Table IV. BMD and other characteristics for postmenopausal women (n=816) according to *FOXC2* genotype.[a]

Characteristic	CC	CT	TT	CT+TT
Number (%)	384 (47.1)	352 (43.1)	80 (9.8)	736 (90.2)
Age (years)	64.0±0.4	63.8±0.5	64.1±1.0	63.9±0.3
Height (cm)	150.0±0.3	150.2±0.3	151.0±0.7	150.1±0.2
Body weight (kg)	51.9±0.4	51.9±0.4	52.4±0.9	51.9±0.3
BMD measured with pQCT (mg/cm^3)				
D50	165.4±3.3	164.9±3.4	149.1±7.2	165.2±2.3[b]
D100	442.7±4.7	447.0±4.8	426.7±10.4	444.8±3.4
P100	1079.4±7.8	1083.7±8.0	1063.0±17.2	1081.4±5.6
BMD measured with DXA (g/cm^2)				
Total body	0.921±0.004	0.922±0.005	0.901±0.010	0.92±0.003[c]
L2-L4	0.810±0.007	0.811±0.007	0.795±0.014	0.810±0.005
Femoral neck	0.646±0.004	0.645±0.004	0.629±0.009	0.646±0.003
Trochanter	0.543±0.004	0.539±0.004	0.531±0.009	0.541±0.003

[a]BMD is adjusted for age, height and body weight. Data are means ± SE. [b]P = 0.0342, [c]P = 0.0492 versus TT.

Table V. BMD and other characteristics for all men (n=1122) according to *PLIN* genotype.[a]

Characteristic	CC	CT	TT	CC+CT
Number (%)	630 (56.1)	418 (37.3)	74 (6.6)	1048 (93.4)
Age (years)	58.9±0.4	59.7±0.5	59.0±1.3	59.2±0.3
Height (cm)	164.7±0.3	164.4±0.3	164.8±0.7	164.6±0.2
Body weight (kg)	62.6±0.4	62.5±0.4	61.1±1.1	62.6±0.3
BMD measured with pQCT (mg/cm^3)				
D50	265.2±2.7	267.2±3.2	276.1±7.7	266.0±2.1
D100	537.8±3.7	542.6±4.5	554.0±10.6	539.7±2.8
P100	1178.3±5.6	1193.7±6.8	1187.7±16.1	1184.5±4.3
BMD measured with DXA (g/cm^2)				
Total body	1.083±0.004	1.090±0.004	1.107±0.010	1.086±0.003[b]
L2-L4	0.975±0.006	0.988±0.007	1.017±0.017	0.980±0.005[c]
Femoral neck	0.749±0.004[d,h]	0.752±0.005	0.790±0.011	0.750±0.003[e]
Trochanter	0.663±0.004[f]	0.671±0.005	0.696±0.011	0.666±0.003[g]

[a]BMD is adjusted for age, height, and body weight. Data are mean ± SE. [b]P=0.0461, [c]P=0.0396, [d]P=0.0021, [e]P=0.0007, [f]P=0.0140, [g]P=0.0106 versus TT; [h]P=0.0052 versus CT.

Because of their small number (n=17), perimenopausal women were excluded from this analysis. The distribution of -512C→T genotypes of *FOXC2* was in Hardy-Weinberg equilibrium in premenopausal (Table III) and postmenopausal (Table IV) women. Age and body weight did not differ among genotypes for premenopausal or postmenopausal women. Height was greater in premenopausal women with the CC genotype that in those with the CT genotype (Table III), but it

Table VI. BMD and other characteristics for all women (n=1112) according to *PLIN* genotype.[a]

Characteristic	CC	CT	TT	CC+CT
Number (%)	609 (54.8)	415 (37.3)	88 (7.9)	1024 (92.1)
Age (years)	59.7±0.4	58.8±0.5	57.9±1.2	59.4±0.3
Height (cm)	151.3±0.2	151.4±0.3	151.8±0.6	151.3±0.2
Body weight (kg)	52.3±0.3	53.1± 0.4	52.7±0.9	52.6±0.3
BMD measured with pQCT (mg/cm^3)				
D50	185.2±2.5	186.3±3.0	180.6±6.5	185.7±1.9
D100	483.4±3.6	486.5±4.4	497.9±9.5	484.7±2.8
P100	1151.2±5.9	1154.4±7.1	1168.2±15.3	1152.5±4.5
BMD measured with DXA (g/cm^2)				
Total body	0.966±0.003	0.963±0.004	0.968±0.009	0.965±0.003
L2-L4	0.864±0.005	0.868±0.006	0.863±0.014	0.866±0.004
Femoral neck	0.680±0.003	0.677±0.004	0.672±0.009	0.678±0.003
Trochanter	0.572±0.003	0.572±0.004	0.561±0.009	0.572±0.003

[a]BMD is adjusted for age, height, and body weight. Data are means ± SE.

Table VII. Effects of genotypes for FOXC2 and PLIN on BMD.[a]

Genotype	D50	D100	P100	Total body	L2-L4	Femoral neck	Trochanter
Men							
FOXC2	**0.0027** (0.0083)	**0.0170** (0.0053)	0.0661	0.3055	0.0787	0.7315	0.4283
PLIN	0.2709	0.2305	0.7867	0.1800	0.2188	**0.0207** (0.0048)	0.1239
All women							
FOXC2	0.0780	0.0526	0.2333	0.1683	0.5003	0.2771	0.3362
PLIN	0.8510	0.0566	0.0919	0.2873	0.6016	0.8517	0.8361
Premenopausal women							
FOXC2	0.7208	0.1008	0.2751	0.2353	0.8690	0.8614	0.4129
PLIN	0.1660	0.8853	0.8262	0.9462	0.6911	0.1224	0.1015
Postmenopausal women							
FOXC2	**0.0372** (0.0055)	0.0947	0.2346	0.1393	0.4126	0.1810	0.4181
PLIN	0.9305	0.2306	0.4186	0.7807	0.8573	0.7448	0.8836

[a]Data were analyzed by single regression analysis of genotype for *FOXC2* (CC=0, CT=TT=1 for men; CC=CT=0, TT=1 for women) or *PLIN* (CC=CT=0, TT=1). Data are P values (R^2). P values of <0.05 are shown in bold.

did not differ among genotypes for postmenopausal women (Table IV). For premenopausal women, BMD for D100 or P100 was greater in individuals with the CC genotype or in the combined group of CC and CT genotypes than in subjects with the TT genotype (Table III). The differences in BMD for D100 and P100 between individuals with the CC genotype and those with the TT genotype were 6.9 and 4.3%, respectively. For postmenopausal women, BMD for D50 or the total body was greater in the combined group of CC and CT genotypes than in individuals with the TT genotype (Table IV).

The differences in BMD for D50 and the total body between the combined group of CC and CT genotypes and individuals with the TT genotype were 9.7 and 2.2%, respectively.

