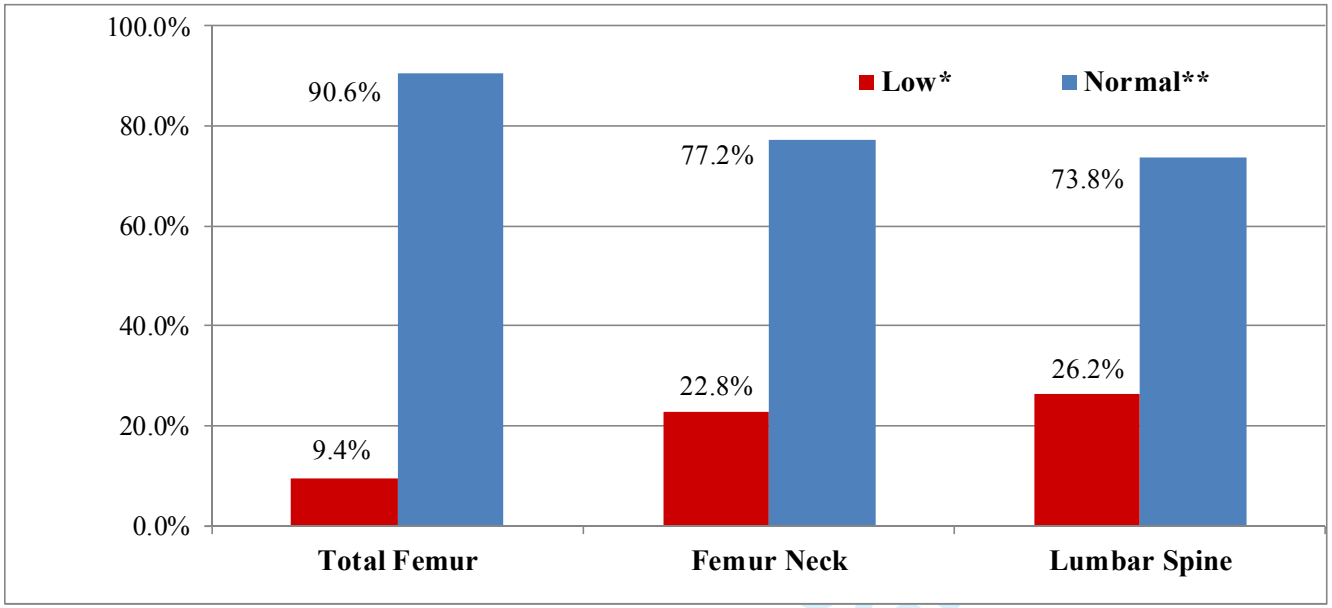
STATISTICAL ANALYSIS

Complex sample analysis was used in this study to correct the distributions of the cluster sample regarding the primary sampling unit, covariance and significance to correspond with those of the general Korean population. In order to compare means between the normal group (T-Score≥-1) and the low BMD group (T-score < -1, osteopenia or osteoporosis) at each 3 site, TF, TN and LB, the t-test was used and to compare proportions, the chi-square test was used. Analysis of covariance (ANCOVA) was used to compare the BMD levels (T-score) of subjects at the three sites by drinking patterns after adjusting for covariates. The covariates included age, height, BMI, starting age of smoking and drinking, blood creatinine and alkaline phosphatase. Logistic regression analysis was conducted to calculated odds ratio (OR) and 95 % confidence intervals (CI) for the association between AUDIT scores and the binary variable of BMD (T-score≥-1: Normal, T-score< -1: Low BMD) at the three sites. All statistical tests were two-tailed, and statistical significance was defined as $p<0.05$. The statistical calculation was performed with SPSS Statistics ver.18 (SPSS, Chicago., IL).

RESULTS

In this sample of 1,183 Korean young women, the mean age was 24.68(±0.12), height 161.38 (±0.21) and BMI 21.50 (±0.13). Among them, 78.9% are drinkers and 14.1% are binge drinkers. Both starting age of smoking and drinking was around 18 years old. The average BMD T-score (±SE) were 0.223(±0.032), -0.273(±0.036) and -0.399(±0.034) at TF, FN and LB, respectively. 9.4% of them have low BMD (either osteopenia or osteoporosis) in TF, 22.8% in FN and 26.2% in LB. (Fig.1)

Figure 1. Distribution of Low BMD of Total Femur, Femur Neck and Lumbar Spine among the subjects



	Total Femur			Femur Neck			Lumbar Spine		
	N	%	S.E	N	%	S.E	N	%	S.E
Low*	106	9.4%	1.0%	241	22.8%	1.4%	299	26.2%	1.5%
Normal**	1,049	90.6%	1.0%	815	77.2%	1.4%	845	73.8%	1.5%
Total	**1,155**	**100.0%**	**0.0%**	**1,056**	**100.0%**	**0.0%**	**1,144**	**100.0%**	**0.0%**

* Low T-score<-1 indicating osteopenia or osteoporosis

**Normal T-score≥-1

General Characteristics of the subjects according to Bone status (Low: T-score<-1 vs Normal: T-score≥-1)

In Table 1, the anthropometric and behavioral characteristics of Korean young women aged 19 to 30 are presented according to bone health status. Low BMD was more frequent in younger women at both TF and LB but in older women at FN. Those who were shorter had significantly low BMD at both FN and LB. Lower weights and BMI were found in those women who had low BMD at all three sites, TF, FN and LB.

The blood tests revealed significantly higher levels of alkaline phosphatase among those with low BMD but no association was found between the levels of vitamin D and BMD at all three sites. Lower levels of blood creatinine were found in the subjects with low BMD at all the sites but the difference in LB was not statistically significant.

The behavioral variables demonstrated that, low BMD at the TF was significantly more common in the women who took in less vitamin A and carotene and started smoking at an earlier age. The portion of subjects who practiced intermediate physical activity was also lower among those with low BMD at TF. Unlike BMD at TF and FN, BMD at LB was associated with age of menarche, indicating that those who started their first period at a later age tend to have low BMD at LB. No association was found between BMD at all sites and daily total energy, protein, fat, carbohydrate, calcium, phosphorus, sodium, and potassium dietary intakes, family history of osteoporosis or fractures, intense physical activity practice and use of oral contraceptives and female hormones.

Table 1. Anthropometric and Behavioral Characteristic of Korean Young Women aged 19 to 30 according to Bone status (Low :T-score<-1 vs Normal:T-score≥-1)

