factors to diabetes mellitus in men and to obesity via aberrant fat metabolism in women.

Introduction

In order to study how to overcome obesity, diabetes mellitus and metabolic syndrome, we recruited 235 subjects with a body mass index (BMI) >28.3 (upper quintile) from examinees who appeared for periodic medical check-up in the Human Doc at Saku Central Hospital (Nagano, Japan). They were asked to participate in the weight loss intervention program named the Saku Control Obesity Program (SCOP). The details of the SCOP were described in elsewhere [1,2]. At the baseline study, we investigated the association among the SNPs, BMI, and other clinical parameters measured at the start point. We had previously reported their 10 SNPs in metabolic syndrome-related genes. Although many population studies have reported on the associations of BMI and other clinical parameters with the SNPs in UCP1/2/3 or β2AR/β3AR genes, few of these associations were confirmed in the obese subjects in the SCOP [3]. In the present study, we reported the effect of preproghrelin gene polymorphisms on the diabetes and obesity.

The human preproghrelin (GHRL) gene is located at locus 3p25–26 and consists of 4 exons and 3 introns. The gene is expressed as a prohormone, preproghrelin, which generates ghrelin and obestatin after posttranslational processing (for review, see NCBI OMIM 605353; www.ncbi.nlm.nih.gov/sites/entrez). O-n-octanoylation at serine-3 is essential for ghrelin activity. Mature ghrelin is secreted from the stomach and stimulates the pituitary to release the growth hormone and also up-regulates eating appetite via the hypothalamus. (Obestatin has the opposite effect; treatment of rats with obestatin suppressed food intake and decreased body-weight gain [4].) Ghrelin is suggested to play an important role in regulating energy balance, insulin signalling, and control of serum glucose concentration. Indeed, administration of ghrelin caused weight gain by reducing fat utilization in mice and rats [5]. Accordingly, dysfunction or insufficient generation of ghrelin may cause growth incompetence. Conversely, overexpression may result in obesity or metabolic syndrome.

Relationships between preproghrelin gene SNPs and susceptibility to metabolic syndrome have been intensively investigated. Leu72Met is the most frequently studied SNP of the gene, which is located in the inter-region of mature ghrelin and obestatin (neighboring the putative convertase cleavage site of obestatin) [4], thereby causing incompetence of the processing due to the polymorphism, possibly affecting the active ghrelin and/or obestatin concentration. However, conflicting results are reported. Ukkola et al. [6] first found this SNP in obese subjects, and reported that the age at onset of self-reported weight problems tended to be lower among 72Met carriers. Korbonits et al. [7] noted that children carrying the 72Met allele had a significantly higher BMI as compared to those carrying only the wild type allele. Hinney et al. [8] also identified this variant but at a similar frequency in both extremely obese children and adolescents and normal weight students. Comprehensive studies examining and comparing the maximum number of SNPs and parameters possible are further required for explaining these disagreements.

Here, we conducted the genotyping of 5 SNPs in the preproghrelin gene −1500C>G, −1062G>C, −994C>T, Leu72Met (+408C>A), and +3056T>C in SCOP. Their frequencies were compared with those of healthy people that were published in the HapMap Project or those analysed in East Asians by other research groups. Additionally, the associations between these SNPs and the clinical parameters related to diabetes mellitus or metabolic syndrome were studied.

HapMap is a public database of common gene variations (human genome) maintained by The International HapMap Consortium [9]. The map includes information on more than 1 million SNPs obtained in 269 DNA samples from 4 populations: Yoruba in Ibadan, Nigeria; Utah, USA; Beijing, China; and Tokyo, Japan.

Subjects and methods

Subjects

Japanese obese subjects aged 40–64 years with a BMI greater than 28.3 were selected from examinees undergoing a medical check-up at the Saku Central Hospital. They were asked to partici-

pate in the intervention program for weight loss named the Saku Control Obesity Program (SCOP); they were divided into two groups and received different instructions on food intake and daily exercise to evaluate the outcome with single nucleotide polymorphisms (SNPs) [1,2]. The participants underwent an anthropometric and clinical examination (height, weight, body fat percentage, waist circumference, visceral fat area, and biochemical markers of blood and urine) (Table 1) and were assessed for present illness, physical activity and dietary habits at the start of this program.

The ethics committees of The National Institute of Health and Nutrition and the Saku Central Hospital approved this investigation. All the participants gave their written informed consent before the start of this program.

Medical examination and measurements

The height (cm) and weight (kg) of the subjects were measured using an automatic scale (Tanita, BF-220, Tokyo, Japan). The percentage body fat was evaluated by the bioelectric impedance method using the same scale. Visceral and subcutaneous fat areas were assessed by a computed tomography scan at the level of the umbilicus, with the subjects in the supine position, and calculated using commercially available software (Fat Scan; N2 System Corp., Osaka, Japan).

Adipocytokines (i.e. leptin, TNF-α, adiponectin, and free fatty acid), C-peptide, and insulin concentrations were examined using the laboratory testing services provided by SRL Inc. (Tokyo, Japan). Leptin (ng/mL) was measured by a radioimmunoassay (Human Leptin RIA Kit, LINCO Research, St. Charles, MO, USA) with a sensitivity of 0.5 ng/mL. TNF-α (pg/mL) was measured by an enzyme-linked immunoassay (ELISA; Quantikine TNF-α HS Immunoassay Kit, R&D Systems Inc., Minneapolis, MN, USA) with a sensitivity of 0.12 pg/mL. The high-molecular-weight form adiponectin (μg/mL) was determined using ELISA (Fujirebio Inc., Tokyo, Japan) with a detection limit of 0.18 μg/mL. Free fatty acid (mequiv./L) was determined using an enzymatic assay (NEFA-SS 'Eiken', Eiken Chemical Co. Ltd., Tokyo, Japan) with a sensitivity of 0.005 mequiv./L. C-peptide (ng/mL) was measured by a chemiluminescent enzyme immunoassay (Lumipulse Presto C-peptide, Fujirebio Inc.) with a minimal detection limit of 0.1 ng/mL. Insulin (μIU/mL) was measured by a chemiluminescent

Table 1 Basic characteristics of the subjects in SCOP.

	All (n=223)	Male (n=115)	Female (n=118)	p-Value*
Age (years)	53.9 ± 6.5	53.3 ± 6.6	54.5 ± 6.4	0.171
Height (cm)	161.8 ± 8.7	168.4 ± 5.8	155.3 ± 5.5	<0.001
Weight (kg)	80.7 ± 12.1	86.5 ± 11.8	75.1 ± 9.6	<0.001
BMI (kg/m^2)	30.78 ± 3.36	30.44 ± 3.55	31.10 ± 3.13	0.134
Body fat percentage (%)	34.97 ± 7.66	29.04 ± 4.44	40.69 ± 5.43	<0.001
Waist circumference (cm)	102.6 ± 8.6	101.5 ± 8.7	103.7 ± 8.4	0.052
Total fat area (cm^2)	441.9 ± 124.4	414.4 ± 133.2	468.7 ± 109.2	<0.001
Subcutaneous fat area (cm^2)	297.3 ± 104.4	255.2 ± 102.9	338.3 ± 88.7	<0.001
Visceral fat area (cm^2)	144.6 ± 52.5	159.1 ± 54.3	130.4 ± 46.7	<0.001
Visceral fat ratio (%)	33.3 ± 10.2	38.8 ± 9.0	27.9 ± 8.3	<0.001
Total cholesterol (mg/dL)	210.3 ± 34.8	204.2 ± 28.0	216.3 ± 39.6	0.007
HDL cholesterol (mg/dL)	52.87 ± 11.10	49.80 ± 9.68	55.86 ± 11.61	<0.001
LDL cholesterol (mg/dL)	125.7 ± 31.8	120.4 ± 28.6	130.8 ± 34.0	0.012
Triacylglycerol (mg/dL)	161.5 ± 101.9	174.9 ± 120.3	148.4 ± 78.3	0.047
HbA1c (%)	5.85 ± 1.06	5.81 ± 0.97	5.89 ± 1.14	0.556
Fasting glucose (mg/dL)	112.0 ± 25.7	111.7 ± 24.9	112.3 ± 26.6	0.875
HOMA-IR	3.3 ± 2.5	3.4 ± 3.1	3.1 ± 1.9	0.380
Free fatty acids (mequiv./L)	0.54 ± 0.20	0.51 ± 0.18	0.57 ± 0.21	0.017
Leptin (ng/mL)	14.83 ± 11.02	8.21 ± 5.63	21.34 ± 11.16	<0.001
Tumor necrosis factor-α (pg/mL)	1.25 ± 0.47	1.29 ± 0.50	1.22 ± 0.43	0.263
Adiponectin (μg/mL)	4.14 ± 2.84	2.79 ± 1.77	5.45 ± 3.06	<0.001
C-peptide (ng/mL)	2.70 ± 1.11	2.86 ± 1.25	2.55 ± 0.92	0.034
Insulin (μIU/mL)	11.62 ± 8.20	12.11 ± 10.01	11.14 ± 5.92	0.373
Creatinin (mg/dL)	0.77 ± 0.17	0.87 ± 0.13	0.67 ± 0.13	<0.001

Values are mean ± SD.
* p-Values are given by Student's t-test between male and female.

enzyme immunoassay (Lumipulse Presto Insulin, Fujirebio Inc.) with a minimal detection limit of 0.3 μIU/mL. Other biochemical markers were examined in the clinical laboratory of the Saku Central Hospital by Hitachi Automated Analyser (Hitachi).