Relationsip between the 1243C→T polymorphism of PLIN and BMD. The distribution of 1243C→T genotypes of *PLIN* was in Hardy-Weinberg equilibrium, and age, height, and body weight did not differ among genotypes, for all men (Table V). Among all men, BMD for the total body or lumbar spine, with adjustment for age, height, and body weight, was significantly greater in individuals with the TT genotype than in the combined group of CC and CT genotypes (Table V). BMD for the femoral neck or trochanter was greater in individuals with the TT genotype than in those with the CC genotype or in the combined group of CC and CT genotypes. BMD for the femoral neck was also greater in individuals with the CT genotype than in those with the CC genotype. The differences in BMD for the total body and lumbar spine between individuals with the TT genotype and the combined group of CC and CT genotypes were 1.9 and 3.6%, respectively. The differences in BMD for the femoral neck and trochanter between individuals with the TT genotype and those with the CC genotype were 5.2 and 4.7%, respectively.

There was no significant relationship between *PLIN* genotype and BMD for all women (Table VI). For premenopausal women, BMD for D100 was greater in the combined group of TT and CT genotypes than in individuals with the CC genotype (data not shown). For postmenopausal women, no relationship was detected between *PLIN* genotype and BMD (data not shown).

Effects of genotypes for FOXC2 and PLIN on BMD. The effects of -512C→T genotype for *FOXC2* and 1243C→T genotype for *PLIN* on BMD at various sites were evaluated by single regression analysis (Table VII). This analysis revealed that -512C→T genotype for *FOXC2* affected BMD for D50 and D100 in men and BMD for D50 in postmenopausal women, and that 1243C→T genotype for *PLIN* affected BMD for the femoral neck in men.

Discussion

We have examined the relationship of the -512C→T polymorphism of *FOXC2* and the 1243C→T polymorphism of *PLIN* with BMD at various sites in community-dwelling Japanese women and men. Our results show that the T allele of *FOXC2* is associated with reduced BMD in both men and women, and that the C allele of *PLIN* is associated with this condition in men.

Association of the -512C→T polymorphism of FOXC2 with BMD. *FOXC2*-deficient mice show multiple defects of skeletal tissue. In the craniofacial skeleton of these animals, for example, the supraoccipital bone is missing and other bones are reduced in size (31,32). *FOXC2* is expressed in the early stage of chondrogenic differentiation both *in vivo* and *in vitro*, and bone morphogenetic proteins regulate *FOXC2* expression in skeletal precursor cells (33). Expression of *FOXC2* in mesenchymal condensation and subsequently in cartilaginous tissue, as well as the phenotype of *FOXC2*-deficient mice, indicate that *FOXC2* contributes to the proliferation and differentiation of skeletal cells.

The T allele of the -512C→T polymorphism in the 5' untranslated region of *FOXC2* was shown to be associated with enhanced insulin sensitivity and lower plasma triglyceride concentration in women (25). A higher level of expression of *FOXC2* in visceral fat than in subcutaneous fat was also apparent only in individuals homozygous for the T allele. These observations suggest that increased expression of *FOXC2* may protect against insulin resistance, and that the -512C→T polymorphism of this gene may influence insulin sensitivity (25). We have now shown that this polymorphism of *FOXC2* is associated with BMD in men and women, with the T allele being related to reduced bone mass. The mechanism responsible for the association of the T allele both with enhanced insulin sensitivity and lower plasma triglyceride concentrations in women (25) and with reduced bone mass in men and women (the present study) remains to be elucidated. The molecular mechanism of the effect of this polymorphism on bone remodeling also remains unclear.

Association of the 1243C→T polymorphism of PLIN with BMD. The AA genotype of the 11,482G→A polymorphism of *PLIN* (rs894160) was shown to be associated with a decreased *PLIN* content and increased lipolytic activity in adipocytes of women (34). Individuals with the 11,482A variant of *PLIN* were also found to manifest both a lower baseline body weight and resistance to weight loss in response to a low-energy diet (35). Haplotypes of several polymorphisms of *PLIN* have been related to the risk of obesity, but the extent of this relationship differs between men and women (36) and among ethnic groups (37). The 1243C→T polymorphism of *PLIN* was associated with total cholesterol levels in Chinese (26). Men with the 1243T variant (CT or TT genotype) had higher plasma concentrations of total cholesterol, high density lipoprotein (HDL)-cholesterol, and LDL-cholesterol than did male CC homozygotes, suggesting that the 1243C→T polymorphism of *PLIN* may affect lipid metabolism. Our present results show that the 1243C→T polymorphism of *PLIN* was associated with BMD in men, with the C allele being related to reduced bone mass. This polymorphism was not associated with body weight or body mass index (data not shown) in the present study, making it unlikely that its association with BMD in men was attributable to an effect on these parameters. The mechanism responsible for the association of the C allele with both lower plasma cholesterol concentrations in men (26) and reduced bone mass in men (the present study) remains to be elucidated. The effects of the 1243C→T polymorphism of *PLIN* on gene expression, the function of the encoded protein, or bone remodeling have not been determined.

Given the multiple comparisons of genotypes with BMD at various sites in the present study, it is not possible to exclude potential statistical errors such as false positives. It is also possible that the polymorphisms associated with reduced BMD in our study are in linkage disequilibrium with polymorphisms of other nearby genes that are actually responsible for the development of this condition. Furthermore, the relevance of the polymorphisms to gene transcription or to protein structure or function and their effects on bone remodeling were not

determined in the present study. Despite these limitations, our present results suggest that *FOXC2* is a susceptibility locus for reduced BMD in Japanese men and women and that *PLIN* constitutes such a locus in Japanese men. Determination of genotypes for these polymorphisms may prove informative for assessment of the genetic risk for reduced BMD.

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研究論文●40

栄養摂取と骨密度減少との関連への遺伝子の影響に関する研究

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1 背景および目的

加齢に伴う骨密度の減少は，高齢者の骨粗鬆症や骨折の最も重要な要因であり，骨密度の減少に影響を与える因子についての検討，そしてその結果から予防医療への展開は，老年医学の大きな課題の１つである。骨粗鬆症は遺伝的な素因も重要であるが，素因があっても必ずしも発症するわけではない。遺伝的要素とともに，環境因子や生活習慣など多くの要因が複雑に関与する[1]。

本研究の目的は，生活要因のうちでも重要な要因の１つである栄養摂取の骨密度との関連と，骨密度に対する栄養と遺伝子との相互作用について明らかにすることである。

2 方法

１．対象

対象は，「国立長寿医療センター・老化に関する長期縦断疫学研究(NILS-LSA)」[2,3]の第１次調査参加者2,267名である。調査参加者は，40歳から79歳までの地域住民から年齢，性別に層化無作為抽出されて選ばれている。本研究では，参加者のうち遺伝子検査と栄養調査，骨密度検査のすべてを実施できた2,051名(59.3±10.8歳)について検討を行った。