	Total Femur					Femur Neck					Lumbar Spine				
	Low	±SE	Normal	±SE	P-value	Low	±SE	Normal	±SE	P-value	Low	±SE	Normal	±SE	P-value
Mean(±SE) Age (years)	22.96	(0.45)	24.85	(0.12)	0.000***	25.68	(0.22)	25.08	(0.13)	0.014*	24.19	(0.22)	24.89	(0.14)	0.000***
Mean(SE) Height (cm)	160.52	(0.61)	161.52	(0.22)	0.122	159.93	(0.38)	161.83	(0.25)	0.000***	160.57	(0.36)	161.62	(0.25)	0.020*
Mean(SE) Weight (kg)	49.35	(0.82)	56.67	(0.39)	0.000***	51.14	(0.48)	57.39	(0.44)	0.000***	51.02	(0.4)	57.85	(0.45)	0.000***
Mean (SE) BMI (kg/m^2)	19.17	(0.34)	21.71	(0.14)	0.000***	20.00	(0.18)	21.90	(0.16)	0.000***	19.79	(0.15)	22.13	(0.16)	0.000***
Mean (SE) Creatinine (mg/dL)	0.67	(0.01)	0.70	(0.00)	0.006**	0.68	(0.01)	0.70	(0.00)	0.026*	0.69	(0.01)	0.70	(0.00)	0.080
Mean (SE) Vitamine D (ng/mL)	14.28	(0.75)	15.11	(0.23)	0.265	15.28	(0.43)	15.01	(0.25)	0.576	15.24	(0.45)	14.99	(0.24)	0.580
Mean (SE) Alkaline Phosphatase (IU/L)	211.81	(7.2)	186.36	(1.85)	0.001***	196.79	(4.43)	184.48	(2.1)	0.009**	198.82	(3.6)	185.61	(2.17)	0.000***
Mean (SE) Energy intake (Kcal)	1,632.13	(77.9)	1,739.65	(27.19)	0.197	1,718.18	(52.31)	1,752.34	(30.7)	0.562	1,727.77	(49.72)	1,736.64	(29.88)	0.880
Mean (SE) Protein (g)	60.53	(3.55)	63.99	(1.26)	0.363	63.65	(2.22)	64.66	(1.43)	0.692	63.55	(2.09)	63.92	(1.42)	0.880
Mean (SE) Fat (g)	41.13	(2.49)	44.89	(1.09)	0.169	42.11	(1.63)	45.48	(1.25)	0.101	44.12	(1.78)	44.79	(1.22)	0.760
Mean (SE) Carbohydrate (g)	251.34	(12.68)	261.53	(3.98)	0.447	267.52	(8.57)	262.27	(4.45)	0.570	263.58	(8.01)	260.31	(4.2)	0.710
Mean (SE) Calcium (mg)	426.38	(35.09)	444.60	(10.24)	0.621	472.24	(22.2)	449.39	(11.9)	0.354	456.07	(19.07)	438.03	(11.03)	0.400
Mean (SE) Phosphorus (mg)	961.01	(52.7)	999.25	(17.19)	0.492	1,032.87	(34.57)	1,008.21	(19.47)	0.520	1,001.97	(30.16)	997.47	(19.17)	0.900
Mean (SE) Sodium (mg)	3,637.97	(246.4)	4,102.15	(103.81)	0.089	4,216.99	(249.91)	4,093.89	(111.01)	0.646	3,988.63	(167)	4,101.63	(118.51)	0.580
Mean (SE) Potassium (mg)	2,395.65	(127.99)	2,520.04	(46.17)	0.370	2,600.93	(89.98)	2,536.60	(49.88)	0.507	2,490.08	(78.19)	2,526.49	(50.93)	0.690
Mean (SE) Vitamine A (μg)	571.16	(47.16)	715.32	(28.26)	0.008**	700.38	(56.13)	712.51	(29.38)	0.841	674.45	(49.87)	711.13	(30.93)	0.520
Mean (SE) Carotene (μg)	2,789.47	(266.72)	3,405.82	(152.47)	0.042*	3,412.39	(322.37)	3,357.77	(154.18)	0.874	3,389.01	(291.31)	3,332.80	(164.65)	0.860
Mean (SE) Menarche age (years)	13.09	(0.17)	13.09	(0.07)	0.989	13.24	(0.12)	13.11	(0.08)	0.312	13.36	(0.14)	12.99	(0.07)	0.020*
Mean (SE) Starting age of drinking (years)	17.87	(0.23)	18.05	(0.08)	0.463	18.42	(0.16)	18.05	(0.09)	0.045*	18.02	(0.13)	18.03	(0.09)	0.915
Mean (SE) Starting age of smoking (years)	16.47	(0.78)	18.13	(0.21)	0.040*	18.02	(0.57)	18.22	(0.22)	0.738	17.84	(0.49)	18.03	(0.23)	0.730
Number (%) Family History	11	(9.4%)	65	(5.2%)	0.082	18	(5.8%)	54	(5.5%)	0.854	25	(7.9%)	52	(5.0%)	0.080
Number (%) Practicing Intense Physical Activity[1)]	10	(10.3%)	137	(12.8%)	0.510	24	(10.6%)	110	(13.3%)	0.331	35	(13.2%)	112	(12.6%)	0.810
Number (%) Practicing Intermediate Physical Activity[2)]	5	(3.5%)	101	(9.2%)	0.035*	20	(7.6%)	80	(9.1%)	0.521	24	(6.8%)	80	(9.3%)	0.200
Number (%) Use of Oral Contraceptive	4	(4.6%)	113	(11.1%)	0.092	26	(11.4%)	89	(11.3%)	0.990	31	(9.8%)	86	(10.7%)	0.690
Number (%) Use of Female Hormone	0	(0%)	14	(1.3%)	0.293	2	(1.0%)	12	(1.4%)	0.730	3	(1.1%)	11	(1.2%)	0.830

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

1) Intense physical activity :those who practice intense physical activity for more than 20 minutes at a time and more than 3 days per week

Examples of Intense physical activity : Running, mountain hiking, fast cycling, fast swimming, Soccer, basketball, squash, single tennis, carrying /moving heavy loads etc

2) Intermediate physical activity:those who practice intermediate physical activity for more than 30 minutes at a time and more than 5 days per week

Examples of Intermediate physical activity : Slow swimming, double tennis, volleyball, badminton, ping-pong, carrying/moving light loads etc

Mean BMD T-score comparisons at the Total Femur, Femur Neck and Lumbar Spine according to drinking patterns and AUDIT scores.

Table 2 presents the average BMD T-scores at the TF, FN and LB according to drinking patterns and AUDIT scores of the subjects after adjustments for age, height, BMI, starting age of smoking and drinking, blood creatinine and alkaline phosphatase.

Lower T-scores were found at all sites in those who drank more frequently and more number of glasses. However, the trend was statistically significant by drinking frequency for the FN alone. In the intergroup comparison, significantly higher FN T-score was found in abstainers (Mean±S.E: 0.147±0.132) than in monthly (-0.309±0.100) and weekly (-0.280±0.118) drinkers and in those who drank 5-6 glasses (-0.333±0.151) and more than 7 glasses (-0.331±0.113) per occasion. At the TF, BMD T-scores of abstainers (0.535±0.158) was also higher than that of weekly drinkers (0.129±0.117).

There was also a decreasing trend in T-scores at all sites of females with greater AUDIT scores. The relationship was, however, statistically significant for the TF and FN but not for the LB. Especially, those in abstinence or low risk drinking had significantly higher T-scores of the TF and FN (TF: 0.432±0.099, FN:-0.031±0.099) than in harmful and hazardous drinking (-0.089±0.097, -0.570±0.131) and in alcohol dependence (-0.121±0.181, -0.648±0.173). Binge drinkers had significantly lower BMD T-scores than abstainers at the TF and FN. No significant association was observed between LB BMD T-scores and any drinking variables used in the study.

Table 2. Mean BMD T-score comparison at Total Femur, Femur Neck and Lumbar Spine according to drinking patterns and AUDIT scores after adjustment [1)]

	Total Femur				Femur Neck				Lumbar Spine			
	N	Avg.	S.E.	P-value	N	Avg.	S.E.	P-value	N	Avg.	S.E.	P-value
Drinking Frequency				**0.221**				**0.040***				**0.754**
Abstainer [2)]	**247**	0.535	0.158	.	232	0.147	0.132	.	243	-0.240	0.191	.
Less than 1 per month	142	0.394	0.215	0.587	126	0.000	0.207	0.537	139	-0.281	0.138	0.862
Monthly(more than 1 per month)	499	0.253	0.082	0.126	444	-0.309	0.100	0.009**	496	-0.318	0.093	0.720
Weekly(more than 2 per week)	129	0.129	0.117	0.041*	119	-0.280	0.118	0.014*	131	-0.411	0.087	0.416
Total N	1,017				921				1,009			
Drinking Amount				**0.127**				**0.053**				**0.858**
Abstainer [2)]	247	0.529	0.157	.	232	0.127	0.135	.	243	-0.247	0.192	.
Less than 4	365	0.407	0.122	0.529	331	-0.076	0.123	0.239	362	-0.281	0.120	0.884
5-6 glasses	185	0.131	0.144	0.065	165	-0.333	0.151	0.026*	185	-0.406	0.124	0.498
More than 7	226	0.172	0.088	0.056	199	-0.331	0.113	0.014*	225	-0.338	0.087	0.679
Total N	1,023				927				1,015			
Binge drinking				**0.031***				**0.013***				**0.366**
Abstainer [2)]	247	0.539	0.151	.	232	0.128	0.127	.	243	-0.228	0.182	.
Binge Drinkers	163	0.125	0.094	0.031*	148	-0.311	0.100	0.013*	164	-0.417	0.085	0.366
Total N	410				380				407			
Audit				**0.001*****				**0.002****				**0.174**
0-7 low risk drinking or abstinence	777	0.432	0.099	.	715	-0.031	0.099	.	767	-0.234	0.105	.
8-15 in excess of low risk drinking	230	0.265	0.103	0.258	208	-0.189	0.112	0.287	231	-0.273	0.093	0.790
16-19 harmful and hazarous drinking	41	-0.089	0.097	0.000***	32	-0.570	0.131	0.001***	41	-0.533	0.141	0.089
20-40 alcohol dependence	37	-0.121	0.181	0.010**	35	-0.648	0.173	0.003**	37	-0.521	0.139	0.121
Total N	1,085				990				1,076			

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

1) Adjusted for Age, Height, BMI, Creatinine, Alkaline Phosphatase, Starting age of smoking and Starting age of drinking

2) Abstainer = Lifetime nondrinkers or those who drank less than 1 per month with 1 or 2 glasses

Association between AUDIT score and Low BMD of Total Femur, Femur Neck and Lumbar Spine

Table 3 presents adjusted odd ratios and 95% confidence intervals for the associations between AUDIT scores and BMD T-score category, normal and low BMD groups. No significant association was found at all three sites, after adjustment for age, height and BMI. When adjusted for age, height, BMI, creatinine, alkaline phosphatase, starting age of smoking and drinking, however the chances of having low BMD (either osteopenia or osteoporosis) particularly, at the FN, significantly increased with higher AUDIT scores. The odds of having either osteopenia or osteoporosis at FN was OR 5.01(95% CI 1.32-18.95) for those in harmful drinking (AUDIT score: 16-19) and 7.47(95% CI 2.07-26.99) for those in alcohol dependence (20-40), compared to those who are in abstinence or low risk drinking categories (0-7).