Genotyping and statistical analysis

DNA was purified from the subjects' blood using QIAamp DNA blood 96 Kit (Qiagen). All the SNPs were genotyped by PCR followed by digestion with a restriction enzyme [PCR-RFLP (restriction fragment length polymorphism) method]. Fragments (80–200 bp) containing the objective SNP were amplified in a 20 μL reaction mixture, including 0.2 mM 4dNTPs, 1.25 units rTaq DNA polymerase (TOYOBO), genomic DNA, and 12.5 pmol of each primer (with/without 1 or 2 bases mismatch). PCR was conducted as follows: 10 min at 94 °C for initial denaturation, 35 cycles of 2 min at 94 °C, 2 min at 52 °C or 61 °C, 1 min at 72 °C, and 10 min at 72 °C as final extension. The PCR product was digested with each restriction enzyme (NEB) and subjected to electrophoresis in a Spreadex EL300 gel (Elchrom Scientific) at 55 °C or a MultiNA microtip electrophoresis DNA/RNA analyser (Shimadzu Biotech) (Table 2).

The database was created in Microsoft Excel file format and converted to SPSS. Statistical analysis was performed by SPSS ver14.0.1 (SPSS Japan, Inc., Tokyo, Japan). Linkage disequilibrium (LD) and haplotype block were predicted using Haploview (www.broad.mit.edu/haploview/haploview) based on an accelerated expectation-maximization algorithm similar to the partition-ligation method [10]. Using the software, the haplotype frequencies in case/control data were also estimated, and their associations were computed.

Table 2 Genotyping of Preproghrelin genes by PCR-RFLP.

SNP	dbSNP	PCR primer F (5′ to 3′)	PCR primer R (5′ to 3′)	Anneal temp.	PCR buffer[a]	Restriction enzyme	Generated frag. (bp)
−1500C>G	rs3755777	CCCAGTTGGATGAAGCACTC	TCATACCAGGCCCATAGGAC	52	A	Sau96 I	91, 12 → 41 + 50, 12(G)
−1062G>C	rs26311	GCCACTGGCTGAAGTTATCC	CTGTTGCTGCTCTGGCCACT	52	A	Nci I	128, 30 → 67 + 61, 30(G)
−994C>T	rs26312	GCCACTGGCTGAAGTTATCC	CTGTTGCTGCTCTGGCCACT	52	A	HpyCH4 III	158 → 139 + 19(C)
Leu72Met(C>A)	rs696217	GTCGAAGAAGCCACCAGCC	AGGTACCGACCCGGACTTAC	52	B	HpyCH4 III	116 → 20 + 96(C)
Arg51Gln(G>A)	rs34911341	GTCGAAGAAGCCACCAGCC	AGGTACCGACCCGGACTTAC	52	B	Sac I	116 → 37 + 79(G)
+3056T>C	rs2075356	GAGAATGCTGGGCAGACCC	GATAAAAGCTGTGGTGCCCAC	61	B	Hpy188 I	122 → 69 + 53(C)
+3083A>G	rs35682	GAGAATGCTGGGCAGACCC	GATAAAAGCTGTGGTGCCCAC	61	B	Ale I	122 → 99 + 23(G)

[a] PCR buffer A: 50 mM Tris–HCl (pH 9.0), 20 mM $(NH_4)_2SO_4$, 0.7 mM $MgCl_2$, 10% DMSO. PCR buffer B: 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$.

Results

Comparison of preproghrelin SNPs frequencies between men and women, with healthy Asian populations

We measured the frequencies of 5 SNPs in the preproghrelin gene in SCOP, −1500C>G (rs3755777), −1062G>C (rs26311), −994C>T (rs26312) (these 3 locate in the promoter), Leu72Met (+408C>A) (rs696217) (2nd exon), and +3056T>C (rs2075356) (2nd intron). Also, Arg51Gln (+346 G>A, rs34911341) (2nd exon) and +3083T>C (rs35682) (2nd intron) were analysed (Table 2). The frequencies of the

Table 3 Genotype frequencies of preproghrelin gene.

SNP	Cohort (population)	Genotype (%)						
		n	Major homo	Hetero	Minor homo	MAF	HWP	*p*-Value
−1500C>G	SCOP	233	61 (26.2)	115 (49.4)	57 (24.5)	0.491	0.85	
	Male	115	30 (26.1)	51 (44.3)	34 (29.6)	0.517	0.23	0.168**
	Female	118	31 (26.3)	64 (54.2)	23 (19.5)	0.466	0.33	
	HapMap-JPT(ss68852548)	45	11 (24.4)	20 (44.4)	14 (31.1)	0.533	0.47	0.643*
	Choi et al. (Korean) [11]	641	167 (26.1)	316 (49.3)	158 (24.6)	0.493	0.73	0.998*
−1062G>C	SCOP	233	84 (36.1)	109 (46.8)	40 (17.2)	0.406	0.65	
	Male	115	41 (35.7)	49 (42.6)	25 (21.7)	0.430	0.16	0.164**
	Female	118	43 (36.4)	60 (50.8)	15 (12.7)	0.381	0.40	
	HapMap-JPT(ss68852547)	45	19 (42.2)	21 (46.7)	5(11.1)	0.344	0.82	0.537*
	Choi et al. (Korean) [11]	640	257 (40.2)	289 (45.2)	94 (14.7)	0.373	0.39	0.468*
−994C>T	SCOP	233	78 (33.5)	110 (47.2)	45 (19.3)	0.429	0.58	
	Male	115	39 (33.9)	50 (43.5)	26 (22.6)	0.443	0.20	0.375**
	Female	118	39 (33.1)	60 (50.8)	19 (16.1)	0.415	0.61	
	JBIC-allele(ss4941811)	454	Not applicable			0.427		0.900*
	Choi et al. (Korean) [11]	639	235 (36.8)	306 (47.9)	98 (15.3)	0.393	0.92	0.336*
Leu72Met (+408C>A)	SCOP	223	143 (64.1)	75 (33.6)	15 (6.7)	0.225	0.23	
	Male	115	74 (64.3)	33 (28.7)	8 (7.0)	0.213	0.12	0.526**
	Female	118	69 (58.5)	42 (35.6)	7 (5.9)	0.237	0.86	
	HapMap-JPT(ss68852544)	45	28 (62.2)	17 (37.8)	0 (0.0)	0.189	0.12	0.196*
	Ando et al. (Japanese female) [12]	300	205 (68.3)	84 (28.0)	11 (3.7)	0.177	0.52	0.149*
	Kuzuya et al. (Japanese male) [13]	2228	1412 (63.4)	728 (32.7)	88 (3.9)	0.203	0.63	0.195*
	Tang et al. (Chinese) [14]	323	195 (60.4)	112 (34.7)	16 (5.0)	0.223	0.99	0.668*
	Zou et al. (Chinese) [15]	125	77 (61.6)	43 (34.4)	5 (4.0)	0.212	0.74	0.611*
	Choi et al. (Korean) [11]	636	429 (67.5)	185 (29.1)	22 (3.5)	0.180	0.71	0.080*
	Kim et al. (Korean) [16]	80	54 (67.5)	23 (28.8)	3 (3.8)	0.181	0.78	0.515*
+3056T>C	SCOP	233	111 (47.6)	92 (39.5)	30 (12.9)	0.326	0.12	
	Male	115	58 (50.4)	43 (37.4)	14 (12.2)	0.309	0.18	0.701**
	Female	118	53 (44.9)	49 (41.5)	16 (13.6)	0.343	0.39	
	HapMap-JPT(ss44387483)	45	22 (48.9)	19 (42.2)	4 (8.9)	0.300	0.97	0.751*
	Ando et al. (Japanese female) [12]	300	162 (54.0)	112 (37.3)	26 (8.7)	0.273	0.30	0.182*

MAF = minor allele frequency, HWP = Hardy Weinberg plot, JBIC = Japanese Biological Informatics Consortium's data.
* *p*-Values are given by the chi-square test between SCOP and another cohort.
** *p*-Values are given by the chi-square test between male and female.

latter 2 were very low (minor allele frequency <0.05): therefore, further analysis was not performed. First, the 5 SNP frequencies between men and women in SCOP were compared (Table 3), but no specific differences were observed. We also compared the SNP frequencies in SCOP with those of healthy people published in the HapMap Project or those analysed in East Asians by other research groups (Table 3) [11–16]: no statistically significant differences were observed among these frequencies. HapMap also introduces these SNP frequencies in Han Chinese healthy people in Beijing (www.hapmap.org/cgi-perl/gbrowse/), and these are very similar to HapMap-JPT and to the results in SCOP, suggesting that the Japanese, Chinese, and Koreans are not quite genetically diverse in the preproghrelin gene polymorphism.

Associations between preproghrelin SNPs and anthropometric or clinical parameters

The phenotypes between men and women were rather different. In men, although the leptin and insulin were not elevated (Table 4), higher concentrations of fasting glucose and HbA1c were observed in the +3056C/C minor homozygotes (Table 5). Additionally, 72Met/Met minor homozygotes also showed the trend of higher concentrations of fasting glucose and HbA1c (Table 5). In these homozygotes, however, the other parameters were not so different.

In contrast, women showed a significant correlation not with diabetes but with fat metabolism and obesity. The −1062C/C minor homozygotes had higher values of C-peptide, insulin (Table 4), total and visceral fat area, waist circumference, and BMI (Table 6). Additionally, 72Met/Met minor homozygotes showed reduced concentrations of leptin (Table 4), total, HDL and LDL cholesterol (Table 5), and increased of visceral fat area (Table 6). Also, in the other SNPs, minor homozygotes showed a similar trend as above and heterozygotes had the intermediate values.

After Bonferroni adjustment for multiple comparisons, almost all of the associations mentioned above remained significant between the major and minor homozygotes. In comparison with the total cholesterol concentration and visceral fat ratio in women, the 72Leu/Met heterozygotes were also significant against Met/Met minor homozygotes (Tables 5 and 6). Similarly, the −1500C/G and −1062G/C heterozygotes have significant values of visceral fat area against their minor homozygotes (Table 6).