２．骨密度

１）末梢骨骨定量CT(pQCT)

スイスScanco社のDensiscan 1000を使用して測定を行った。スキャンは橈骨遠位端で10スキャン，橈骨骨幹部で６スキャン，合計16スキャンである。スキャン厚は１mm，スキャンの間隔は1.5 mmである。橈骨遠位端は海綿骨と皮質骨からなり，全断面の骨密度D100と，中央部の海綿骨部の骨密度D50を計算している。また，橈骨骨幹部は皮質骨であり，全断面の骨密度P100を計算している。

２）二重X線吸収法(DXA)

米国Hologic社の二重X線吸収装置QDR4500にて，全身骨，左右の大腿骨(頸部，大転子部，ワード三角)，腰椎の計４スキャンの各測定を行った。本研究では，大腿骨については右側の測定値のみを使用した。

３）栄養調査

秤量法と写真記録法を併用した３日間の食事調査から，エネルギー，カルシウム，ビタミンD，蛋白質摂取量を推定し，解析に用いた。

４）遺伝子検査

参加者のEDTA採血血漿からDNAを分離し，凍結保存している。この保存DNAを用いて，蛍光法によるアレル特異DNAプライマー測定システム(東洋紡)を用いてタイピングを行った。現在までにタイピングの終了した約130種類の遺伝子多型のうち，骨密度との関連があった17の遺伝子多型[4-14]と，それに関連した候補遺伝子多型16の計33遺伝子多型について，栄養摂取との関連を網羅的に検討した(表１)。

５）解析方法

男性，未閉経女性，閉経女性の３群に分けて，一般線形モデルGLMにて栄養素が骨密度に及ぼす影響につい

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表1　解析を行った候補遺伝子多型

	略　号	遺伝子多型
○	MMP1	Matrix metalloproteinase-1 (1G/2G at −1607)
	MMP3	Stromelysin promoter (5A/6A at −1612)
○	MMP9	Matrix metalloproteinase-9 (C-1562T)
	MMP12	Matrix metalloproteinase-12 (A-82G)
	ACE	Angiotensin converting enzyme (D/I)
○	ADR	Androgen receptor (CAG repeat)
○	ESR1	Estrogen receptor α (PP/pp)
○	ESR2	Estrogen receptor α (XX/xx)
○	CCR	Chemokine receptor 2 (G190A(Val64Ile))
	CASR	Calcium-sensing receptor (Arg990Gly)
○	COL	Collagen type 1 (G-1997T)
○	OST	Osteocalcin (C298T)
○	OPG1	Osteoprotegerin (T245G)
○	OPG2	Osteoprotegerin (T-223C)
○	VDR1	Vitamin D receptor (T2C)
	VDR2	Vitamin D receptor (A-3731G)
○	TGF1	Transforming growth factor-β_1 (T29C)
	TGF2	Transforming growth factor-β_1 (C-509T)
	IL1A	Interleukin-1 α (C-889T)
	IL1B	Interleukin-1 β (C-511T)
	IL4R	Interleukin-4 receptor (G1902A(Q576R))
○	IL6	Interleukin-6 (C-634G)
	IL10	Interleukin-10 (A-592C)
	WNR	Werner helicase (c.4330TC(1367Cys/Arg))
○	KLOT	Klotho (G-395A)
○	PON1	Paraoxonase-1 (Gln192Arg)
○	PON2	Paraoxonase-1 (Met55Leu)
	LEP	Leptin (A19G)
	LEPR	Leptin receptor (Gln223Arg)
	VEGF1	Vascular endothelial growth factor (C936T)
	VEGF3	Vascular endothelial growth factor (C-2578A)
	VEGF4	Vascular endothelial growth factor (G-1154A)
○	DRD4	Dopamine D_4 receptor (C-521T)

○印はわれわれが骨密度との関連を既に報告している多型[4-14]。

て，各遺伝子多型の野生型と変異型(ヘテロおよびホモ変異)で有意な差があるかどうかを年齢および肥満度(BMI)，エネルギー摂取量について調整し検討した。男性，未閉経女性，閉経女性の3群，33候補遺伝子，4種類の栄養素，8種類の骨密度，計3,168の組み合わせの網羅的検討結果を解析し，遺伝子多型と栄養の相互作用についてのパターンを解析した。

3. 結果

男性では，ESR2およびOSTはカルシウムと，VDR1はエネルギーおよび蛋白質と，VDR2はエネルギーおよび蛋白質と，PON2はエネルギーおよびカルシウムと，LEPRはエネルギー，蛋白質およびカルシウムと，OPG1，OPG2，LEPは腰椎骨密度のみにカルシウムとの間で相互作用が認められた(表2)。未閉経女性では，MMP9はカルシウムおよびビタミンDと，ACEはビタミンDと，ESR2はエネルギー，カルシウム，ビタミンDと，CASRはビタミンDと，OPG2はビタミンDと，VDR1はエネルギーと，IL1AはカルシウムおよびビタミンDと，LEPRは腰椎骨密度のみビタミンDと骨密度との間に相互関係が認められた。閉経女性では，MMP9はビタミンDと，MMP12はエネルギーおよびカルシウムと，VDR2はビタミンDおよび蛋白質と，IL6はエネルギーおよびカルシウムと，WNRはエネルギー，蛋白質およびビタミンDと，LEPはビタミンDと，LEPRは蛋白質と骨密度との間に相互関係が認められた。

図1は，男性でのLEPR多型別にみた蛋白質摂取量による大腿骨頸部骨密度との関連を示している。GA/AA型群では蛋白質摂取量が多ければ骨密度も高いが，GG型群では蛋白質摂取量による骨密度の有意な違いはない。遺伝子多型での2つの直線は，1日の蛋白質摂取量の平均値に近い80 g付近で交差しており，蛋白質摂取量が多い群ではGA/AA型の方が骨密度が高いが，蛋白質摂取量が少ない群ではGG型の方が骨密度は高くなっており，逆転現象がみられる。

4. 考察

骨粗鬆症の候補遺伝子の多型により，エネルギー，蛋白質，カルシウム，ビタミンD摂取量が骨密度に及ぼす影響に差が認められたが，性別および閉経の有無によって遺伝子多型の影響に違いがあった。また，遺伝子多型の種類により，影響を受ける骨の部位が異なっており，骨密度減少のリスクを判定するためには，多くの遺伝子多型について様々な検討を行っていく必要があると思われた。

遺伝子多型と骨密度，骨粗鬆症との関連については，同じ多型であっても対象集団によって有意差があったり，なかったり，場合によっては全く逆の結果が報告される

表 2　骨粗鬆症候補遺伝子多型と骨密度との関連における栄養素摂取の影響(男性における結果の一部)

		MMP1	MMP3	MMP9	MMP12	ACE	ADR	ESR1	ESR2	CCR	CASR	COL	OST	OPG1	OPG2	VDR1	VDR2	TGF1
エネルギー	B_L24															*	*	
	B_RFN															**		
	B_Rtro															***	**	
	B_Rward									*						**		
	B_TOT									**						***		
	D100															*		
	D50															***		
	P100																	
カルシウム	B_L24	*							***				*	**	**			
	B_RFN																	
	B_Rtro						*											
	B_Rward							*	*				*					
	B_TOT												***				*	
	D100												*					
	D50																	
	P100																	
ビタミンD	B_L24																	
	B_RFN																	
	B_Rtro																	
	B_Rward																	
	B_TOT					*												
	D100																	
	D50																	
	P100																	
蛋白質	B_L24															*	*	
	B_RFN															*		
	B_Rtro															**	**	
	B_Rward															*		
	B_TOT									**			*			*	*	
	D100																	
	D50															**		
	P100								*									

年齢，BMI，エネルギー摂取量で調整。***：p<0.001，**：p<0.01，*：p<0.05.