Table 3. Association between AUDIT and Low BMD of Total Femur, Femur Neck and Lumbar Spine

Audit score	N	Adjusted Odd Ratio [1)]	95% CI (Low, Upper)		P-value	Adjusted Odd Ratio [2)]	95% CI (Low, Upper)		P-value
Total Femur					**0.860**				**0.932**
0-7 low risk drinking or abstinence	777	**Reference**				**Reference**			
8-15 in excess of low risk drinking	230	0.860	0.422	1.754	0.679	0.995	0.207	4.782	0.995
16-19 harmful and hazarous drinking	41	1.483	0.518	4.248	0.463	1.084	0.160	7.360	0.934
20--40 alcohol dependence	37	1.186	0.326	4.313	0.796	1.591	0.350	7.222	0.547
Femur Neck					**0.756**				**0.01****
0-7 low risk drinking or abstinence	715	**Reference**				**Reference**			
8-15 in excess of low risk drinking	208	0.910	0.569	1.457	0.695	2.401	0.773	7.456	0.129
16-19 harmful and hazarous drinking	32	1.485	0.562	3.928	0.425	5.011	1.325	18.954	0.02**
20--40 alcohol dependence	35	1.334	0.508	3.504	0.558	7.466	2.065	26.992	0.00***
Lumber Spine					**0.147**				**0.140**
0-7 low risk drinking or abstinence	767	**Reference**				**Reference**			
8-15 in excess of low risk drinking	231	0.681	0.449	1.031	0.069	0.385	0.133	1.120	0.080
16-19 harmful and hazarous drinking	41	1.421	0.666	3.032	0.363	1.232	0.427	3.554	0.699
20--40 alcohol dependence	37	1.469	0.657	3.284	0.348	1.435	0.400	5.144	0.579

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

Adjusted 1) : Age, Height, BMI

Adjusted 2) : Age, Height, BMI, Creatinine, Alkaline Phosphatase, Starting age of smoking and Starting age of drinking

DISCUSSION

Osteoporosis is the direct consequence of the failure to attain sufficient PBM in youth, typically before in the mid 30's, and/or excessive rate of bone loss in later years, suggesting that the risk of fragility fractures in the elderly can start from the first two decades of life. According to a previous study, sufficient bone accrual has even more effect on the probability of frangibility fracture in old age than the rate of bone loss.[15] Although PBM attainment is mainly attributable to genetic factors,[17] it can be affected by environmental factors such as alcohol consumption. The purpose of this study was to examine bone status (of the TF, FN and LB) of healthy Korean young women aged from 19 to 30 years of age by their drinking patterns.

We found that BMD at the three sites were different by age, weight, BMI and level of alkaline phosphatase. Lower level of creatinine was related to low BMD of TF and FN and lower height to that of LB and FN. Among 3 sites, only low BMD of TF showed strong correlation with low level of Vitamin A and carotene intake, less intermediate physical activity and earlier starting age of smoking, and older age at menarche was associated with low BMD at LB. No significant difference in BMD at 3 sites was found by the level of vitamin D and calcium and intense physical activity, three of well-known risk factors for osteoporosis. However, insufficient level of Vitamin D (15.02 ng/ml$\pm$0.23) and calcium (442.17mg$\pm$9.54) was found all over the subjects, according to the Vitamin D Council and the Institute of Medicine standard.[18-19] This observed low level of calcium intake can be part of the reason for the insignificant relation between physical activities and BMD with the possibility that the beneficial effect of physical activity on bone is manifest only through synergistic interaction with high calcium intake--over 1,000mg/day.[20]

After adjustment for eligible covariates, strong associations were found between the BMD T-scores of the TF and FN and alcohol use in young Korean women while no association was found at the LB. The BMD of the TF and FN tends to get lower with higher AUDIT scores and binge drinking. Those who drink more frequently are more likely to have lower BMD at the FN. This difference in FN BMD became more significant between abstainers and young women who were weekly, monthly and binge drinkers, and drank more than 5 glasses per occasion. Although not statistically significant, the study results also indicated that abstainers had higher BMD T-scores than any other group of drinkers at all three sites, indicating no beneficial effect of moderate drinking on bone.

Unlike our result, previous studies observed higher hip or spine BMD in women who drank moderately than in those who were abstainers and heavy drinkers.[6, 21-25] However, the optimal drinking amount for beneficial effect on bone cannot be defined since the threshold varies among studies; 8g alcohol/day,[23] 28-57g/week,[24] 11-29g/day,[25] more than 2 drinks/day[6], and more than 29 drinking occasions/month.[21] Additionally, this beneficial effect was observed mostly in postmenopausal but not in premenopausal women.[21, 26] A previous study also revealed increased hip and forearm fracture risk in premenopausal women with 5-24g per day drinking.[27] These conflicting results can be explained by the earlier reports that moderate alcohol intake can increase BMD levels indirectly by elevating estrogen levels whose dramatic decrease after menopause is major contributor to the rapid rate of bone loss in postmenopausal women.[28-29] The misclassification of abstainers can be another reason for this inconsistent result. The beneficial effect of alcohol use on BMD can be exaggerated by integrating lifetime abstainers with past drinkers who may have stopped drinking due to health concerns.[30]: A meta-analysis suggested that this benefit was observed in most studies with insufficient adjustment for major potential confounders, reflecting

confounding by unmeasured healthy behaviors.[31] Since the majority of previous studies have been conducted with postmenopausal women, more studies are needed with more precise and appropriate designs, to confirm our findings, especially effects of moderate drinking on the bone health of young women in their twenties.

In odd ratio analysis, the tendency to have osteopenia or osteoporosis at the FN was found more commonly in the women with higher AUDIT scores while this correlation was not observed at either the TF or LB. Particularly, those who were harmful drinkers (16-19) and alcohol dependent (20-40) were 5 and 7 times more likely to have low FN BMD than those who were low risk drinkers or abstinent (0-7), respectively. This finding should be considered critical because low BMD of the FN is highly related to increased risk of hip fractures[32-33] which is the most serious of all osteoporotic fractures, leading to high premature mortality and morbidity.[1] Its medical cost is also substantial with inevitable surgery and long hospital stays similar to the number of stays for cardiovascular disease, breast cancer and chronic obstructive pulmonary disease,[34] accounting for 63% of the total cost of all the osteoporotic fractures.[35]

Among Asian countries, Japan has the highest annual expenditures of over $4.9 billion for hip fracture care alone and the total cost for hip fractures within the first year after fracture in Singapore is projected to be $145 million in 2050.[36] Globally, aging populations continue to have increasing incidence of hip fractures, making hip fractures one of the most serious social and economic burdens in most countries, including Korea. The worldwide number of osteoporotic hip fractures is estimated to grow threefold from 1.7 million in 1990 to 6.3 million by 2050,[37] and over 50% of the hip fractures are expected to occur in Asia by 2050.[38] Hip fracture among Koreans have also been on the rise, especially in women over 50 years of age, with a 4.7% increase from 2001 to 2004 with a remarkable sixfold increase in

Honam province in the southern part of Korea for the last 13 years--1991 to 2004.[39] The reason for these rising trends in hip fractures, however, cannot be explained by the aging of the population alone, as many former studies reported that age-specific incidence is also growing.[40-42]

The detrimental effects of alcohol we observed, particularly on FN BMD, implies that the growing prevalence of alcohol consumption, especially, high-risk drinking (about 10% from 2005 to 2010) among Korean young women[16, 43] will be a major factor of increasing hip fracture incidence in near future. Compared to the ongoing increase in the prevalence of alcohol consumption among young women in Korea, the awareness of alcohol-related harm on women's health, including osteoporosis, is low[43] and the drinking, moreover, is becoming more and more socially acceptable among women: The main social supply of alcohol to Korean female high school students is mothers.[44] Consequently, it is crucial to provide Korean women, from teenagers to adults, with educational programs at the school and community levels to promote the awareness of alcohol-harm on bone health focusing on the attainment of PBM. At the same time, the deficiency of vitamin D and calcium among Korean young women also suggests that appropriate dietary guidelines need to be established for young people to prevent its adverse impact on bone health in later years.