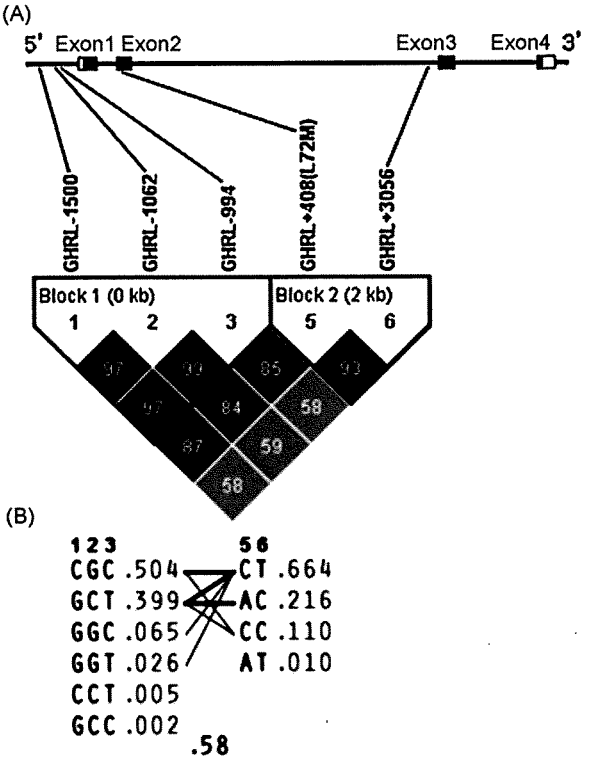

Figure 1 (A) SNP positions and LD block structure of the preproghrelin gene in SCOP. *D'* (100×) values are displayed in the squares. (B) Haplotype blocks and their population frequencies. If haplotypes in 2 blocks occur together with a frequency >10%, a thick line connects them. If >1%, a thin line does so. Beneath these lines, a recombination rate between the 2 blocks estimated using Haploview was shown.

Haploview analysis of haplotype frequencies in case/control subgroups to detect causative loci

To know about the causative loci of the above abnormalities, we depicted the LD map beforehand, using Haploview (Fig. 1A). In this region, two haplotype blocks were found: block 1, −1500 - −1062 - −994 and block 2: Leu72Met - +3056. Haplotype block 1 expands in the promoter region and block 2, in the exon–intron region. Fig. 1B shows the haplotypes and their estimated frequencies.

Next, we divided the 115 male subjects into 2 subgroups of diabetic and non-diabetic and estimated the frequencies of each haplotype in the subgroups, using Haploview (Table 7a). Based on the criterion of The Japan Diabetes Society, the diabetic subgroup comprised 59 subjects diagnosed with diabetes mellitus and under medication for diabetes, with a fasting glucose value >126 mg/dL, 2 h 75 g oral glucose tolerance test (OGTT) value >200 mg/dL, or HbA1c value >6.5%. The +408C -

Table 4 Preproghrelin genotypes and plasma biochemical parameters.

SNP	Genotype	*n*	Free fatty acids	Leptin	TNF-α	Adiponectin	C-peptide	Insulin	Creatinin
Male									
−1500C/G	C/C	30	0.46 ± 0.16	9.58 ± 7.95	1.44 ± 0.58	2.72 ± 1.58	2.88 ± 1.27	12.00 ± 9.20	0.90 ± 0.14
	C/G	51	0.50 ± 0.19	7.56 ± 3.88	1.26 ± 0.48	2.92 ± 1.91	2.99 ± 1.31	12.98 ± 11.44	0.87 ± 0.13
	G/G	34	0.55 ± 0.20	7.96 ± 5.36	1.19 ± 0.45	2.66 ± 1.76	2.62 ± 1.15	10.90 ± 8.41	0.85 ± 0.12
−1062G/C	G/G	41	0.47 ± 0.15	9.09 ± 7.33	1.40 ± 0.59	2.61 ± 1.49	2.74 ± 1.20	11.63 ± 8.50	0.89 ± 0.14
	G/C	49	0.51 ± 0.19	7.62 ± 3.82	1.21 ± 0.44	2.99 ± 1.93	3.05 ± 1.31	12.85 ± 11.41	0.87 ± 0.13
	C/C	25	0.56 ± 0.21	7.91 ± 5.46	1.25 ± 0.48	2.68 ± 1.91	2.67 ± 1.23	11.44 ± 9.66	0.84 ± 0.12
−994C/T	C/C	39	0.47 ± 0.15	9.26 ± 7.46	1.43 ± 0.58	2.64 ± 1.52	2.77 ± 1.21	11.72 ± 8.66	0.89 ± 0.14
	C/T	50	0.51 ± 0.19	7.37 ± 3.62	1.21 ± 0.44	2.91 ± 1.91	3.00 ± 1.31	13.00 ± 11.42	0.87 ± 0.13
	T/T	26	0.55 ± 0.21	8.23 ± 5.57	1.23 ± 0.48	2.77 ± 1.89	2.70 ± 1.22	11.00 ± 9.18	0.86 ± 0.12
Leu72Met	Leu/Leu	74	0.48 ± 0.16	8.21 ± 6.15	1.35 ± 0.55	2.57 ± 1.44	2.91 ± 1.37	12.39 ± 11.20	0.88 ± 0.14
	Leu/Met	33	0.53 ± 0.21	8.22 ± 4.84	1.15 ± 0.43	3.09 ± 2.12	2.81 ± 1.12	11.67 ± 8.00	0.86 ± 0.12
	Met/Met	8	0.63 ± 0.23	8.13 ± 3.87	1.25 ± 0.28	3.53 ± 2.65	2.57 ± 0.48	11.32 ± 5.43	0.84 ± 0.12
			$p = 0.084$						
+3056T/C	T/T	58	0.47 ± 0.16	8.40 ± 6.59	**1.40 ± 0.54**	2.70 ± 1.55	2.90 ± 1.33	12.22 ± 11.27	0.89 ± 0.13
	T/C	43	0.52 ± 0.19	8.06 ± 4.85	**1.17 ± 0.48**	2.82 ± 1.97	2.95 ± 1.29	12.77 ± 9.52	0.86 ± 0.13
	C/C	14	0.59 ± 0.23	7.88 ± 3.36	**1.17 ± 0.28**	3.04 ± 2.08	2.38 ± 0.55	9.63 ± 4.55	0.86 ± 0.12
			$p = 0.080$		$p = 0.049$				
Female									
−1500C/G	C/C	31	0.57 ± 0.16	19.42 ± 7.84	1.17 ± 0.39	6.01 ± 3.20	2.21 ± 0.70	**9.13** ± 4.28	0.67 ± 0.10
	C/G	64	0.56 ± 0.22	23.19 ± 12.71	1.23 ± 0.45	5.28 ± 3.12	2.64 ± 0.75	**11.45** ± 4.66	0.66 ± 0.14
	G/G	23	0.58 ± 0.27	18.87 ± 9.82	1.25 ± 0.43	5.16 ± 2.73	2.72 ± 1.43	**13.04** ± 9.43	0.68 ± 0.13
							$p = 0.060$	$p = 0.046$	
−1062G/C	G/G	43	0.57 ± 0.16	18.63 ± 7.47	1.17 ± 0.37	5.89 ± 3.07	**2.22** ± 0.67	**9.13** ± 4.05	0.66 ± 0.10
	G/C	60	0.57 ± 0.23	23.37 ± 13.02	1.26 ± 0.47	5.29 ± 3.12	**2.66** ± 0.75	**11.66** ± 4.56	0.66 ± 0.14
	C/C	15	0.57 ± 0.29	21.15 ± 11.07	1.17 ± 0.46	4.82 ± 2.81	**3.02** ± 1.66	**14.90** ± 11.27	0.71 ± 0.15
							$p = 0.006$	$p = 0.003$	
−994C/T	C/C	39	0.58 ± 0.16	**18.30** ± 7.65	1.16 ± 0.38	5.96 ± 3.05	**2.23** ± 0.70	**9.20** ± 4.22	0.66 ± 0.11
	C/T	60	0.56 ± 0.22	**23.97** ± 12.69	1.26 ± 0.46	5.23 ± 3.17	**2.67** ± 0.74	**11.54** ± 4.60	0.66 ± 0.14
	T/T	19	0.59 ± 0.29	**19.41** ± 10.66	1.19 ± 0.44	5.07 ± 2.74	**2.81** ± 1.54	**13.89** ± 10.17	0.69 ± 0.14
				$p = 0.033$			$p = 0.028$	$p = 0.013$	
Leu72Met	Leu/Leu	69	0.59 ± 0.21	**20.18** ± 9.64	1.24 ± 0.44	5.98 ± 3.18	2.39 ± 0.76	10.30 ± 5.06	0.66 ± 0.12
	Leu/Met	42	0.53 ± 0.22	**24.30** ± 12.98	1.15 ± 0.43	4.75 ± 2.71	2.81 ± 1.09	12.54 ± 6.88	0.67 ± 0.14
	Met/Met	7	0.53 ± 0.19	**14.91** ± 9.74	1.33 ± 0.35	4.49 ± 3.21	2.53 ± 1.04	10.98 ± 6.87	0.70 ± 0.15
				$p = 0.048$		$p = 0.082$	$p = 0.065$		
+3056T/C	T/T	53	0.57 ± 0.19	20.89 ± 10.10	1.23 ± 0.40	5.98 ± 3.38	2.41 ± 0.77	10.41 ± 5.26	0.67 ± 0.13
	T/C	49	0.57 ± 0.24	20.48 ± 9.95	1.18 ± 0.46	5.10 ± 2.82	2.69 ± 1.10	11.85 ± 6.37	0.65 ± 0.11
	C/C	16	0.55 ± 0.23	25.47 ± 16.68	1.27 ± 0.43	4.80 ± 2.50	2.56 ± 0.79	11.37 ± 6.61	0.68 ± 0.18

p-Values are given by ANOVA, and $p < 0.05$ are bold.

Table 5 Preproghrelin genotypes and lipidemia- or diabetes- related parameters.