B_L24：腰椎骨密度，B_RFN：右大腿骨頸部骨密度，B_Rtro：右大腿骨大転子部骨密度，B_Rward：右大腿骨ワード三角骨密度，B_TOT：全身骨密度，D100：橈骨遠位端全断面骨密度，D50：橈骨遠位端中央部骨密度，P100：橈骨骨幹部全断面骨密度。

遺伝子多型の略号に関しては表 1 を参照。

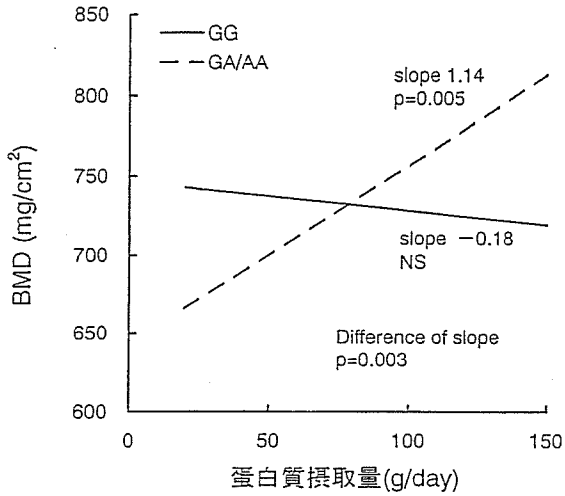

図1　Leptin receptor遺伝子Gln223Arg多型別にみた蛋白質摂取量と大腿骨頸部骨密度との関連(男性)
年齢，BMI，エネルギー摂取量で調整。

こともある。今回の結果で示されたように，遺伝子多型と疾病や疾病マーカーとの関連については栄養摂取など生活習慣の影響が強く，対象集団の設定によって結果が大きく異なってしまう。例えばカルシウムの摂取量は，乳製品を大量に摂っている欧米と，乳製品の摂取が少なく，食事中のカルシウム量が少ない日本では，未閉経女性のIL1Aと大腿骨頸部骨密度との関連は，欧米人では変異型の方が骨密度は高いのに，日本人では逆に野生型の方が骨密度が高い結果になる。したがって，こうした詳細な栄養調査，さらには喫煙や飲酒，運動などの生活習慣について，網羅的に相互作用についての検討を行っていかなければ，遺伝子多型を利用したテーラーメイドの医療や予防を進めていくことができない。

5．結語

骨粗鬆症の候補遺伝子の多型により，エネルギー，蛋白質，カルシウム，ビタミンD摂取量が骨密度に及ぼす影響に差が認められたが，性別および閉経の有無によって遺伝子多型の影響に違いがあった。また，遺伝子多型の種類により，影響を受ける骨の部位が異なっており，骨密度減少のリスクを判定するためには，多くの遺伝子多型について様々な検討を行っていく必要があると思われた。

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研究論文●42

閉経後女性の体力と骨密度の関連に matrix metalloproteinase(MMP)-12 (A-82G)遺伝子多型が及ぼす影響

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1．緒言

骨密度には遺伝的要因が50～70%関与し，生活習慣や環境要因が30～50%関与するといわれている。遺伝的要因は直接骨代謝に影響を与えるとともに，生活習慣や環境要因と骨密度との関連にも影響を及ぼすと考えられる[1,2]。しかし，生活習慣と骨量との関係に遺伝子多型が与える影響についての研究は，まだほとんど行われていない。

Matrix metalloproteinase(MMP)は，破骨細胞が骨を再吸収する際に必要とされる酵素[3]であるが，どのような種類のMMPが骨吸収の決定要因かは明らかになっていない。

本研究では，MMP-12(A-82G)遺伝子多型が体力と骨密度との関連に及ぼす影響を横断的に検討した。

2．対象および方法

対象は，国立長寿医療センターで行われている「老化に関する長期縦断疫学研究(NILS-LSA)」[4]の第1回調査参加者2,267人(40～79歳)の中で，MMP-12(A-82G)遺伝子多型が同定された閉経女性820人(平均年齢63.9±8.6歳)である。末梢骨骨定量CT(pQCT)を用いて橈骨遠位端の骨密度D50，D100，P100(mg/cm³)を，また二重X線吸収装置(DXA)で全身骨，右大腿骨(頸部，大転子部，ワード三角)，腰椎の骨密度(g/cm²)を測定した。体力や日常生活での活動度の指標として，握力，脚筋力，上体起こし回数(30秒間)，青年期の定期的運動の有無，速歩時の歩幅，万歩計計測による総消費エネルギーを用いた。MMP-12遺伝子多型(A-82G)はASP-PCR法でタイピングした。骨密度と体力・運動関連要因との関係がMMP-12遺伝子多型によってどのように異なるかを，年齢，BMIを調整した一般線形化モデルで検討した。統計解析にはSAS 8.2を用い，$p<0.05$を統計的有意とした。

表1　MMP-12(A-82G)遺伝子多型の分布

	AA	AG	GG
人　数	790	30	0
(%)	(96.3)	(3.7)	(0.0)

GG型はこのコホートでは認められなかった。
Hardy-Weinbergの平衡は保たれていた。

3．結果

MMP-12(A-82G)遺伝子多型のAA群は790人(96.3%)，AG群は30人(3.7%)であり，GG群は認められなかった(表1)。AA群とAG群との間に，年齢，体格，骨密度，体力・運動関連要因の有意差は認められなかった(表2)。骨密度と脚筋力，上体起こし，青年期の定期的運動の有無，握力，万歩計計測による総消費エネルギーとの関係は，AG群で有意に強かった(表3)。

例えば，脚筋力と大腿骨大転子部骨密度との間には

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表2　MMP-12(A-82G)遺伝子多型別の対象者の特性