The present study has several limitations. Firstly, this cross-sectional study cannot evaluate the causality between alcohol use and low BMD. Prospective studies are needed to clarify the relationship. Secondly, for drinking frequency and amount, only the past year's experience was considered and, therefore, the present results cannot fully reflect alcohol's effect on bones by the extent and duration of alcohol exposure. Lastly, our definition of abstainers can lead to biased result from previous studies. According to the threshold of moderate drinking in previous studies, however, less than one per month with 1-2 glasses at a

sitting, are small enough to be categorized into abstainers. Despite these limitations, the study has several strengths. Our study is the first study to investigate the effect of alcohol on young female bone health at the TF, FN and LB, using a sample population representative of Korean young women in their twenties. Additionally, the study was able to assess the adverse role of alcohol in bone development more accurately than previous studies by selecting only healthy Korean young female adults free of any disease, which can deteriorate BMD by influencing bone metabolism, such as diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, and various cancers.

In conclusion, low BMD of young Korean women was related with drinking frequency, amounts consumed and AUDIT scores, after adjusting for covariates. Of the three sites, this association was most evident in the FN: The more drinks, the lower BMD at the FN and with higher AUDIT scores the higher the chance of osteopenia or osteoporosis. Since alcohol's effect on bone is complex with cumulative effects of many factors on bone health over the years, and there is scarcity of studies with young women in twenties, rigorous prospective studies are needed focusing on the effects of alcohol on optimal bone mass attainment with carefully measured cofounders.

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Contributorship Statement: SS contributed to the study concept, analysis and interpretation. SS was the leading writer while SC and MY provided contributions to intellectual content for alcohol and nutrition variables, respectively, and MN assisted the writing of the manuscript and reviewed overall content. SS, SC, MN and MY approved the final version of the manuscript.

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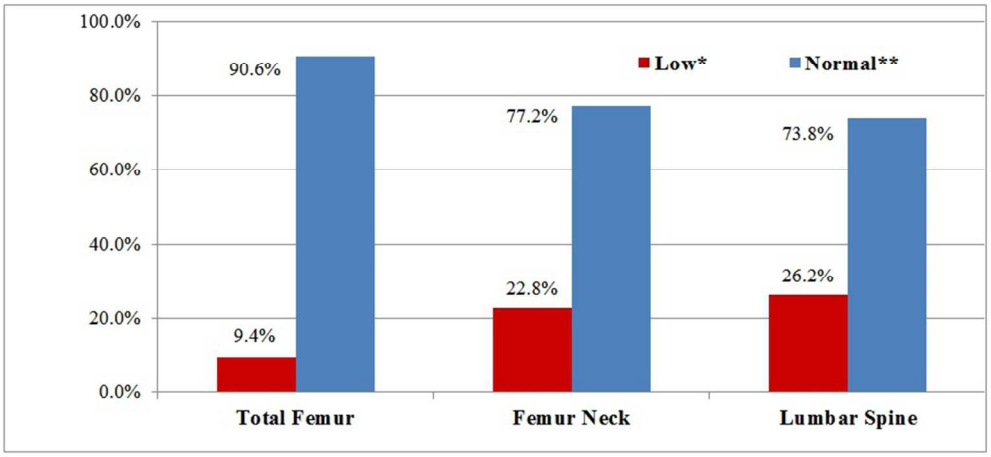
Figure 1. Distribution of Low BMD of Total Femur, Femur Neck and Lumbar Spine among the subjects



	Total Femur			Femur Neck			Lumbar Spine		
	N	%	S.E	N	%	S.E	N	%	S.E
Low*	106	9.4%	1.0%	241	22.8%	1.4%	299	26.2%	1.5%
Normal**	1,049	90.6%	1.0%	815	77.2%	1.4%	845	73.8%	1.5%
Total	**1,155**	**100.0%**	**0.0%**	**1,056**	**100.0%**	**0.0%**	**1,144**	**100.0%**	**0.0%**

* Low T-score<-1 indicating osteopenia or osteoporosis

**Normal T-score≥-1

242x180mm (96 x 96 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Confirmation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Y
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Y
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Y
Objectives	3	State specific objectives, including any prespecified hypotheses	Y
Methods			
Study design	4	Present key elements of study design early in the paper	Y
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Y
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Y
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Y
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Y
Bias	9	Describe any efforts to address potential sources of bias	Y
Study size	10	Explain how the study size was arrived at	Y
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Y
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Y
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Y
		(*e*) Describe any sensitivity analyses	N/A

Continued on next pag

Results			Confirmation
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Y
		(b) Indicate number of participants with missing data for each variable of interest	Y
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Y
		(*b*) Report category boundaries when continuous variables were categorized	Y
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Y
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Y
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Y
Generalisability	21	Discuss the generalisability (external validity) of the study results	Y
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

BMJ Open

The Association between alcohol consumption and Korean young women's bone health: Cross Sectional study from 2008-2011 Korea National Health and Nutrition Examination Survey

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Keywords:	AUDIT, PUBLIC HEALTH, EPIDEMIOLOGY

The Association between Alcohol Consumption and Korean Young Women's Bone Health: Cross-Sectional data from 2008-2011 Korea National Health and Nutrition Examination Survey

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Keywords: Alcohol, Bone Health, Osteoporosis, Femur Neck, Peak Bone Mass, Young women

Text Word Count: 3903

ABSTRACT

Objectives: To assess the association between alcohol and healthy Korean young women bone by AUDIT scores and drinking consumption; frequency and amount

Design: Cross-sectional study composed of three parts; health interview, health examination, nutrition survey

Setting: 2008-2011 Korea National Health and Nutrition Examination Survey

Participants: Of the 21,303 subjects whose bone mineral density (BMD) was assessed, n=1,176 healthy women aged 19 to 30 years old were selected.

Primary and secondary outcome measures: Mean BMD T-scores of total femur (TF), femur neck (FN) and lumbar spine (LB) by drinking consumption and AUDIT scores, and the odds of having low BMD (T-score <-1) at the sites by AUDIT scores

Results: After adjustment, the lower BMD were found at three sites in those who drank more and had higher AUDIT scores. These associations were significant by AUDIT scores at the TF (P=0.002) and FN (p=0.004) and by drinking frequency and amount at FN (p=0.029, p=0.039). The adjusted odds ratio of having low BMD increased significantly, particularly at FN, in those who had higher AUDIT scores such as 16-17 harmful drinking (OR 4.31; 95%CI 1.16 to 16.06) and 20-40 alcohol dependence (OR 5.99; 95%CI 1.69 to 21.21), compared with young women who scored 0-7 low risk drinking or abstinence. No beneficial effect of moderate drinking was observed at any of the sites and the association between alcohol and bone health was most evident at the FN.

Conclusions: It is crucial to promote the awareness of alcohol harm on Korean young women's bone health. At the same time, since alcohol's effect on bone is complex with cumulative effects of various factors over the years and there is an absence of studies with young women in their twenties, more studies, in particular for FN, are needed with more precise and appropriate design to confirm our findings.

STRENGTHS AND LIMITATIONS OF THIS STUDY

- The first study to investigate the association between alcohol and young female bone health at the total femur, femur neck and lumbar spine, using a nationwide sample data representative of Korean young women in their twenties.
- Only healthy Korean young women, free of diseases known to influence bone metabolism, were considered in the study.
- The study is limited by its cross-sectional nature.
- Drinking variables based on the past year's experience were limited in their ability to fully reflect the effect of alcohol on bones by the extent and duration of alcohol exposure.
- The small number of outcome cases decreased the precision of the association between alcohol use and Korean young women bone heath.

INTRODUCTION

Osteoporosis is a skeletal disorder characterized by the reduction of bone density and quality, leading to weakness of the skeleton and increased risk of fractures, especially of the wrist, spine and hip.[1-2] Osteoporotic fractures are an important cause of mortality and morbidity and a considerable financial burden on economies.[1] With the trend toward aging populations, osteoporosis is a major public health concern in many countries, including Korea.