SNP	Genotype	*n*	Total-cho.	HDL-cho.	LDL-cho.	Triacylglycerol	HbA1c	Fasting Glc.	HOMA-IR
Male									
−1500C/G	C/C	30	198.7±30.8	47.27±9.40	117.8±30.4	184.7 ± 185.6	5.61±0.87	107.8±21.1	3.25±2.72
	C/G	51	209.0±25.3	49.24±9.27	122.5±29.5	186.5 ± 89.5	5.84±0.83	113.5±26.1	3.62±3.11
	G/G	34	201.7±28.7	52.88±9.98	119.5±26.1	148.9 ± 81.2	5.95±1.22	112.6±26.4	3.25±3.32
				$p=0.057$					
−1062G/C	G/G	41	202.6±28.6	48.10±9.71	122.8±27.1	158.7 ± 90.5	5.62±0.76	107.5±19.1	3.12±2.47
	G/C	49	208.2±28.5	51.29±10.49	120.7±31.1	181.2 ± 93.0	5.92±0.92	114.9±26.1	3.62±3.11
	C/C	25	198.8±25.6	49.68±7.66	115.9±26.2	189.0 ± 192.6	5.90±1.32	112.4±30.3	3.47±3.83
−994C/T	C/C	39	202.2±29.2	48.26±9.87	122.1±27.6	159.6 ± 92.6	5.66±0.76	107.9±19.5	3.15±2.52
	C/T	50	206.6±27.8	50.10±10.21	120.1±30.4	181.7 ± 91.5	5.87±0.88	114.6±25.9	3.65±3.10
	T/T	26	202.5±26.9	51.54±8.25	118.4±27.4	184.7 ± 189.8	5.94±1.36	112.0±29.8	3.34±3.73
Leu72Met	Leu/Leu	74	200.7±26.6	49.20±9.54	118.2±25.6	166.7 ± 93.1	5.68±0.77	108.8±19.2	3.43±3.37
	Leu/Met	33	212.6±29.5	51.64±9.91	125.8±33.8	192.5 ± 173.8	5.95±0.98	115.2±29.0	3.26±2.16
	Met/Met	8	201.6±29.9	47.75±10.18	118.4±32.5	177.6 ± 72.2	6.46±2.00	125.1±44.9	3.84±3.51
							$p=0.061$		
+3056T/C	T/T	58	202.0±29.2	48.40±9.71	122.4±27.5	156.1 ± 79.4	**5.58±0.65**	**106.8±16.0**	3.31±3.19
	T/C	43	207.1±27.2	50.51±8.32	119.6±29.2	187.0 ± 106.4	**5.93±0.95**	**113.3±27.3**	3.62±3.03
	C/C	14	204.2±26.1	53.43±12.65	114.5±32.2	215.8 ± 242.6	**6.37±1.70**	**127.6±38.7**	3.20±2.71
							$p=0.013$	**$p=0.016$**	
Female									
−1500C/G	C/C	31	222.5±38.1	59.81±12.72	135.3±33.1	137.0 ± 84.4	5.67±0.68	112.1±26.2	2.67±2.04
	C/G	64	215.2±40.6	54.55±10.24	131.8±33.9	144.3 ± 71.3	6.00±1.34	113.8±28.4	3.18±1.36
	G/G	23	211.2±39.5	54.17±12.89	122.1±35.3	175.3 ± 85.7	5.90±1.02	108.3±22.2	3.55±2.66
				$p=0.086$					
−1062G/C	G/G	43	223.8±43.0	58.81±12.25	137.1±35.3	139.3 ± 89.4	5.74±0.71	111.2±24.6	**2.62±1.81**
	G/C	60	214.4±35.7	54.20±10.56	130.1±31.3	150.6 ± 71.0	6.02±1.39	114.5±29.3	**3.25±1.33**
	C/C	15	202.9±42.5	54.00±12.81	115.9±37.7	165.7 ± 73.8	5.82±1.06	106.7±20.4	**4.03±3.17**
				$p=0.062$					**$p=0.030$**
−994C/T	C/C	39	223.4±42.8	59.18±12.46	137.2±35.5	134.8 ± 78.3	5.76±0.73	112.2±25.5	2.67±1.89
	C/T	60	214.9±37.5	53.58±10.05	130.8±32.3	153.1 ± 81.4	5.97±1.36	112.9±28.4	3.19±1.35
	T/T	19	206.4±38.8	56.21±13.26	117.9±34.3	161.5 ± 67.1	5.95±1.10	110.4±23.9	3.83±2.85
				$p=0.062$					$p=0.080$
Leu72Met	Leu/Leu	69	**223.2±39.0**	**58.06±11.47**	**137.5±32.6**	138.4 ± 70.9	5.81±0.98	111.4±27.2	2.89±1.84
	Leu/Met	42	**211.0±38.4**	**52.88±10.87**	**124.9±33.5**	165.9 ± 92.4	5.99±1.33	115.0±26.1	3.54±1.93
	Met/Met	7	**181.1±33.2**	**52.00±13.95**	**101.0±32.8**	141.9 ± 28.2	6.13±1.52	104.6±24.4	2.79±1.60
			$p=0.014$	**$p=0.048$**	**$p=0.008$**				
+3056T/C	T/T	53	**224.5±40.6**	**59.30±12.03**	**137.5±34.4**	138.2 ± 76.1	5.73±0.71	110.9±24.3	2.96±2.02
	T/C	49	**214.0±34.1**	**52.51±10.18**	**128.6±29.9**	164.8 ± 86.2	6.03±1.37	113.0±26.9	3.25±1.65
	C/C	16	**196.6±46.1**	**54.69±11.76**	**115.5±40.3**	132.3 ± 49.2	6.03±1.48	114.8±33.7	3.24±2.08
			$p=0.040$	**$p=0.011$**	**$p=0.062$**				

p-Values are given by ANOVA, and $p<0.05$ are bold.

Table 6 Preproghrelin genotypes and obesity-related parameters.

SNP	Genotype	*n*	BMI	Body fat (%)	Waist circumf.	Total fat area	Subcutaneous f.a.	Visceral f.a.	Visceral fat (%)
Male									
−1500C/G	C/C	30	30.53±4.04	28.83 ± 5.00	101.6±9.6	414.3±144.3	255.3 ± 105.3	159.0±62.5	38.70±9.54
	C/G	51	29.81±2.04	29.05 ± 4.11	100.4±6.3	392.1±89.5	237.2 ± 63.9	154.9±47.1	39.52±7.84
	G/G	34	31.31±4.64	29.21 ± 4.50	103.1±10.8	447.9±170.0	282.3 ± 139.3	165.6±57.8	37.98±10.24
−1062G/C	G/G	41	30.31±3.59	28.31 ± 4.57	101.3±8.6	410.7±130.1	253.4 ± 93.5	157.2±59.2	38.47±9.00
	G/C	49	30.22±2.93	29.52 ± 4.58	101.1±7.5	407.4±120.8	248.5 ± 84.1	158.9±58.6	38.91±9.09
	G/C	25	31.09±4.55	29.34 ± 3.91	102.8±11.1	434.0±162.1	271.4 ± 145.8	162.6±36.0	39.37±9.13
−994C/T	C/C	39	30.41±3.65	28.46 ± 4.62	101.6±8.8	412.8±132.5	253.2 ± 95.6	159.6±59.6	38.91±9.01
	C/T	50	30.10±2.90	29.36 ± 4.59	100.7±7.4	403.0±118.9	247.4 ± 83.2	155.6±57.9	38.51±9.15
	T/T	26	31.15±4.48	29.32 ± 3.90	103.0±10.9	438.5±160.0	273.4 ± 142.8	165.1±38.0	39.41±8.98
Leu72Met	Leu/Leu	74	30.39±3.42	28.90 ± 4.59	101.1±8.2	410.3±130.5	251.4 ± 91.5	158.9±59.0	38.85±8.81
	Leu/Met	33	30.28±3.97	29.38 ± 4.09	101.8±9.8	415.6±148.9	258.1 ± 131.4	157.6±47.7	38.98±9.83
	Met/Met	8	31.58±3.07	28.95 ± 4.89	104.0±9.3	446.6±91.7	279.1 ± 73.9	167.5±36.9	38.29±8.06
+3056T/C	T/T	58	30.37±3.63	28.88 ± 4.92	101.5±8.7	411.1±141.7	254.3 ± 100.7	156.8±59.0	38.52±8.75
	T/C	43	30.57±3.74	29.24 ± 3.82	101.7±9.1	419.0±136.6	254.2 ± 117.2	164.9±51.0	40.01±9.13
	C/C	14	30.36±2.73	29.11 ± 4.33	101.1± 8.0	413.6±84.5	262.4 ± 63.8	151.1±45.1	36.62±9.71
Female									
−1500C/G	C/C	31	30.45±2.71	40.20 ± 5.32	102.3±7.9	**453.1±101.1**	330.9 ± 87.7	**122.2±44.7**	27.08±9.07
	C/G	64	31.14±3.19	40.34 ± 4.84	103.2±7.9	**455.6±95.1**	330.5 ± 77.3	**125.1±45.3**	27.41±7.99
	G/G	23	31.87±3.44	42.33 ± 6.90	107.1±9.5	**526.0±138.7**	370.0 ± 113.9	**156.0±46.5**	30.17±8.02
					p=0.087	***p*=0.018**		***p*=0.012**	
−1062G/C	G/G	43	**30.11±2.55**	39.90 ± 5.09	**101.3±7.7**	**439.4±94.2**	321.8 ± 79.2	**117.6±42.0**	26.73±8.33
	G/C	60	**31.49±3.10**	40.75 ± 4.82	**104.3±7.7**	**472.2±98.1**	341.2 ± 80.5	**131.0±46.2**	27.78±8.02
	C/C	15	**32.40±4.09**	42.73 ± 8.01	**108.1±10.8**	**538.4±156.4**	373.8 ± 132.1	**164.6±47.3**	31.44±8.86
			***p*=0.019**		***p*=0.016**	***p*=0.009**		***p*=0.003**	
−994C/T	C/C	39	**30.09±2.67**	39.60 ± 5.18	101.4±7.8	**438.5±97.8**	321.3 ± 82.0	**117.2±43.2**	26.70±8.54
	C/T	60	**31.52±3.08**	41.06 ± 4.82	104.3±7.8	**472.6±98.1**	341.4 ± 79.5	**131.2±46.3**	27.77±7.94
	T/T	19	**31.86±3.79**	41.78 ± 7.38	106.6±10.1	**518.0±145.4**	363.4 ± 122.1	**154.6±47.5**	30.55±8.72
			***p*=0.042**		*p*=0.059	***p*=0.030**		***p*=0.015**	
Leu72Met	Leu/Leu	69	30.69±2.91	40.34 ± 5.27	102.6±8.0	458.4±106.3	334.1 ± 87.7	**124.3±40.8**	**27.22±7.52**
	Leu/Met	42	31.83±3.39	41.27 ± 5.63	105.0±8.9	480.0±112.5	347.4 ± 90.2	**132.5±54.1**	**27.55±9.27**
	Met/Met	7	30.83±3.30	40.71 ± 6.30	106.7±7.5	502.1±120.2	325.2 ± 98.8	**176.9±27.3**	**36.08±5.52**
								***p*=0.016**	***p*=0.024**
+3056T/C	T/T	53	30.63±2.99	40.60 ± 5.50	103.2±8.1	466.4±111.1	339.0 ± 92.6	127.4±39.9	27.51±7.35
	T/C	49	31.35±3.20	40.96 ± 5.57	103.2±8.9	462.0±105.9	334.6 ± 86.6	127.4±50.4	27.51±9.25
	C/C	16	31.93±3.36	40.18 ± 5.04	106.8±7.2	496.8±115.0	347.4 ± 86.7	149.3±54.2	30.12±8.34