	AA(n=790)	AG(n=30)	P value
年齢(歳)	64.0±8.6	63.7±7.6	n.s.
体　格			
身長(cm)	150.1±6.1	149.8±6.9	n.s.
体重(kg)	51.8±8.2	50.0±8.3	n.s.
BMI(kg/m^2)	23.0±3.3	22.3±3.4	n.s.
体脂肪率(%)	32.2±5.1	31.2±5.3	n.s.
骨密度			
全身骨(g/cm^2)	0.9187±0.1101	0.9159±0.1260	n.s.
大腿骨頸部(g/cm^2)	0.6431±0.1022	0.6602±0.1610	n.s.
大腿骨大転子部(g/cm^2)	0.5390±0.1017	0.5411±0.1404	n.s.
大腿骨ワード三角(g/cm^2)	0.4516±0.1441	0.4772±0.2118	n.s.
腰椎(g/cm^2)	0.8095±0.1502	0.7862±0.1813	n.s.
D50(mg/cm^3)	164.1±68.6	145.3±70.8	n.s.
D100(mg/cm^3)	443.5±108.2	418.5±100.3	n.s.
P100(mg/cm^3)	1,080.3±187.3	1,042.1±197.0	n.s.
体力・運動関連要因			
握力(kg)	23.7±4.7	23.6±5.1	n.s.
脚筋力(kg)	25.2±6.5	24.1±7.5	n.s.
上体起こし(回/30 sec)	4.6±4.6	5.4±4.9	n.s.
青年期の定期的運動(あり，%)	269(34.1)	9(30.0)	n.s.*
速歩時の歩幅(cm)	68.6±7.3	65.3±9.6	n.s.
総消費エネルギー(kcal/day)	1,496.2±295.4	1,416.3±561.4	n.s.

Mean±s.d., n.s.：not significant (Student's t-test)，n.s.*：not significant (χ^2 test).
AA群とAG群との間に年齢，体格，骨密度，体力・運動関連要因の有意差は認められなかった。

表3　骨密度に対する体力・運動関連要因とMMP-12(A-82G)遺伝子多型の交互作用

	体力・運動関連要因					
	握　力	脚筋力	上体起こし	青年期の定期的運動	速歩時の歩幅	総消費エネルギー
全身骨		*	*			
大腿骨頸部	*	***	*	*		*
大腿骨大転子部		***				
大腿骨ワード三角		***	*	*		
腰　椎		**				
D50						
D100						
P100		**				

骨密度に対してMMP-12(A-82G)との交互作用が有意であった体力・運動関連要因を示した(*：p<0.05，**：p<0.01，***：p<0.001，年齢・BMIを調整)。交互作用が有意であった場合は，すべて「AG群での体力・運動関連要因による骨密度変化はAA群より大きい」という方向性を示していた。

AA群でもAG群でも有意な関連が認められたが(AA群：p<0.05，AG群：p<0.001)，AG群でグラフの傾きはより強く，脚筋力と遺伝子多型との交互作用は有意であった(p<0.001)。AA群では脚筋力10 kg当たり大転子部骨密度は12.9 mg/cm^{2}多いと推定されたが，AG群ではその約10倍の104 mg/cm^{2}多いという結果であった(図1)。

同様に，青年期の定期的運動の有無はAA群，AG群い

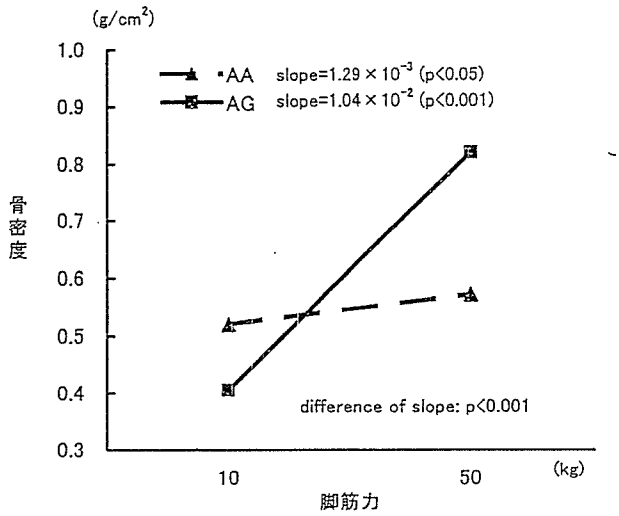

図1　脚筋力と大転子部骨密度との関係にMMP-12(A-82G)遺伝子多型が及ぼす影響(年齢・BMIで調整)

AA群とAG群のグラフの傾きは有意に異なっており(交互作用：p<0.001)，脚筋力の大転子部骨密度に対する影響は，AG群ではAA群の約10倍と推定された(各スロープの比較による)。

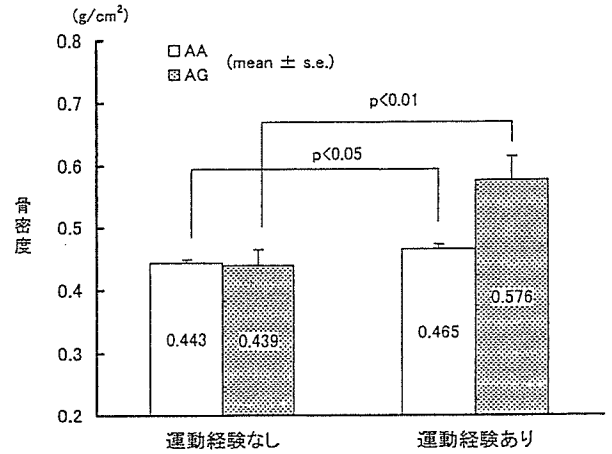

図2　青年期の定期的運動経験とワード三角部骨密度との関係にMMP-12(A-82G)遺伝子多型が及ぼす影響(年齢・BMIで調整)

AA群，AG群ともに，青年期に定期的運動経験がある群では有意に骨密度が高かった(p<0.05，p<0.01)。しかし，運動経験の影響はAG群でより大きく(交互作用：p<0.05)，AA群では経験あり群の骨密度が経験なし群の5.0%増であったのに対して，AG群では31.2%増であった。

ずれの骨密度にも影響を及ぼしていた。定期的運動と遺伝子多型との交互作用は有意であり(p<0.05)，運動経験の影響はAG群でより大きかった(図2)。

4 考察

MMP-12(A-82G)多型は体力・運動関連要因と骨密度との関係に影響を及ぼしており，AG多型を有する群ではAA多型群と比較して体力・運動関連要因の骨密度への影響が大きいと考えられた。

MMP-12は組織マクロファージなどに限局的に発現が確認されており，いままでに血管新生や血管のリモデリングなどとの関連が報告されている[5]。しかし，骨代謝における役割は不明である。一方，MMPの中でもMMP-14やMMP-9は破骨細胞中に存在が証明されており，破骨細胞中でMMPやcathepsin Kが骨の再吸収に重要な役割を果たすことは知られているが，どのMMPが酵素反応の律速段階となっているかは不明である。