In Korea, the proportion of people over 65 years of age was 7.2% in 2000 and is expected to reach 32.3% by 2040.[3] According to the recent 5-year (2007-2011) Korean osteoporosis patients statistics released in 2013 by the Health Insurance Review Agency (HIRA), Korea, 93.7% of the patients were 50 years or older and the growing rate of the number of the total patients was 44.3% with an annual growth rate of 9.7%. There was a particularly substantial increase in elderly patients over 70 years old with 75.2% increase during the same period with associated medical care costs of about 72 billion won (approximately 7.1million dollars) in 2011, an increase of 35% from 2007 with 7.9% annual growth.[4]

Even though osteoporosis is considered an age-related disease, it is also affected by many other factors such as weight, dietary factors, family history of osteoporosis, menopausal status, exercise, smoking and drinking. Heavy drinking, particularly, is known to have detrimental effects on bone density while the effect of light or moderate drinking on bones remains mixed: It can be beneficial for postmenopausal women but no benefit was found for premenopausal women.[5-7] Many human and animal studies indicate that alcohol consumption interrupts bone growth and replacement of bone tissue, causing increased bone fragility and susceptibility to fractures.[8-13] Influencing directly or indirectly on bone metabolism, alcohol consumption during adolescence and young adulthood, before the mid

30's, prevents the attainment of optimal Peak Bone Mass(PBM) which is a major contributor to the development of strong and healthy bones in later years.[14-15]

The Annual Korea National Health and Nutrition Examination Survey (KNHANES), however, suggests that alcohol consumption among Korean young women in their twenties was outstanding in every drinking indicator: High risk drinking (on average more than 5 glasses per occasion more than twice per week) and weekly binge drinking (on average more than 5 glasses at a sitting with more than once per week) rates in this group are 10.6% and 17.4%, higher than the 8%, 14.8% of all women aged over 19 years old respectively. The rates of their yearly and monthly drinking were also higher at 86.5% and 57.7%, respectively, than any other female age groups.[16] Besides, alcohol use in this life stage, particularly the formation of unhealthy drinking habits, may have deleterious effects on health in later years.

Most research on the effects of alcohol on bone health has focused on middle-aged females over the age of 40 or postmenopausal women, when the excessive bone loss is the key concern rather than adequate PBM attainment. There are few studies on the association of alcohol use with the bone health of Korean women younger than 35 years of age, even though optimal bone growth and development typically occur in this life stage. The aim of this study, therefore, is to assess the association between alcohol use and Korean young female adults bone health by drinking patterns, using national based data from the Korea National Health and Nutrition Examination Survey.

METHODS

The KNHANES is a cross-sectional survey conducted by the Korea Centers for Disease Control and Prevention and the Korean Ministry of Health and Welfare since 1998.

The survey was made up of three parts: a health interview survey, a health examination survey, and a nutrition survey. KNHANES represents a nationwide study of non-institutionalized civilians and used a stratified and multistage probability sampling design with a rolling survey-sampling model. Using a structured questionnaire, trained interviewers conducted face to face interviews.

Subjects

We used KNHANES data collected between 2008 and 2011. A total of 37,753 people (80.7% of the total target population of 46,777), all of whom provided written consent, participated in the survey and, 21,303 of them had their bone mineral density (BMD) measured. Among them, only female respondents aged from 19 to 30 years old and who completed the interview survey related to female health (n=1,315) were included in the present analysis. Those diagnosed with hypertension (n=5), hyperlipidemia (n=7), cardiac infarction/angina (n=1), arthritis (n=22), osteoarthritis (n=14), rheumarthiritis (n=10), osteoporosis (n=5), tuberculosis (n=21), asthma (n=35), renal failure (n=2), diabetes (n=7), thyrosis (n=29), stomach cancer (n=2), liver cancer (n=2), breast cancer (n=2), cervical cancer (n=2), other cancers (n=3), hepatitis B (n=6), hepatitis C (n=2), and thyroid gland cancer (n=4) were excluded. Pregnant women (n=2) were also excluded. Finally, a total of 1,176 subjects were selected for analysis in the present study.

Variables

Bone status variables

T-scores of bone mineral density of the total femur (TF), femur neck (FN) and lumbar spine (LB) was used as a continuous variable or as a binary variables (T-score≥-1, T-score< -1), to determine bone health status and characteristics of the subjects by bone status. According to WHO's standard, T-scores of ≥-1 are considered normal, -2.5<T-score<-1,

osteopenia, and T-score≤-2.5, osteoporosis but, in this study, we categorized them into 2 groups: The normal (T-score≥-1) and low BMD groups (T-score<-1, osteopenia or osteoporosis). In order to measure BMD at these three sites, whole body dual-energy X-ray absorptiometry (DXA) was performed with a QDR Discovery (formerly known as the QDR 4500A) fan beam densitometer (Hologic, Inc., Bedford, MA, USA) following procedures recommended by the manufacturer. The results of DXA were analyzed using the standard techniques of the Korean Society of Osteoporosis and Hologic Discovery software (version 13.1).

Drinking variables

Drinking variables were assessed through items which inquired about whether they had ever drunk at least a glass of alcohol in their lifetime or not, frequency of alcohol consumption and amounts of alcohol consumed per occasion in the past year. In this study, abstainers were defined as those who never drank in her lifetime or who drank less than one per month with 1-2 glasses per occasion in the past year. Those who had not drunk at all only in the past year were excluded as missing values since the reason they stopped drinking could have been due to health problems which could have had confounding effect on our analysis if included in the study. The drinking frequency was divided into 3 groups: less than once per month, monthly (more than once per month), weekly& daily (more than twice per week). For drinking amount, the number of glasses people drank per occasion was categorized into less than 4, 5-6 glasses and more than 7 glasses. In this study, 1 glass is equivalent to roughly 8g of pure alcohol which can be found in 220ml of regular beer with about 4.5% alcohol and 50ml of distilled spirits (soju) with about 19% alcohol. The amount of alcohol was computed as {amount of drink (ml) x volume of alcohol (%) x density of ethanol at room-temperature (0.8)}/100. With 8g of pure alcohol per glass, less than 4 glasses were considered equal to

less than 32g of pure alcohol. In the analysis, those who drank either less than once per month or less than 4 glasses were regarded as moderate drinkers. AUDIT (Alcohol Use Disorders Identification Test) scores were also considered. The subjects were grouped according to their AUDIT scores: Abstinence or low risk drinking (0-7 points), more than low risk drinking (8-15 points), harmful and hazardous drinking (16-19 points) and alcohol dependence (20-40 points).

Other variables

We considered age, height, weight, BMI, age of initiation of smoking, physical activity, nutritional intake, age of menarche, family history of osteoporosis, oral contraceptive and female hormone use as potential confounding factors. The KNHANES health examination measured height and body weight, and body mass index (BMI) was calculated from the measured weight and height measurements as weight/height^2 (kg/m^2). Information for age, age of initiation of smoking and drinking, physical activity, age of menarche, family history of osteoporosis, oral contraceptive and female hormone use was examined through the health interview survey. Lifetime smoking also was examined by asking "How many cigarettes have you smoked in your lifetime" (under or more than 100 or Never). All data for nutritional intake was collected by using a 24-hour dietary recall. Part of the health examination survey included the collection of blood samples which were used for biochemical measurements.

STATISTICAL ANALYSIS

Complex sample analysis was used in this study to correct the distributions of the cluster sample regarding the primary sampling unit, covariance and significance to

correspond with those of the general Korean population. In order to compare means between the normal group (T-Score≥-1) and the low BMD group (T-score < -1, osteopenia or osteoporosis) at each of the three sites, TF, TN and LB, the t-test was used and to compare proportions, the chi-square test was used. Analysis of covariance (ANCOVA) was used to compare the BMD levels (T-score) of subjects at the three sites by drinking patterns after adjusting for covariates. The covariates included age, height, BMI, age of initiation of smoking, blood creatinine and alkaline phosphatase. Logistic regression analysis was conducted to calculate odds ratio (OR) and 95 % confidence intervals (CI) for the association between AUDIT scores and the binary variable of BMD (T-score≥-1: Normal, T-score< -1: Low BMD) at TF and FN. All statistical tests were two-tailed, and statistical significance was defined as $p<0.05$. The statistical calculation was performed with SPSS Statistics ver.18 (SPSS, Chicago., IL).