p-Values are given by ANOVA, and $p < 0.05$ are bold.

Table 7 Association check of preproghrelin gene polymorphisms and haplotype.

SNP	Allele	Freq.	Freq. in case/control	*p*-Value	Block	Haplotype	Freq.	Freq. in case/control	*p*-Value
(a) Diabetes mellitus in male (case = 59/control = 56)									
GHRL − 1500	G	0.517	0.576/0.455	0.067	Block 1	CGC	0.473	0.423/0.526	0.118
GHRL − 1062	C	0.430	0.492/0.366	0.055		GCT	0.417	**0.482**/0.348	**0.038**
GHRL − 994	T	0.443	0.492/0.393	0.132		GGC	0.079	0.077/0.081	0.907
GHRL + 408 (L72M)	A	0.213	0.237/0.188	0.357		GGT	0.017	0.009/0.027	0.291
GHRL + 3056	C	0.309	**0.390**/0.223	**0.006**	Block 2	CT	0.687	0.610/**0.767**	**0.010**
						AC	0.208	0.237/0.178	0.272
						CC	0.100	**0.153**/0.045	**0.007**
(b) Visceral fat area ≥100 cm² in female (case = 84/control = 34)									
GHRL − 1500	G	0.466	0.506/0.368	0.054	Block 1	CGC	0.534	0.494/0.632	0.054
GHRL − 1062	C	0.381	**0.423**/0.279	**0.040**		GCT	0.381	**0.423**/0.279	**0.040**
GHRL − 994	T	0.415	**0.458**/0.309	**0.035**		GGC	0.051	0.048/0.059	0.723
GHRL + 408 (L72M)	A	0.237	0.256/0.191	0.289		GGT	0.034	0.036/0.029	0.809
GHRL + 3056	T	0.657	0.661/0.647	0.841	Block 2	CT	0.643	0.647/0.631	0.814
						AC	0.223	0.242/0.175	0.260
						CC	0.120	0.097/0.178	0.083
						AT	0.014	0.014/0.016	0.880
(c) Total-cho. ≥220 mg/dL in female (case = 54/control = 64)									
GHRL − 1500	C	0.534	0.537/0.531	0.929	Block 1	CGC	0.534	0.537/0.531	0.929
GHRL − 1062	G	0.619	0.648/0.594	0.391		GCT	0.381	0.352/0.406	0.391
GHRL − 994	C	0.585	0.602/0.570	0.624		GGC	0.051	0.065/0.039	0.370
GHRL + 408 (L72M)	C	0.763	**0.824**/0.711	**0.042**		GGT	0.034	0.046/0.023	0.334
GHRL + 3056	T	0.657	0.713/0.609	0.095	Block 2	CT	0.643	**0.711**/0.584	**0.043**
						AC	0.223	0.174/0.264	0.099
						CC	0.120	0.113/0.126	0.747
						AT	0.014	0.002/0.025	0.133
(d) BMI ≥30 kg/m² in female (case = 67/control = 52)									
GHRL − 1500	G	0.466	**0.538**/0.375	**0.013**	Block 1	CGC	0.534	0.462/**0.625**	**0.013**
GHRL − 1062	C	0.381	**0.462**/0.279	**0.004**		GCT	0.381	**0.462**/0.279	**0.004**
GHRL − 994	T	0.415	**0.492**/0.317	**0.007**		GGC	0.051	0.045/0.058	0.671
GHRL + 408 (L72M)	A	0.237	0.273/0.192	0.149		GGT	0.034	0.030/0.038	0.731
GHRL + 3056	C	0.343	**0.409**/0.260	**0.016**	Block 2	CT	0.643	0.582/**0.720**	**0.028**
						AC	0.223	0.263/0.172	0.094
						CC	0.120	0.146/0.088	0.174
						AT	0.014	0.009/0.020	0.475

p-Values are given by the chi-square test between case and control, and $p < 0.05$ are bold.

+3056C haplotype was more frequent in the diabetic subgroup (case) than in the non-diabetic subgroup (control) (Table 7a). Simultaneously, the +408C - +3056T haplotype was more frequent in the non-diabetic subgroup, suggesting that the causative locus is located around not 72Met but +3056C.

Similarly, 118 female subjects were grouped into the subgroup of higher visceral fat area (100 cm^2 and more) and the normal one. A visceral fat area $\geq$100 cm^2 is the criterion for the metabolic syndrome, indicated by The Japanese Society of Internal Medicine. The higher visceral fat area subgroup (84 subjects) had the −1500G - −1062C - −994T haplotype more frequently (Table 7b). Particularly, the −1062C and −994T were highly significant (Table 7b).

The female subjects were also divided into two subgroups of higher total cholesterol ($\geq$220 mg/dL) and normal concentration. A value $\geq$220 mg/dL for total cholesterol is the criterion for lipidemia, according to The Japan Atherosclerosis Society. A higher total cholesterol subgroup (54 subjects) had a higher ratio of the +408C - +3056T haplotype than the lower subgroup (Table 7c).

Furthermore, the female subjects were grouped into a higher BMI (30 kg/m^2 and more) subgroup and relatively lower BMI subgroup. The higher BMI subgroup (67 subjects) had the −1500G - −1062C - −994T haplotype more frequently (Table 7d). Conversely, the +408C - +3056T haplotype was more frequent in a subgroup with a relatively lower BMI (52 subjects) (Table 7d), implying that there are more than 2 loci relevant in adiposity. Thus, the susceptibility to obesity is a mixed feature of the above-mentioned higher visceral fat area subgroup with the lower total cholesterol subgroup.

Discussion

Phenotypes of the preproghrelin gene SNPs are particularly complex, since this study revealed them to be quite different between men and women. In obese men, the +3056C/C minor homozygotes were demonstrated to be susceptible to diabetes mellitus, which is not accompanied by insulin or leptin accumulation.

In contrast, in obese women, the correlation of preproghrelin polymorphism with diabetes may be insignificant, i.e., although the −1062C/C minor homozygotes (those of −1500C>G and −994C>T also) had higher concentrations of insulin and C-peptide, their fasting glucose and HbA1c values were normal. Instead, these preproghrelin polymorphisms may contribute to the development of obesity via aberrant fat storage; female subjects with a −1500G - −1062C - −994T haplotype had a higher BMI and a tendency of fat mass accumulation, particularly visceral. This is notable because generally, women tend to accumulate subcutaneous rather than visceral fat mass. Likewise, in SCOP, females had a lower visceral fat area (130.4 cm^2 on an average) than males (159.1 cm^2) (Table 1). To the contrary, females with the above haplotype tended to have a higher visceral fat area comparable to male average. (This is because estrogen influences body fat distribution; the estradiol concentration is inversely associated with visceral fat accumulation in menstruating females [17]; and body-fat distribution shifts toward the upper part of the body (viscera) after menopause [18].)

Additionally, preproghrelin polymorphism also affected serum cholesterol homeostasis: female subjects with the 72Met allele showed lower serum concentrations of total, HDL and LDL cholesterol. Thus, reduced cholesterol are probably linked to the accumulation of body fat: however, interestingly, the causative loci may be distinct; lower cholesterols, 72Met; accumulation of visceral fat, −1062C and −994T.

This study suggested that the contribution of the preproghrelin gene SNPs to metabolic syndrome should be estimated separately in women and men. This is a reasonable proposition because androgens regulate ghrelin secretion or catabolism in a gender-specific manner; androgens and ghrelin have similar effect in males but opposite effects in females [19]. Conversely, ghrelin may down-regulate the concentrations of testosterone [20] or estrogen; Misra and his group reported that adolescent athletes, who are possibly in the negative energy balance state, showed high ghrelin and decreased estradiol concentrations [21,22].

Choi et al. identified the 9 SNPs in the promoter region and compared their frequencies in type 2 diabetic patients with non-diabetic controls in a Korean population [11]. They found the association of the −1062C allele with lower HDL cholesterol, higher fasting glucose, and higher homeostasis model assessment of insulin resistance (HOMA-IR) values. Their results were similar to ours. Possibly, gender-specific estimation may uncover the contribution of these SNPs to diabetes or obesity.

In the study examining the risk of metabolic syndrome in an Amish population, a Caucasian cohort of European descent, Steinle et al. [23] found the association of the 72Met variant with increased prevalence of metabolic syndrome and higher fasting glucose, lower HDL cholesterol, and higher triglyceride concentrations. Their results partly agree with our data. In the cohort, metabolic syn-

drome as well as type 2 diabetes mellitus was more prevalent among women as compared with men. Because their results were not gender-specific, it is not known whether 72Met variant was strongly associated with the above phenotypes in women.