本研究では，閉経女性の骨量に体力・運動が及ぼす効果がMMP-12(A-82G)のAG型でAA型より強いことが示唆されたが，その機序はまだ不明であり，今後細胞レベルでの基礎的研究や機能解析などで検証する必要がある。また，MMP-12(A-82G)遺伝子多型により体力・運動と骨量減少との関連が異なるかどうかについて，縦断的に検討する必要性もある。

しかし，本研究で遺伝子多型により体力・運動関連要因と骨密度との関連が異なる可能性が示唆されたことは，今後，運動習慣などの生活習慣への介入による骨粗鬆症予防戦略を考える際に，遺伝子多型を考慮することによって，より効果的，効率的な介入ができることを示唆するものであり，予防医学や厚生労働行政における意義は大きいと考えられる。

5 結論

地域在住中高年女性820人を対象に，MMP-12(A-82G)遺伝子多型が体力・運動関連要因と骨密度との関連に及ぼす影響を横断的に検討した。MMP-12(A-82G)遺伝子多型のAG多型を有する群では，AA多型群に比較して，体力・運動関連要因と骨密度との関連はより強く，この遺伝子多型を有する中高年女性では運動の骨密度への影響がより大きいと考えられた。

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Dietary Supplement Use by Community-living Population in Japan: Data from the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA)

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JEA

Original Article

Dietary Supplement Use by Community-living Population in Japan: Data from the National Institute for Longevity Sciences Longitudinal Study of Aging (NILS-LSA)

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BACKGROUND: There are few studies about dietary supplement use and nutrient intake from these products in Japan. The purpose of this study was to clarify (1) the prevalence of dietary supplement use, (2) the characteristics of dietary supplement users, (3) nutrient intake from dietary supplements, and (4) the existence of dietary supplement users who took excessive nutrients from these products.
METHODS: To collect the information on dietary supplement use in the previous year and nutrient intake from these products, we conducted a self-administered dietary supplement frequency questionnaire. The subjects were 2,259 people aged 40-82 years. Dietary supplements were grouped into 8 major categories. A dietary supplement database was developed to estimate nutrient intake from these products. Excess users were defined as people who consumed more nutrient than the tolerable upper intake level of the Dietary Reference Intakes for Japanese.
RESULTS: In the previous year, 55 % of males and 61 % of females consumed dietary supplements. Dietary supplement use was especially prevalent in females, subjects who felt unhealthy, and subjects who were more careful of maintaining an appropriate weight, though the association was affected by the frequency of dietary supplement use. The most common dietary supplements were drink type in males and vitamins in females. Some nutrient values obtained from dietary supplements were higher than those from food. Excess users were found for intake of vitamin A, B_6, K, niacin, iron, and magnesium.
CONCLUSIONS: It is important to clarify dietary supplement use and to estimate nutrient intake from these products.
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Key words: Dietary Supplements, Nutrition Surveys, Cohort Studies, Minerals, Vitamins.

Because sales of dietary supplements have increased in Japan,[1] it is conceivable that striking growth in the use of dietary supplements will occur in Japan, as it has in the USA and other developed countries. Assessing nutrient intake from dietary supplements, especially micronutrient intake, is very important. Because the levels of some micronutrients contained in these products are much higher than those contained in food,[2-4] people can easily consume such nutrients at toxic levels.[5-9] To monitor nutrient intake from dietary supplements is an important issue for public health. Furthermore, to assess nutrient intake from dietary supplements is essential for the development of nutritional epidemiologic studies. Lack of inclusion of dietary supplements in nutrient intake could possibly cause misclassification of individuals with regard to their total nutrient intake.[2-4,10,11] However, there have been very few studies on dietary supplement use in Japan. There is still uncertainty about the prevalence of dietary supplement use, nutrient intake from these products, and existence of users who consume extremely high levels of nutrients. One reason for the delay in the study of dietary supplement use in Japan might be due to the lack of a dietary supplement database. An extensive database that includes nutrient contents of dietary supplements is necessary for evaluating nutrient intake from these products; how-

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ever, such a database has not been established or distributed in Japan. In contrast, several studies have attempted to estimate quantitatively the amount of nutrient intake from these products in the United States and European countries.[2-11]

Therefore, we conducted a self-administered dietary supplement frequency questionnaire to collect information on dietary supplement use in the National Institute for Longevity Sciences-Longitudinal Study of Aging (NILS-LSA), and developed a database of dietary supplements to estimate the amount of nutrient intake from these products. The purpose of this study was to clarify the following four points: (1) the prevalence of dietary supplement use, (2) the characteristics of dietary supplement users, (3) nutrient intake from dietary supplements, and (4) the existence of dietary supplement users who took excessive nutrients from these products.

METHODS

Subjects

The subjects were 2,259 males and females aged 40 to 82 years who participated in the second wave examination of the NILS-LSA (from April 2000 through May, 2002). The NILS-LSA is a comprehensive population-based longitudinal study of aging, which started in 1997. The participants were stratified by both age and sex, and were randomly selected from resident registrations in the city of Obu and town of Higashiura in central Japan. The numbers of males and females recruited were similar and the baseline age was 40 to 79 years, with the similar numbers of participants in each decade of age (40s, 50s, 60s, 70s). At the first wave examination, we sent an invitation letter to 7,790 people and 3,434 people replied. A total of 2,267 people participated in the first wave examination. All participants gave their informed consent before they participated in the study. Details of the study purpose, design, and examination procedures have been described elsewhere.[12]

Definition and Categories of Dietary Supplements in the NILS-LSA

Dietary supplements were defined as supplements to meals containing any dietary ingredients from unnatural food forms such as capsules, tablets, powders, or liquid. Dietary supplements included vitamins, minerals, herbs, botanical products, and other sub-

Table 1. Categorization of dietary supplements by the National Institute for Longevity Sciences Longitudinal Study for Aging.

Category	Description or sub-category
1. Vitamin *	14 sub-categories Multivitamin, Vitamin A, Vitamin D, Vitamin E, Vitamin K, Vitamin B_1, Vitamin B_2, Vitamin B_6, Vitamin B_{12}, Niacin, Vitamin C, Folic acid, Biotin, and Pantothenic acid
2. Mineral *	4 sub-categories Calcium, Iron, Magnesium, and Other minerals
3. Fatty acid *	6 sub-categories Linoleic acid, Linolenic acid, Stearic acid, Docosahexaenoic acid, Eicosapentaenoic acid, and Other fatty acids
4. Amino acid	Formulations containing mainly of single amino acids and some proteins
5. Dietary fiber	Water soluble and water insoluble dietary fibers
6. Drink type	Liquid type dietary supplement for recovery from tiredness, or health promotion, etc. The amount consumed at one time is about 30 mL to 200 mL. Includes quasi-drugs and medicinal drugs but does not include beverages.
7. Medicine	Prescription and non-prescription medicines which contain some nutrients, except medicines which are classified into categories 1 to 6. Example : remedies for cold which contain vitamin C.
8. Others	These formulations included compounds that do not fit into any other category. Example: flavonoids, carotenoids other than beta-carotene, catechin, and herbal products (propolis, royal jelly, chlorella, garlic, etc.)