RESULTS

In this sample of 1,176 Korean young women, the mean age was 24.68(±0.12), height 161.38 (±0.21) and BMI 21.50 (±0.13). Among them, 95.07% are lifetime drinkers and 22.04% lifetime smokers. Age of initiation of both drinking and smoking was around 18 years old. The average BMD T-score (±SE) were 0.223(±0.032), -0.273(±0.036) and -0.399(±0.034) at TF, FN and LB, respectively. 9.4% of them have low BMD (either osteopenia or osteoporosis) at TF, 22.8% at FN and 26.2% at LB. (Fig.1)

General Characteristics of the subjects according to Bone status (Low: T-score<-1 vs Normal: T-score≥-1)

In Table 1, the anthropometric and behavioral characteristics of Korean young women aged 19 to 30 are presented according to bone health status. Low BMD was more frequent in younger women at both TF and LB but in older women at FN. Those who were shorter had significantly low BMD at both FN and LB. Lower weights and BMI were found in those women who had low BMD at all three sites.

The blood tests revealed significantly higher levels of alkaline phosphatase among those with low BMD but no association was found between the levels of vitamin D and BMD at all three sites. Lower levels of blood creatinine were found in the subjects with low BMD at all the sites but the difference in LB was not statistically significant.

The behavioral variables demonstrated that, low BMD at the TF was significantly more common in the women who took in less vitamin A and carotene and started smoking at an earlier age. The portion of subjects who practiced intermediate physical activity was also lower among those with low BMD at TF. Unlike BMD at TF and FN, BMD at LB was associated with age of menarche, indicating that those who started their first period at a later age tend to have low BMD at LB. No association was found between BMD at all sites and calcium, phosphorus, sodium, and potassium dietary intakes, family history of osteoporosis or fractures, intense physical activity practice and use of oral contraceptives and female hormones.

Table 1. Anthropometric and Behavioral Characteristic of Korean Young Women aged 19 to 30 according to Bone status (Low :T-score<-1 vs Normal:T-score≥-1

	Total Femur					Femur Neck					Total Femur				
	Low	±SE	Normal	±SE	P-value	Low	±SE	Normal	±SE	P-value	Low	±SE	Normal	±SE	P-value
Mean(±SE) Age (years)	22.96	(0.45)	24.85	(0.12)	0.000***	25.68	(0.22)	25.08	(0.13)	0.014*	24.19	(0.22)	24.89	(0.14)	0.000***
Mean(SE) Height (cm)	160.52	(0.61)	161.52	(0.22)	0.122	159.93	(0.38)	161.83	(0.25)	0.000***	160.57	(0.36)	161.62	(0.25)	0.020*
Mean(SE) Weight (kg)	49.35	(0.82)	56.67	(0.39)	0.000***	51.14	(0.48)	57.39	(0.44)	0.000***	51.02	(0.4)	57.85	(0.45)	0.000***
Mean (SE) BMI (kg/m^2)	19.17	(0.34)	21.71	(0.14)	0.000***	20.00	(0.18)	21.90	(0.16)	0.000***	19.79	(0.15)	22.13	(0.16)	0.000***
Mean (SE) Creatinine (mg/dL)	0.67	(0.01)	0.70	(0.00)	0.006**	0.68	(0.01)	0.70	(0.00)	0.026*	0.69	(0.01)	0.70	(0.00)	0.080
Mean (SE) Vitamine D (ng/mL)	14.28	(0.75)	15.11	(0.23)	0.265	15.28	(0.43)	15.01	(0.25)	0.576	15.24	(0.45)	14.99	(0.24)	0.580
Mean (SE) Alkaline Phosphatase (IU/L)	211.81	(7.2)	186.36	(1.85)	0.001***	196.79	(4.43)	184.48	(2.1)	0.009**	198.82	(3.6)	185.61	(2.17)	0.000***
Mean (SE) Calcium (mg)	426.38	(35.09)	444.60	(10.24)	0.621	472.24	(22.2)	449.39	(11.9)	0.354	456.07	(19.07)	438.03	(11.03)	0.400
Mean (SE) Phosphorus (mg)	961.01	(52.7)	999.25	(17.19)	0.492	1,032.87	(34.57)	1,008.21	(19.47)	0.520	1,001.97	(30.16)	997.47	(19.17)	0.900
Mean (SE) Sodium (mg)	3,637.97	(246.4)	4,102.15	(103.81)	0.089	4,216.99	(249.91)	4,093.89	(111.01)	0.646	3,988.63	(167)	4,101.63	(118.51)	0.580
Mean (SE) Potassium (mg)	2,395.65	(127.99)	2,520.04	(46.17)	0.370	2,600.93	(89.98)	2,536.60	(49.88)	0.507	2,490.08	(78.19)	2,526.49	(50.93)	0.690
Mean (SE) Vitamine A (μg)	571.16	(47.16)	715.32	(28.26)	0.008**	700.38	(56.13)	712.51	(29.38)	0.841	674.45	(49.87)	711.13	(30.93)	0.520
Mean (SE) Carotene (μg)	2,789.47	(266.72)	3,405.82	(152.47)	0.042*	3,412.39	(322.37)	3,357.77	(154.18)	0.874	3,389.01	(291.31)	3,332.80	(164.65)	0.860
Mean (SE) Menarche age (years)	13.09	(0.17)	13.09	(0.07)	0.989	13.24	(0.12)	13.11	(0.08)	0.312	13.36	(0.14)	12.99	(0.07)	0.020*
Mean (SE) Starting age of drinking (years)	17.87	(0.23)	18.05	(0.08)	0.463	18.42	(0.16)	18.05	(0.09)	0.045*	18.02	(0.13)	18.03	(0.09)	0.915
Mean (SE) Starting age of smoking (years)	16.47	(0.78)	18.13	(0.21)	0.040*	18.02	(0.57)	18.22	(0.22)	0.738	17.84	(0.49)	18.03	(0.23)	0.730
Number (%) Family History	11	(9.4%)	65	(5.2%)	0.082	18	(5.8%)	54	(5.5%)	0.854	25	(7.9%)	52	(5.0%)	0.080
Number (%) Practicing Intense Physical Activity[1)]	10	(10.3%)	137	(12.8%)	0.510	24	(10.6%)	110	(13.3%)	0.331	35	(13.2%)	112	(12.6%)	0.810
Number (%) Practicing Intermediate Physical Activity[2)]	5	(3.5%)	101	(9.2%)	0.035*	20	(7.6%)	80	(9.1%)	0.521	24	(6.8%)	80	(9.3%)	0.200
Number (%) Use of Oral Contraceptive	4	(4.6%)	113	(11.1%)	0.092	26	(11.4%)	89	(11.3%)	0.990	31	(9.8%)	86	(10.7%)	0.690
Number (%) Use of Female Hormone	0	(0%)	14	(1.3%)	0.293	2	(1.0%)	12	(1.4%)	0.730	3	(1.1%)	11	(1.2%)	0.830

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

1) Intense physical activity :those who practice intense physical activity for more than 20 minutes at a time and more than 3 days per week
Examples of Intense physical activity : Running, mountain hiking, fast cycling, fast swimming, Soccer, basketball, squash, single tennis, carrying /moving heavy loads etc

2) Intermediate physical activity:those who practice intermediate physical activity for more than 30 minutes at a time and more than 5 days per week
Examples of Intermediate physical activity : Slow swimming, double tennis, volleyball, badminton, ping-pong, carrying/moving light loads etc

Mean BMD T-score comparisons at the Total Femur, Femur Neck and Lumbar Spine according to drinking consumption and AUDIT scores.

Table 2 presents the average BMD T-scores at the TF, FN and LB according to drinking consumption and AUDIT scores of the subjects after adjustments.

Lower T-scores were found at all sites in those who drank more frequently and more number of glasses. However, the trend was statistically significant by both drinking frequency ($p=0.029$) and amount ($p=0.039$) for the FN alone. Although the decreasing trend by drinking frequency was not significant at TF, BMD T-scores of abstainers (0.519 ± 0.152) was higher than that of weekly drinkers (0.141 ± 0.117) in its intergroup comparison.

There was also a decreasing trend in T-scores at all sites with greater AUDIT scores. The relationship was, however, statistically significant for the TF and FN but not for the LB. Especially, those in abstinence or low risk drinking had significantly higher T-scores of the TF and FN (TF: 0.414 ± 0.096, FN:-0.050 ± 0.099) than in harmful and hazardous drinking (-0.077 ± 0.100, -0.568 ± 0.133) and in alcohol dependence (-0.110 ± 0.189, -0.626 ± 0.176). No significant difference was observed in LB BMD T-scores by any drinking variables used in the study.