Kuzuya et al. [13] reported a significantly higher frequency of the 72Met allele in the higher BMI (25 and more) group of middle-aged men than in the normal-weight group, suggesting that not only the gender but also the extent of obesity (obese or non-obese) should be considered.

Recently, Zavarella et al. [24] reported on the protective effect of ghrelin in insulin resistance by the 72Met allele in a Caucasian (Italian) population consisting of obese and normal weight subjects; the Met/Met minor homozygotes had lower values of triglycerides, fasting insulin, HOMA-IR, and a higher total ghrelin level. We could not observe such effects in Leu72Met. They also reported that the T/T minor homozygotes of −604C>T had decreasing values of fasting insulin and HOMA-IR. We have not yet analysed −604T>C (rs27647): however, if LD is maintained between −604T>C and −994C>T, our results in the −994 minor homozygotes are not in agreement with theirs. Preproghrelin gene polymorphism may affect insulin metabolism differently according to the race and gender of the subjects or extent of obesity (obese or non-obese).

We are particularly interested in the relationship existing among the SNPs, plasma ghrelin concentration, and susceptibility to diabetes or obesity. Unfortunately, the plasma ghrelin was not measured in the present study. Ando and his colleague [25] reported on the association of higher acylated ghrelin (active form) concentration and body weight, BMI, fat area, waist circumference, skinfold thickness, and a lower HDL cholesterol in Japanese women with the +3056C allele. They stated that the 72Met carriers also had a higher acylated ghrelin but to a lesser extent than the +3056C carriers. Some groups reported a higher ghrelin concentration in the 72Met carriers [24]. These results predict that a higher ghrelin predisposes to obesity in the subjects with the −1500G - −1062C - −994T haplotype as well. Above mentioned Choi et al. [11] used biochemical experiments and found that a transcription factor, myoD, preferentially binds to the region with −1062C, although they described that the region with −1062G had a 1.7-fold higher promoter activity of preproghrelin than that with −1062C.

About the reason why preproghrelin gene SNP may relate to diabetes in obese men, we suppose that higher ghrelin affects serum glucose homeostasis via androgens. Dezaki et al. described that besides effects on appetites and energy homeostasis, ghrelin is also involved in regulating insulin release and glucose metabolism: ghrelin inhibits insulin release [26]. As shown in Table 4, male +3056C/C homozygotes indeed had lower insulin and C-peptide levels on an average, though these were not significant.

Haploview enables the study of LD among SNPs and the estimation of frequencies of the analysed haplotype. Our D' values of the LD are mostly in agreement with those of Ando et al. [12] or of Choi et al. [11]. We observed 2 haplotype blocks in this region: block 1, −1500C>G - −1062G>C - −994C>T and block 2: Leu72Met - +3056T>C. These SNPs shows a strong LD in each haploblocks. This is probably because not only the coding-rich region but also the promoter region also has functional importance.

In conclusion, the +3056C/C minor homozygotes were associated with predisposition to diabetes mellitus in obese men. In obese women, the −1500G - −1062C - −994T haplotype was correlated with visceral adiposity, and the 72Met - +3056C haplotype was associated with susceptibility to obesity via aberrant fat metabolism (reduction of serum cholesterol). Thus, polymorphisms of the preproghrelin gene are suggested to be closely related to diabetes and obesity in obese Japanese. Further studies are required to evaluate these findings and to elucidate the underlying biological mechanisms.

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Modification of Diet in Renal Disease MDRD Studyを考える

渡邊　昌

「医と食」編集長

過去50年来、尿毒症の進行停止に低たんぱく食が効果を上げることが知られていた。日本では慶應義塾大学の大森憲太、浅野誠一らが草分けとなり、最近では昭和大学の出浦照国らが普及を図っている。しかし、日本においては低たんぱく食に懐疑的な意見もあり、低たんぱく食をめざす方向は一定していない。そのひとつの理由に米国で行われた大がかりな臨床試験Modification of Diet in Renal Disease (MDRD) Studyが期待したようなよい結果を出せなかったことがある。本稿ではMDRDの問題点を精査し、最近の長期追跡調査の結果をあわせて論じたい。

MDRD Study

MDRD Studyはランダム化多施設共同研究として食事性たんぱく質とリンの摂取、血圧のコントロールによって慢性腎臓病（CKD）の進行を遅らせられないかという臨床試験である。その最初の結果は、Klahrら[1]により1994年にN Eng J Medに掲載され、超低たんぱく食群の死亡が増えたことが報告されたために、一気に低たんぱく食は無効でむしろ危険という誤解が広まった。

研究は米国腎臓病学会の関係者によって周到に準備され、ベースラインにおいて患者登録は18歳～70歳の範囲で、血清クレアチニン（Cr）が男性では1.4～7.0 mg/dl、女性では1.2～7.0 mg/dlの者とされた[1,2]。インスリン治療をしている糖尿病患者や腎移植を受けているものは除外された。

2,507名の患者がスクリーニングされ、ベースライン期に1,785人がGFR評価、食事摂取、血圧の検査に回された。GFRが25 ml/min/1.73m^{2}以上ある者は食事性たんぱく質の摂取量を1.3または0.6g/kg/day以上にするように振り分けられた。またGFRが24 ml/min/1.73m^{2}以下のものはたんぱく質摂取量を0.6 g/kg以上摂るようにいわれた。他の条件は、体重はNational Health and Nutrition Evaluation Surveyに記載される標準体重の80～120%、血清アルブミンは3.0g/dl以上、尿中たんぱく質は10g/day以下であった。3ヵ月後にたんぱく質摂取量、GFR、血圧が再検査され、GFRが25-55 ml/min/1.73m^{2}範囲のもの585名をA群、GFRが13-24 ml/min/1.73m^{2}のもの255名をB群に振り分けた。

A群は通常たんぱく質量（1.3g/kg/day）と低たんぱく食（0.28g/kg/day）群に振り分けられた。B群はA群と同じ低たんぱく食群と超低たんぱく食（0.28g/kg/day）群に振り分けられた。超低たんぱく食群は同時に0.28g/kg/dayの割合でアミノ酸—ケト酸混合物をサプリメントとして服用した（表1）。また、マルチビタ

表1　超低たんぱく食に使用されたケト酸・アミノ酸サプリメント

Ketoacid-aminoacid mixture		umol/kg/day
L-tyrosine	271	
L-threonine	119	
calcium	17	
D,L-hydroxymethylthiobutyrate	34	
L-tryptophan	4	
L-ornithine	491	塩基性アミノ酸
L-lysine	273	
L-histidine	68	
ketoisocaproate	305	
ketoisovalerate	254	
R,S-ketomethylvalerate	237	

ミン・ミネラルの錠剤を毎日1錠服用した。

エネルギー源摂取の目安は体重が標準の115%までは30kcal/kg、それより肥満のもの、高血圧や脂質異常症のあるもの、2型糖尿病患者は25-30 kcal/kgとされた。

栄養コーディネーター

Nutrition Coordinating Center (NCC) はピッツバーグ大学の臨床栄養センターが担当し、介入プログラム「Protein Wise」を作成した。このプログラムは行動理論にもとづき、それぞれのグループで差があるが、基本的にはどの食事グループにも同じようにすることとされた。このプログラムのゴールは自分で長期間食材を選び、食事を用意する技術を身につけ、自分でできるのだ、という感覚を持たせることである。NCCはこの研究に参加する栄養士を教育し、介入の素材を用意し、使用マニュアルを作成し、個々の臨床試験をしている栄養士と電話で相談にのり、個々の患者が摂取基準を守れるようにカンファレンスを呼び掛けた。また、各センターに低たんぱく食品や高カロリー低たんぱくサプリメントなどを送った。

たんぱく質摂取量は毎月、尿中尿素から計算した。摂取量(g)=6.25 x [UUN (g/day)+0.31 x 標準体重(kg)]である。ケト酸・アミノ酸サプリメントの摂取状況は血漿alloisoleucine濃度で判定した。

GFRは、^{125}I-iothalamateにより測定した。

栄養状態は血清アルブミン、トランスフェリン、体重、体脂肪率（二頭筋、三頭筋、肩甲骨下の皮下脂肪から計算)、上腕筋、尿中クレアチニンから判定した。追跡期間は平均2.2年（0-44ヵ月）である。

その途中で以下のような変化がみられた場合には対処方法が細かく決められた。体重がベースラインから2

表2　追跡2ヵ月までの低たんぱく食達成率と死亡、入院、中止例

		A群 N	A群 患者人年当たり%		B群 N	B群 患者人年当たり%
通常たんぱく食	死亡	9	1.3	低たんぱく食	1	0.3
N=294	入院	66	11.1	N=129	32	12.7
	中止	46	6.7		55	19
低たんぱく食	死亡	2	0.3	超低たんぱく食	4	1.4
N=291	入院	63	10.3	N=126	28	11.6
	中止	38	5.5		48	17.3
達成たんぱく摂取4分位				達成たんぱく摂取4分位		
< 0.74	死亡	1	0.3	< 0.61	0	0
	入院	31	9.9		15	11.5
	中止	20	5.7		26	17.5
	小計	145			64	
0.75 - 0.93	死亡	1	0.3	0.62－0.67	1	0.7
	入院	31	10.8		13	10.3
	中止	19	5.9		22	14.9
	小計	144			63	
0.94 - 1.12	死亡	5	1.5	0.68－0.75	1	0.7
	入院	35	12.3		19	16
	中止	26	7.9		23	16
	小計	144			64	
> 1.13	死亡	3	0.9	> 0.76	3	2.4
	入院	32	10.5		13	11.2
	中止	18	5.1		31	24.5
	小計	145			63	