Dietary supplements were defined as supplements to meals including any dietary ingredients from unnatural food forms such as capsules, tablets, powders, or liquids. Dietary supplements included prescription medicine, and non-prescription medicine, but functional foods and modified foods were not included in the category of dietary supplement.
*: Further classified into sub-categories shown in the Table.

stances (e.g., enzymes, organ tissues, metabolites, concentrates, and constituent extracts of these substances). Dietary supplements also included prescription medicine and non-prescription medicine, but functional foods and modified foods were not included in the category of dietary supplements.

Dietary supplements were grouped into eight major categories on the basis of primary nutrient content or similarity in overall ingredients and rationale for use.[13] In addition, we defined "drink type" separately, because Hakura et al[14] reported that "drink type" dietary supplements were widely consumed in Japan. The major categories of dietary supplements used in the NILS-LSA were (1) vitamin, (2) mineral, (3) fatty acid, (4) amino acid, (5) dietary fiber, (6) drink type (liquid type dietary supplement for recovery from tiredness, health promotion, etc., with a serving size of about 30 mL to 200 mL. The drink type category included quasi-drugs, but did not include beverages), (7) medicine (prescription and non-prescription medicines which contained some nutrients, except medicines which were classified into categories 1 to 6, e.g. cold remedies with vitamin C), and (8) others (These formulations included compounds that did not fit into any other category and were not described in the Standard Tables of Food Composition in Japan, Fifth Revised Edition,[15] for example, flavonoids, carotenoids expect beta-carotene, propolis, and so on) (Table 1). In addition, vitamins, minerals, and fatty acids were further classified into sub-categories. We use the term "all" dietary supplements which consisted of eight categories, when we did not consider the categories of the dietary supplements.

Assessment of Dietary Supplement Intake

A self-administered questionnaire was used to assess dietary supplement intake. First, it was mailed to the participants and the participants were asked to record it by themselves at home before the study examination. Then, the participants came to our center to get the study examination. At the examination, the questionnaire was reviewed by trained dietitians through an interview that took approximately 10 minutes. In the questionnaire, participants were asked whether they had taken any dietary supplement in the previous year. In case they had taken any dietary supplement, the name of the product, manufacturer or distributor, serving size and frequency of intake in the previous year (6 categories, i.e., less than once per week, 1-2 times per week, 3-6 times per week, one per day, 2 times per day, and 3 or more times per day) were also recorded.

Definition of Dietary Supplement Users

Dietary supplement users in the present study were defined as persons who took any dietary supplement at least once in the previous year. Users of dietary supplements were categorized into three groups: "daily users": those who reported any dietary supplement use once a day or more for the past 12 months, "weekly users": those who reported any dietary supplement use once a week or more but less than once a day for the past 12 months, and "seldom users": those who reported any dietary supplement use once a year or more but less than once a week for the past 12 months. When a participant had taken multiple dietary supplements in a major category or in a sub-category, the user category was defined based on the dietary supplement with the highest frequency of use. We used the term "any users" when we did not consider the frequency of use. "Weekly users" and "daily users" were considered to be "regular users". "Seldom users" were excluded when we calculated the amount of nutrient intake from dietary supplements.

Development of Dietary Supplement Database

A new dietary supplement database was developed for the NILS-LSA based on information obtained from the study participants and additional intensive investigation. We asked dietary supplement users to bring the products to the study visit. Then, the labels of the products were transcribed or photocopied to get information on the nutrient contents. In case dietary supplement users did not bring the products or could not provide enough information about the products at the visit, we asked them to send the labels of the products by mail. In addition, when information on nutrient content was not available from users, we tried to get it directly from the manufacturer or distributor of the products. We created a database of dietary supplements that included the names of products, manufacturer and/or distributor and nutrient contents in standardized units such as a tablet or a capsule.

Some products in which nutrient content was not described were excluded when we developed the database, and we did not calculate the nutrient intake from these products (62 products). Finally, we succeeded in constructing a database of 902 dietary supplement products in May 2002.

Assessment of Nutrient Intake from Dietary Supplements

Energy and nutrient intake from "all" dietary supplements among "regular users" was estimated using the frequency, amount of intake and nutrient contents in the dietary supplement database. The frequency of dietary supplement intake per day was quantified during the calculation (0.2 for 1-2 per week, 0.6 for 3-6 per week). If a participant reported uncertainty about the information on dietary supplement intake, that dietary supplement was excluded from the calculation of nutrient intake (7 males and 22 females were excluded from the analysis because they reported uncertainty about the information on dietary supplement intake). When a participant had taken various kinds of dietary supplements, energy and nutrient intake were summed across all dietary supplements. Nutrient intakes from "all" dietary supplements were compared with those from food according to the results of the National Nutrition Survey in Japan 2002.[16]

Participants who daily consumed some nutrients at more than the tolerable upper intake level (UL) in the 6th Edition[17] or 2005 Edition[18] of Nutrient-Based Dietary Reference Intakes (DRIs) in Japan were defined as "excess users". The ULs for adults in the 6th Edition of DRIs were as follows: 5,000 IU for vitamin A, 2,000 IU for vitamin D, 600 mg α-TE for vitamin E, 30,000 μg

for vitamin K, 30 mgNE for niacin, 100 mg for vitamin B_6, 2,500 mg for calcium (under 70 years old), 40 mg for iron, and 650 mg (50 years old and over) or 700 mg (40 to 49 years old) for magnesium. The UL for adults in the 2005 Edition of DRIs were as follows: 3,000 μgRE (10,000 IU) for vitamin A, 50 μg (2,000 IU) for vitamin D, 600 mg (70 years old and over for females) to 800 mg (40 to 69 years old for males) for vitamin E, 300 mgNE for niacin (the amount of mg of nicotinic acid amide was used), 60 mg for vitamin B_6, 2,300 mg for calcium, and 40 mg (40-49 and 70 years old and over for females) to 55 mg (40 to 49 years old for males) for iron.