Table 2. Mean BMD T-score comparison at Total Femur, Femur Neck and Lumbar Spine according to drinking patterns and AUDIT scores after adjustment [1)]

	Total Femur				Femur Neck				Lumbar Spine			
	N	Avg.	S.E.	P-value	N	Avg.	S.E.	P-value	N	Avg.	S.E.	P-value
Drinking Frequency				**0.245**				**0.029***				**0.935**
Abstainer [2)]	**247**	0.519	0.152	.	232	0.119	0.121	.	243	-0.300	0.183	.
Less than 1 per month	142	0.369	0.211	0.561	126	-0.017	0.202	0.561	139	-0.326	0.136	0.912
Monthly(more than 1 per month)	499	0.257	0.081	0.136	444	-0.305	0.099	0.008**	496	-0.312	0.092	0.954
Weekly(more than 2 per week)	129	0.141	0.117	0.047*	119	-0.269	0.116	0.014*	131	-0.388	0.091	0.670
Total N	1,017				921				1,009			
Drinking Amount				**0.122**				**0.039***				**0.882**
Abstainer [2)]	247	0.517	0.151	.	232	0.104	0.124	.	243	-0.303	0.184	.
Less than 4	365	0.408	0.122	0.570	331	-0.075	0.124	0.285	362	-0.280	0.122	0.918
5-6 glasses	185	0.129	0.142	0.066	165	-0.334	0.150	0.029*	185	-0.407	0.121	0.645
More than 7	226	0.179	0.084	0.052	199	-0.324	0.107	0.009**	225	-0.324	0.087	0.920
Total N	1,023				927				1,015			
Audit				**0.002****				**0.004****				**0.228**
0-7 low risk drinking or abstinence	777	0.414	0.096	.	715	-0.050	0.099	.	767	-0.262	0.104	.
8-15 in excess of low risk drinking	230	0.284	0.100	0.357	208	-0.169	0.112	0.418	231	-0.243	0.098	0.898
16-19 harmful and hazarous drinking	41	-0.077	0.100	0.001***	32	-0.568	0.133	0.002**	41	-0.522	0.144	0.148
20-40 alcohol dependence	37	-0.110	0.189	0.019*	35	-0.626	0.176	0.007**	37	-0.504	0.141	0.198
Total N	1,085				990				1,076			

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

1) Adjusted for Age, Height, BMI, Creatinine, Alkaline Phosphatase and Age of initiation of smoking

2) Abstainer = Lifetime nondrinkers or those who drank less than 1 per month with 1 or 2 glasses

Association between AUDIT scores and LowBMD (T-score<-1) of Total Femur and Femur Neck

Table 3 presents adjusted OR and 95% confidence intervals for the associations between AUDIT scores and BMD T-score category, normal and low BMD groups. No significant association was found at either site, after adjustment for age, height and BMI. When adjusted for age, height, BMI, creatinine, alkaline phosphatase and age of initiation of smoking, however the chances of having low BMD particularly, at the FN, significantly increased with higher AUDIT scores. The odds of having either osteopenia or osteoporosis at FN was OR 4.31(95% CI 1.16-16.06) for those in harmful drinking (AUDIT score: 16-19) and 5.99(95% CI 1.69-21.21) for those in alcohol dependence (20-40), compared to those who are in abstinence or low risk drinking categories (0-7).

Table 3. Association between AUDIT and Low BMD(T-score<-1) of Total Femur and Femur Neck

Audit score	N	Adjusted Odd Ratio [1)]	95% CI (Low, Upper)		P-value	Adjusted Odd Ratio[2)]	95% CI (Low, Upper)		P-value
Total Femur					**0.860**				**0.926**
0-7 low risk drinking or abstinence	777	**Reference**				**Reference**			
8-15 in excess of low risk drinking	230	0.860	0.422	1.754	0.679	0.926	0.233	3.677	0.913
16-19 harmful and hazarous drinking	41	1.483	0.518	4.248	0.463	0.922	0.162	5.237	0.927
20--40 alcohol dependence	37	1.186	0.326	4.313	0.796	1.549	0.355	6.755	0.560
Femur Neck					**0.756**				**0.024***
0-7 low risk drinking or abstinence	715	**Reference**				**Reference**			
8-15 in excess of low risk drinking	208	0.910	0.569	1.457	0.695	1.992	0.672	5.905	0.213
16-19 harmful and hazarous drinking	32	1.485	0.562	3.928	0.425	4.311	1.158	16.059	0.029*
20--40 alcohol dependence	35	1.334	0.508	3.504	0.558	5.990	1.692	21.208	0.006**

Statistical significance *p<=0.05, **p<=0.01, ***p<=0.001

Adjusted 1) : Age, Height, BMI

Adjusted 2) : Age, Height, BMI, Creatinine, Alkaline Phosphatase and Age of initiation of smoking

DISCUSSION

Osteoporosis is the direct consequence of the failure to attain sufficient PBM in youth, typically before in the mid 30's, and/or excessive rate of bone loss in later years, suggesting that the risk of fragility fractures in the elderly can start from the first two decades of life. According to a previous study, sufficient bone accrual has even more effect on the probability of frangibility fracture in old age than the rate of bone loss.[15] Although PBM attainment is mainly attributable to genetic factors,[17] it can be affected by environmental factors such as alcohol consumption. The purpose of this study was to examine bone status (of the TF, FN and LB) of healthy Korean young women aged from 19 to 30 years of age by their alcohol use.

We found that BMD at the three sites was different by age, weight, BMI and level of alkaline phosphatase. Lower level of creatinine were related to low BMD of TF and FN and lower height to that of LB and FN. Of the three sites, only low BMD of TF showed strong correlation with low level of Vitamin A and carotene intake, less intermediate physical activity and earlier age of initiation of smoking, and older age at menarche was associated with low BMD at LB. No significant difference in BMD at the three sites was found by the level of vitamin D and calcium and intense physical activity, three of well-known risk factors for osteoporosis. Overall, insufficient level of Vitamin D (15.02 ng/ml$\pm$0.23) and calcium (442.17mg$\pm$9.54) was found among the subjects, according to the Vitamin D Council and the Institute of Medicine standard.[18-19] This observed low level of calcium intake can be part of the reason for the insignificant relation between physical activities and BMD with the possibility that the beneficial effect of physical activity on bone is manifest only through synergistic interaction with high calcium intake--over 1,000mg/day.[20]

After adjustment for eligible covariates, the different BMD T-scores by alcohol use were found at TF and FN in young Korean women while no difference was found at the LB. The BMD of the TF and FN tends to get lower with higher AUDIT scores. Those who drink more frequently are more likely to have lower BMD at the FN. This difference in FN BMD became more significant between abstainers and young women who were weekly and monthly drinkers, and drank more than 5 glasses per occasion. There was no significantly higher BMD of moderate drinkers at all three sites than that of abstainers.

Unlike our result, previous studies observed higher hip or spine BMD in women who drank moderately than in those who were abstainers and heavy drinkers.[6, 21-25] However, the optimal drinking amount for beneficial effect on bone cannot be defined since the threshold varies among studies; 8g alcohol/day,[23] 28-57g/week,[24] 11-29g/day,[25] more than 2 drinks/day[6], and more than 29 drinking occasions/month.[21] Additionally, this beneficial effect was observed mostly in postmenopausal but not in premenopausal women.[21, 26] A previous study also revealed increased hip and forearm fracture risk in premenopausal women with 5-24g per day drinking.[27] These conflicting results can be explained by the earlier reports that moderate alcohol intake can increase BMD levels indirectly by elevating estrogen levels whose dramatic decrease after menopause is major contributor to the rapid rate of bone loss in postmenopausal women.[28-29] The misclassification of abstainers can be another reason for this inconsistent result. The beneficial effect of alcohol use on BMD can be exaggerated by integrating lifetime abstainers with past drinkers who may have stopped drinking due to health concerns.[30]: A meta-analysis suggested that this benefit was observed in most studies with insufficient adjustment for major potential confounders, reflecting confounding by unmeasured healthy behaviors.[31]

Our findings also support the idea that the skeletal responsiveness to alcohol may differ by site as well as age. [32-33] Compared to TF and FN BMD, no significant difference in LB BMD by alcohol use was found in this study. The different result can be explained by animal studies whose results implied that the alcohol-related bone deficiencies during adolescence and young adulthood may be caused by decreasing the activity of growth plate at the end of femur, Insulin-like Growth Factor 1(IGF-1) levels in the blood and maturity of the bone, rather than a loss of bone itself. [9, 34-35] Since the majority of previous studies have been conducted with postmenopausal women, more studies are needed with more precise and appropriate designs, to confirm our findings, especially the effects of moderate drinking on bone health and the more detrimental effect of alcohol on femur than lumbar BMD of young Korean women in their twenties.