表3　A群、B群のたんぱく質、エネルギー摂取と体重変化

		at the end of baseline		during follow-up		
		usual protein	low protein diet	usual protein	low protein diet	
A群	たんぱく摂取量 M	1.12±0.18	1.12±0.19	1.11 + 0.14	0.77 + 0.13	
	たんぱく摂取量 F	1.13±0.17	1.12±0.22	1.09 + 0.14	0.76 + 0.11	
	エネルギー摂取 M	27.6±7.01 kcal	27.6±7.27 kcal	26.7 + 5.44	23.1 + 5.72	
	エネルギー摂取 F	26.4+6.64	26.9+7.40	24.7 + 5.31	21.9 + 6.26	
	体重 M	89.0 + 14.9	85.4+ 13.5	88.5 + 14.6	83.2 + 12.8	
	体重 F	71.8 + 15.0	70.5+ 14.3	72.2 + 14.9	69.3 + 13.7	
		low protein	very low protein	low protein	very low protein	
B群	たんぱく摂取量 M	0.84±0.20	0.87±0.18	0.72 + 0.11	0.48 + 0.11	0.66 + 0.11 with suppl.
	たんぱく摂取量 F	0.89±0.15	0.87±0.21	0.73 + 0.09	0.47 + 0.11	0.65 + 0.11 with suppl.
	エネルギー摂取 M	25.3±7.04	25.9±7.48	22.5 + 4.83	22.7 + 4.92	
	エネルギー摂取 F	24.1±5.83	23.3±5.81	20.6 + 3.78	21.1 + 4.74	
	体重 M	80.8 + 0.20	81.9 + 11.16	79.6 + 11.5	79.3 + 10.9	
	体重 F	67.6 + 12.4	66.1 + 15.7	65.9 + 11.9	65.0 + 14.3	

.5 kg以上減少した場合、あるいは標準体重の5%減少あるいは標準体重の80%以下になった場合は、エネルギー摂取を上げる。標準体重の5%以上、あるいは糖尿病患者にあっては標準体重の115%以上になったらエネルギー摂取を減らす。血清アルブミンが3g/dl台で0.5以上低下した場合、あるいは3g/dl以下に減った場合は、まずエネルギー摂取量を増やし、それでも駄目な場合はたんぱく質摂取を増やす。どの群でもたんぱく質摂取量が目標とする範囲よりも下回った場合、急いで回復させる。血清トランスフェリンが200mg/dlより50以上下がった場合もたんぱく質摂取量を増やす。

中止の要件として3g/dl以下から改善しないアルブミン値、標準体重の75%以下の痩せ、6mg/dl以上の高リン血症、急速なGFRの低下(A群)や重篤な病気などである。

結果

MDRDは平均2.2年追跡された。最長は44ヵ月である。経過中の死亡や入院、中止等の結果を表に示す。B群の超低たんぱく食群の方が入院や死亡の割合が多いが、摂取たんぱく質の四分位で見てみると、多く摂取している方が予後の悪いことがわかる(表2)。

驚くべきことはたんぱく摂取量が目標の低たんぱく質摂取量に到達していないことである(表3)[2]。超低たんぱく食群ですら、男女ともに0.5 g/kgに近く、ケト酸・アミノ酸サプリメントを入れると0.66 ± 0.11 g/kgである。この時点でこの研究は失敗したと言える。

また、エネルギー摂取もきわめて低く男女ともに22～20kcal/kgしか摂られていない。この量は対象者の体重から私たちの「体重x 0.4」の式で推計すると必要量の70%程度しかまかなえていないことになる。この絶対的なエネルギー不足が開始2ヵ月にしてすでに1-2 kgの体重減として表れて、多数の中止者につながったのであろう。

Menonら[3]はB群について臨床試験終了後9ヵ月の時点でさらに追跡調査をしているが、この時点でのたんぱく質摂取量は0.7 ± 0.1 g/kgで、エネルギー摂取量は低たんぱく食群が20.5、超低たんぱく食群が22.5 kcal/kgであった。低たんぱく食群では129人中、腎不全が117人、腎不全後の死亡が23人、腎不全前の死亡が7人、イベントなしは5人のみであった。また、超低たんぱく食群は126人中、腎不全が110人、腎不全後の死亡が39人、腎不全前の死亡が10人であり、イベントフリーは6人であった。つまり死亡が超低たんぱく食群で増えたという結論である。

表4　低たんぱく食の利益と不利益

	利　益	不 利 益
証明済み	毒素の負荷を減らす	Protein Energy Malnutrition になりやすい
	腎不全の進行を遅らせる	食事が複雑
	血圧をコントロールできる	密接な指導が必要
	リンをコントロールできる	筋肉が減少
	H+をコントロールできる	
	インスリン感受性を改善	
	たんぱく尿を改善	
賛否両論	腎不全終末期への保存期を延ばす	体重が増える
		超低たんぱく食では死亡が増える

これに対し、MenonらのMDRD研究の長期追跡結果をうけて最近のAmer J Kidney Disに編集者の意見として下記のようにまとめたコメントが掲載された。

評価

MDRD研究は設定した低たんぱく食に至らず、目的をかなえることができなかった点で明かに失敗である。また、絶対的に不足なエネルギー摂取によってたんぱくエネルギー栄養障害(PEM)を誘導したようなものである。超低たんぱく食摂取者に腎不全前の死亡が多いことは、アミノ酸・ケト酸サプリメントの毒性も考えねばならない。B群の窒素源の摂取としては低たんぱく食群も、超低たんぱく食群もサプリメントを足せば0.6 g/kg以上となり、窒素平衡からみて不足する量ではない。

アミノ酸・ケト酸サプリメントには塩基性アミノ酸としてオルニチン、リシンの量が多い。オルニチンは尿素回路に介在する遊離アミノ酸であるが、我々はアンモニア負荷のある状態での腎不全患者はシトルリンの蓄積が起きることを観察しており、オルニチン投与はこの回路に過剰負荷をかけるようなものではないか、と考えている。リシンも腎不全患者では尿中への排泄が落ちているし、ヒスチジンもメチル化代謝物しか尿中へ排泄されない。このように各アミノ酸の代謝状態を考えると、特定のアミノ酸を過剰投与することはむ

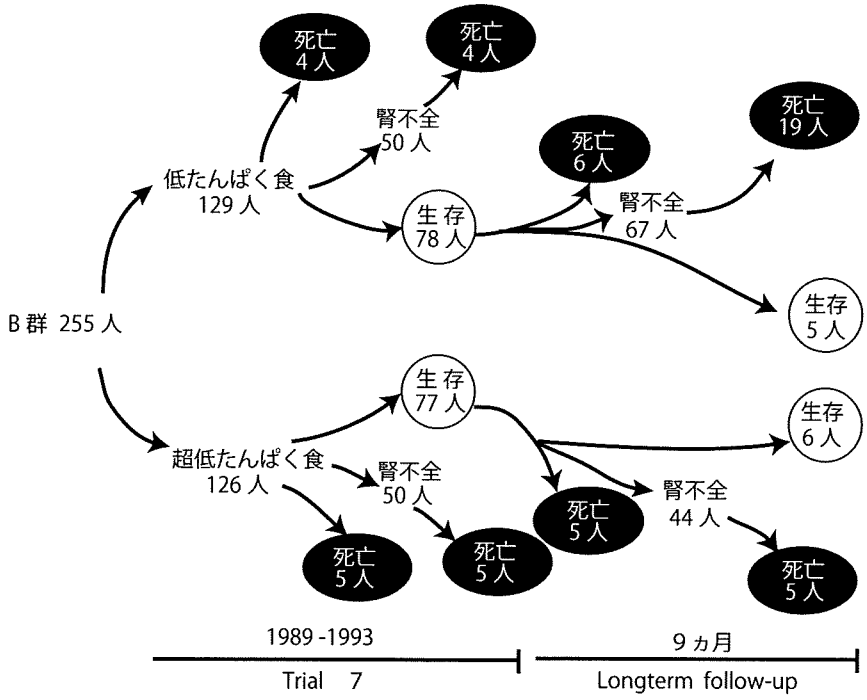

図　B群スタートから5年間の結果

生存は、透析、腎移植に入らなかったもので両群合わせて11人（4.3%）しかいない。

しろリスクの方が大きくなっていたと考えるとMDRDの結果は理解しやすい。

結論

MDRD研究は低たんぱく食の効果をみる面では失敗の研究であった。その後のメタアナリシスでは低たんぱく食の有効性を示すものが多い[4,5]。現在、日本では慢性腎臓病患者の重症化予防のための厚生労働省の戦略研究（FROM-J研究）が進められているが、低たんぱく食の効果のエビデンスとなることが望まれる。

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Evaluation of Modification of Diet in Renal Disease (MDRD) Study

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We have made a review of the Modification of Diet in Renal Disease (MDRD) Study. In this study, 585 patients in group A (GFR 22-55 ml/min/1.73m^2) were divided into two sets, one set prescribed a normal protein intake (1.3 g/kg/day) and the other a low protein diet (LPD, 0.6 g/kg/day); the 255 patients in group B (GFR 13-24 ml/min/1.73m^2) were divided into an LPD set (0.6 g/kg/day) and very low protein diet (VLPD, 0.28 g/kg/day) set. The VLPD set were also prescribed a daily 0.28 g/kg amino acid-keto acid supplement and multivitamin tablet. Energy intake was set at 30 kcal/kg body weight. Follow-up period was 2.2 yrs in average; an additional 9-month follow-up identified poor prognosis in the VLPD group. The actual protein intake was found to be above the specified level, and it is thought that the low energy intake (22-20 kcal/kg) may be the cause of malnutrition. In addition, it is believed that the high concentration of ornithin and lysine may cause toxicity in CKD patients. In our opinion, failure of the MDRD study to demonstrate efficacy of LPD, is due to insufficient energy intake. *Clinical & Functional Nutriology 2009; 1(5): 238-41*