Other Variables

Sociodemographic and lifestyle characteristic data, such as smoking habits, subjective health status, total family annual income, education, marriage status, and care of maintaining appropriate weight, were collected using a questionnaire. The body mass index (BMI) was calculated using the formula (weight (kg)/height $(m)^2$). Energy intake from food, energy intake from fat, and total alcohol intake were assessed through 3-day weighed dietary records (3DR). 3DR was carried out on three continuous days (two weekdays and one weekend day). The average intakes of nutrient per day were calculated according to the 5th Edition Standard Tables of Foods Consumption and other resources.[19]

Statistical Analysis

The prevalences of "all" dietary supplement users among males and females were compared by the chi-squared test by user category (any, seldom, weekly, and daily). Sociodemographic and lifestyle characteristics of "all" dietary supplement users and nonusers were compared by the Cocran-Mantel-Haenszel test adjusted for sex and age by user category. The prevalences of dietary supplement use of each major category of users and of the main sub-categories of users among males and females were compared by the chi-squared test by user category. Energy and nutrient intake from "all" dietary supplements among "regular users" (by sex), and major categories of dietary supplements, including (1) vitamin, (2) mineral, (6) drink type, and (8) others among "regular users" were expressed as percentiles, maximum values, and number of "excess users". All the statistical analyses were performed using the Statistical Analysis System, release 8.2.[20] Differences with p value less than 0.05 were considered significant.

RESULTS

The prevalence of "all" dietary supplement users in each user category and sociodemographic and lifestyle characteristics by user categories are shown in Table 2. In this study, 55 % of males and 61 % of females consumed some kind of dietary supplement ("all" dietary supplements) in the previous year. Among these subjects, females were more likely to take dietary supplements than males ($p<0.01$). "Seldom users" constituted about 20 % of the subjects (males: 23%, females: 19%). "Regular users" constituted about 30 % of males ("weekly users": 14 %, "daily users": 18 %) and 40% of females ("weekly users": 16 %, "daily users": 26 %). "Seldom users" were predominant among males ($p<0.05$) while "daily users" were predominant among females ($p<0.001$). The prevalence of "all" dietary supplement users in each user category varied depending on the age group ($p<0.05$, adjusted for sex). "Seldom users" ($p<0.001$) and "weekly users" ($p<0.05$) were prevalent among middle-aged people, while "daily users" were prevalent among older people ($p<0.001$). "All" dietary supplement users were subjectively less healthy than nonusers after adjustment for sex and age ("any users": $p<0.01$). However, the association was influenced by the frequency of use ("seldom users": not significant, "weekly users": $p<0.01$, and "daily users": $p<0.05$). When dietary supplement use was limited to use without all prescription and non-prescription medicine, subjective health status was significantly associated with the use of dietary supplements in "any users" and "weekly users" ("any users": $p<0.01$, "seldom users": $p=0.57$, "weekly users": $p<0.01$, "daily users": $p=0.07$). "All" dietary supplement users were more careful of maintaining appropriate weight than nonusers in the "any users"category ($p<0.05$); however, the associations of "all" dietary supplements with other characteristics were not significant (i.e., smoking, education, marriage status, BMI, energy intake from food, alcohol intake, etc.) in all user categories.

The prevalence of dietary supplement users by major category and sub-category by user categories are shown in Table 3. Among major categories of dietary supplements, the most widely consumed dietary supplement was drink type (27.0%), the second was vitamin (23.1%), the third was "others" (18.3%) and the fourth was medicine (12.0%) in males. On the other hand, the most widely consumed dietary supplement was vitamin (30.2%), the second was "others" (26.9%), the third was drink type (24.8%), and the fourth was medicine (9.7%) in females. The prevalence of vitamin, "others", and mineral dietary supplement use in females was significantly higher than that in males; however, drink type dietary supplement use in males was significantly higher than that in females in "any users". The prevalence of amino acid, fatty acid, and dietary fiber use was only about 1 % or less in "any users".

About a half of vitamin, "others", and mineral users consumed their respective supplements daily, whereas 60 % of drink type dietary supplement users and most medicine users consumed these supplements less frequently than once a week.

With regard to the prevalence in the sub-category of vitamin users, the prevalence of multivitamin was the highest, the second highest was vitamin C, and the third highest was vitamin E for both sexes. Calcium was the most popular nutrient in the mineral sub-category for both sexes.

Energy and nutrient intake from "all" dietary supplements among "regular users" are shown in Table 4. Median values of energy, macronutrients, minerals, and some fat-soluble vitamins (vitamin A, vitamin D, vitamin E, and vitamin K) intake from

Table 2. Prevalence of "all" dietary supplement users in each user category and sociodemographic and lifestyle characteristics by user categories.

			User category (%)†			
		n	Any‡	Seldom§	Weekly‖	Daily¶
Sex	Males	1,152	55	23	14	18
	Females	1,107	61**	19*	16	26***
Age (year)	40-49	534	65	33	17	14
	50-59	580	55	23	15	17
	60-69	562	55	17	15	24
	70-	583	57*	11***	13*	33***
Smoking	Never	1,268	61	20	15	25
	Past	524	55	21	13	21
	Current	462	54	22	16	17
Subjective health status	Excellent/Good	573	55	26	12	18
	Usual	1,433	58	19	16	23
	Bad/Very bad	244	66**	20	18**	28*
Total sum of family annual income, million yen	-4.49	668	57	15	14	28
	4.50-9.99	1,012	57	24	14	20
	10.00-	513	61	24	18	19
Education	Less than high school	671	58	15	14	28
	High school or equivalent	923	57	20	16	22
	More than high school	655	60	28	15	17
Marriage status	Unmarried	58	50	24	14	12
	Married	1,944	57	21	15	22
	Separated/Divorced	51	67	31	20	16
	Widowed	202	63	13	17	33
Body mass index (kg/m^2)	<18.5	123	56	16	11	29
	18.5-24.9	1,588	59	21	16	22
	25.0-	547	56	21	14	21
Care of maintaining appropriate weight	Yes	1,375	60	20	16	24
	No	876	55*	22	14	20
Energy intake (kcal/day) ††	-1500	201	58	19	13	26
	1500-1999	926	60	19	15	26
	2000-2499	759	56	22	15	20
	2500-	225	58	27	16	15
Energy intake from fat (%) ††	<20	203	59	15	16	28
	20-24	639	56	19	14	24
	25-29	792	58	22	15	21
	30-	477	61	25	15	21
Total alcohol intake (g etanol/day) ††	<10	1,500	60	20	16	24
	10-19	265	56	21	12	23
	20-29	139	52	24	13	15
	30-	207	51	23	12	16

Participants using any dietary supplements were defined as any dietary supplement users during the previous year.

†: Dietary supplement users were categorized into three user groups:
Seldom; seldom users those who reported any dietary supplement use once a year or more but less than once a week for the past 12 months.
Weekly; weekly uses those who reported any dietary supplement use once a week or more but less than once a day for the past 12 months.
Daily; daily users those who reported any dietary supplement use once a day or more for the past 12 months.

‡: n=1,306 (628 males and 678 females)

§: n=470 (260 males and 210 females)

‖: n=335 (158 males and 177 females)

¶: n=501 (210 males and 291 females)

*p<0.05, **p<0.01, ***p<0.001: Sex distribution was tested by chi-squared test. Age distribution was tested by Cocran-Mantel-Haenszel chi-squared test adjusted for sex. Other variables were tested by Cocran-Mantel-Haenszel chi-squared test adjusted for sex and age