In OR analysis, the tendency to have osteopenia or osteoporosis at the FN was found more commonly in the women with higher AUDIT scores while this correlation was not observed at the TF. Particularly, those who were harmful drinkers (16-19) and alcohol dependent (20-40) were 4 and 6 times more likely to have low FN BMD than those who were low risk drinkers or abstinent (0-7), respectively. This finding should be considered critical because low BMD of the FN is highly related to increased risk of hip fractures[36-37] which is the most serious of all osteoporotic fractures, leading to high premature mortality and morbidity.[1] Its medical cost is also substantial with inevitable surgery and long hospital stays similar to the number of stays for cardiovascular disease, breast cancer and chronic obstructive pulmonary disease,[38] accounting for 63% of the total cost of all the osteoporotic fractures.[39]

Among Asian countries, Japan has the highest annual expenditures of over $4.9 billion for hip fracture care alone and the total cost for hip fractures within the first year after

fracture in Singapore is projected to be $145 million in 2050. [40] Globally, aging populations continue to have increasing incidence of hip fractures, making hip fractures one of the most serious social and economic burdens in most countries, including Korea. The worldwide number of osteoporotic hip fractures is estimated to grow threefold from 1.7 million in 1990 to 6.3 million by 2050,[41] and over 50% of the hip fractures are expected to occur in Asia by 2050.[42] Hip fracture among Koreans have also been on the rise, especially in women over 50 years of age, with a 4.7% increase from 2001 to 2004 with a remarkable sixfold increase in Honam province in the southern part of Korea for the last 13 years--1991 to 2004.[43] The reason for these rising trends in hip fractures, however, cannot be explained by the aging of the population alone, as many former studies reported that age-specific incidence is also growing.[44-46]

The detrimental association, we observed, between alcohol and FN BMD implies that the growing prevalence of alcohol consumption, especially, high-risk drinking (about 10% from 2005 to 2010) among Korean young women [16, 47] will be a major factor of increasing hip fracture incidence in near future. Compared to the ongoing increase in the prevalence of alcohol consumption among young women in Korea, the awareness of alcohol-related harm on women's health, including osteoporosis, is low[47] and the drinking, moreover, is becoming more and more socially acceptable among women: The main social supply of alcohol to Korean female high school students is mothers.[48] Consequently, it is crucial to provide Korean women, from teenagers to adults, with educational programs at the school and community levels to promote the awareness of alcohol-harm on bone health focusing on the attainment of PBM. At the same time, the deficiency of vitamin D and calcium among Korean young women also suggests that appropriate dietary guidelines need to be established for young people to prevent its adverse impact on bone health in later years.

The present study has several limitations. Firstly, this cross-sectional study cannot evaluate the causality between alcohol use and low BMD. Prospective studies are needed to clarify the relationship. Secondly, for drinking frequency and amount, only the past year's experience was considered and, therefore, the present results cannot fully reflect alcohol's effect on bones by the extent and duration of alcohol exposure. Thirdly, our definition of abstainers can lead to biased result from previous studies. According to the threshold of moderate drinking in previous studies, however, less than one per month with 1-2 glasses at a sitting, are small enough to be categorized into abstainers. Lastly, self-reported alcohol intake, AUDIT scores and smoking status may be underreported due to recalling and social desirability bias. [49, 50] A relatively small number of smokers and a small number of the outcome cases decreased the precision of the OR estimate in the study. A larger study with more cases should be considered for more precise estimate of the association between alcohol use and young Korean women bone health. Despite these limitations, the study has several strengths. Our study is the first study to investigate the association between alcohol and young female bone health at the TF, FN and LB, using a sample population representative of Korean young women in their twenties. Additionally, the study was able to assess the adverse role of alcohol in bone development more accurately than previous studies by selecting only healthy Korean young female adults free of any disease, which can deteriorate BMD by influencing bone metabolism, such as diabetes mellitus, hypertension, dyslipidemia, chronic kidney disease, and various cancers.

In conclusion, low BMD of young Korean women was related with drinking frequency, amounts consumed and AUDIT scores, after adjusting for covariates. Of the three sites, this association was most evident in the FN: The more drinks, the lower BMD at the FN and with higher AUDIT scores the higher the chance of osteopenia or osteoporosis. Since

alcohol's effect on bone is complex with cumulative effects of many factors on bone health over the years, and there is scarcity of studies with young women in twenties, rigorous prospective studies are needed focusing on the effects of alcohol on optimal bone mass attainment with carefully measured cofounders.

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Competing Interests: There are no competing interests.

Contributorship Statement: SS contributed to the study concept, analysis and interpretation. SS was the leading writer while SC and MY provided contributions to intellectual content for alcohol and nutrition variables, respectively, and MN assisted the writing of the manuscript and reviewed overall content. SS, SC, MN and MY approved the final version of the manuscript.

Data sharing: It is cross-sectional panel data from Korea National Health and Nutrition Examination Survey done by Korea Centers for Disease Control and Prevention and Korean Ministry of Health and Welfare.

The data, therefore, are available to everybody from the following website;

https://knhanes.cdc.go.kr/knhanes/index.do

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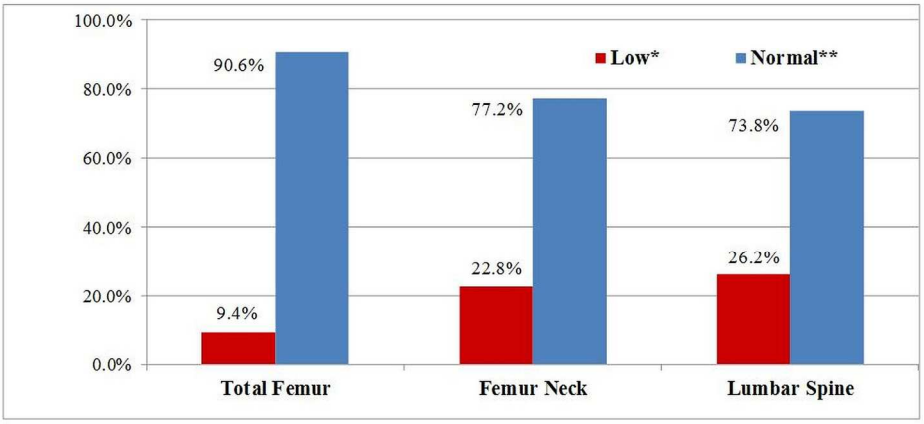
Figure 1. Distribution of Low BMD of Total Femur, Femur Neck and Lumbar Spine among the subjects



	Total Femur			Femur Neck			Lumbar Spine		
	N	%	S.E	N	%	S.E	N	%	S.E
Low*	106	9.4%	1.0%	241	22.8%	1.4%	299	26.2%	1.5%
Normal**	1,049	90.6%	1.0%	815	77.2%	1.4%	845	73.8%	1.5%
Total	**1,155**	**100.0%**	**0.0%**	**1,056**	**100.0%**	**0.0%**	**1,144**	**100.0%**	**0.0%**

* Low T-score<-1 indicating osteopenia or osteoporosis

**Normal T-score≥-1

210x150mm (300 x 300 DPI)

STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Confirmation
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	Y
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	Y
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	Y
Objectives	3	State specific objectives, including any prespecified hypotheses	Y
Methods			
Study design	4	Present key elements of study design early in the paper	Y
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Y
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	Y
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	N/A
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	Y
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Y
Bias	9	Describe any efforts to address potential sources of bias	Y
Study size	10	Explain how the study size was arrived at	Y
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	Y
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	Y
		(*b*) Describe any methods used to examine subgroups and interactions	N/A
		(*c*) Explain how missing data were addressed	N/A
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	Y
		(*e*) Describe any sensitivity analyses	N/A

Continued on next pag

Results			Confirmation
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	N/A
		(b) Give reasons for non-participation at each stage	N/A
		(c) Consider use of a flow diagram	N/A
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	Y
		(b) Indicate number of participants with missing data for each variable of interest	Y
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	N/A
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	N/A
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	Y
		(*b*) Report category boundaries when continuous variables were categorized	Y
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	N/A
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	N/A
Discussion			
Key results	18	Summarise key results with reference to study objectives	Y
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Y
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Y
Generalisability	21	Discuss the generalisability (external validity) of the study results	Y
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	N/A

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.