テーラーメイド・ヌトリションは フードアイコンから

渡邊 昌

食品機能表示研究会代表

Simple Food Icon for the tailor-made nutrition

Shaw Watanabe, Committee for Label of Food Function Claim

糖尿病、高血圧症、脂質異常症などの生活習慣病は内臓肥満が大きな要因とされ、肥満克服が健康日本21の目標のひとつに掲げられた。しかし2005年の中間解析では肥満者増に歯止めがかからず成果を上げていない。メタボリックシンドロームは疾病ではなく、未病の状態であるので、症状がなく自分で動機付けがしっかりしていないと改善は難しい。食事と運動によって肥満を解消すれば高血糖、高血圧、脂質異常はほとんど正常範囲に復することができるので、社会全体としてサポートする体制づくりが重要になっている。

自分に必要なエネルギー摂取

体重を落としたい人はひとりひとりが自分に必要なエネルギー量をまず理解するのが必要であるが、簡便な方法がないために栄養指導の際に実行させるのが難しかった。体が必要とするエネルギーは基礎代謝＋活動量に見合うもので、総エネルギー消費量TEEで示される。[1,2]

糖尿病の食事指導では80・を1単位として扱っている。平成17年の国民健康・栄養調査結果[2]から対象者の体重と摂取エネルギーの関係をみると成長期はその比が1.0前後から成長にともなって低下してくるが、成長後は0.4あたりで一定になる（図1）。これは二重標識水あるいはダグラスバックを用いて測定した値ともほぼ一致する。

私たちは80・を1単位とする単位制を採用すれば自分のエネルギー必要量をだれでも容易に知ることができる方法を発見した。2重標識水やヒューマンカロリメーターを用いて測定したエネルギー消費の実測値から推定式をつくると成人では男女とも体重1・あたり0.4を掛けた値、よく運動する人では0.5を乗じた値が目安になる。単位制をとれば・の表示が国際標準のMJ（メガジュール）に変わっても混乱しないし、多くの食品や食事は80・だと整数にくくりやすいので、食品やメニューに採用することもでき、国民、生産者、提供者が三位一体となって体重コントロールに取り組める。これは特定健診、個別指導の際にも有効と思われる。すでに80・＝1単位制は女子栄養大学では長年使用しているし、糖尿病の食品交換表などで広く使われているので医師、栄養士、患者にとっても違和感が少ない。これをフードアイコンとして三大栄養素のエネルギー比率や野菜や果物の機能マーカーとしての抗酸化価（AOU）と一緒に表示してはどうかと提案している。

体重変化のない成人では、エネルギー消費量とエネルギー摂取量はほぼ等しい。脂肪1・は約7000・であるので、1日に240・ずつ減らせられればひと月に1・ずつ痩せられる筈である。私たちの単位制では、単位を計算する際の体重として必ずしも現在の実体重でなくとも、目標とする体重としてもよいところがミソである。

体重が、100・もある人は80・で計算してもよいし、ひと月に1-2・痩せるように設定してもよい。またBMI

が 18 以下のような痩せの人は太りたい体重を目標値に置いてもよい。肥満者にいきなり 1600 ・の食事にする、というような方法は本人の飢餓感や急激な体重減はリバウンドしやすい、ということがある。また、それまで 3000 ・近くとっているとすると、1600 ・との差は脂肪やたんぱく質が燃やされてエネルギー源とされるので、筋肉崩壊を来しやすくなる可能性がある。これは低たんぱく食の必要がある患者の場合などは最も注意せねばならない点である。

子どもへの適用

この単位法のよい点は子どもにまで単位制を拡張でき

図 1　体重によるエネルギー摂取量と 80 ・＝ 1 単位とした場合の体重当たりの単位数。　平成 17 年度国民健康・栄養調査より計算

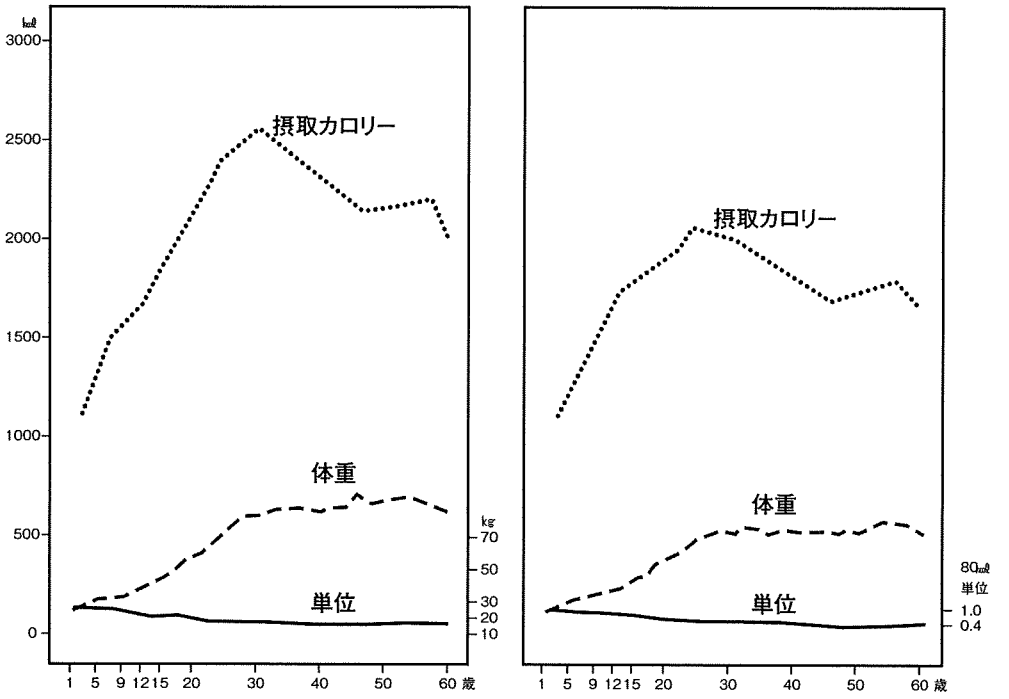
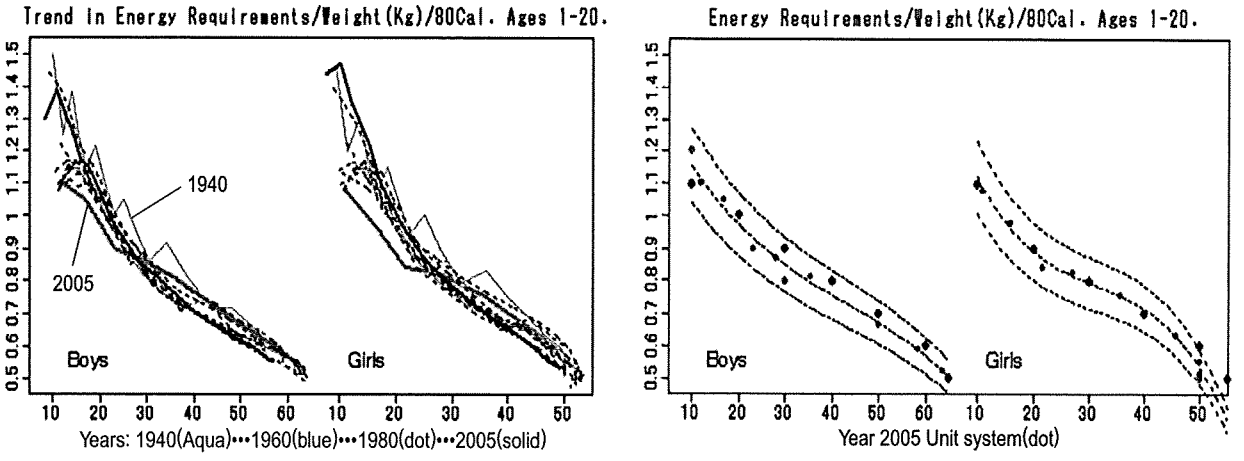


図 2　子どものエネルギー摂取基準の変遷（左）と体重当たりの単位法を適用した場合の摂取量（右）



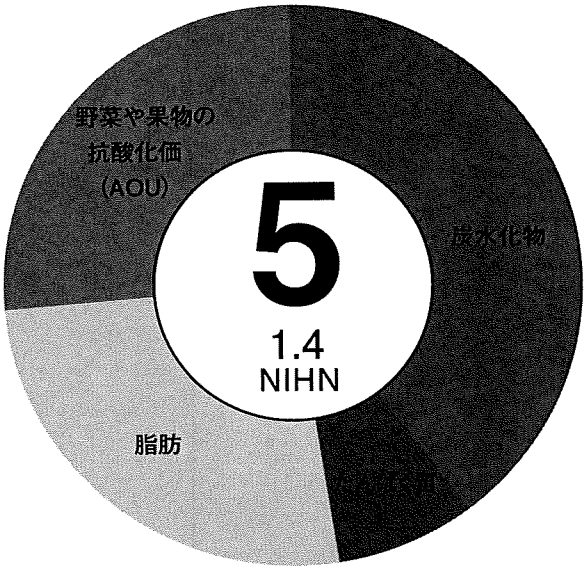


図3　フードアイコン。中央に80・＝1単位とした単位数。周辺に炭水化物、たんぱく質、脂肪のエネルギー比、また野菜・果物の指標としてAOUを円グラフで表し、栄養の量とバランスを直感的に把握できる利点がある。小学校から習う「栄養3食運動」ともつながり、子どもにも理解しやすい。たんぱく質や食塩制限のある人々も利用しやすくするにはグラフの中に数字を印字することも可能である。

ることである。子どものエネルギー摂取基準は時代によって変遷してきた。1995年から2005年までの変化をグラフでみると、幼児や若年層では低下してきており、逆に10歳前後では最近の方が多少増えている（図2左）。

単位法では、10・の子どもは体重 x 1.0でよく、20・までは0.9、30・までは0.8、40・までは0.7、50・までは0.6という具合に、体重10・ごとに0.1を減じてかけていけば、60・になると0.5を掛けることになり、成人につなげることができる（図3右）。この方式で計算すると女児の場合は2005年の摂取基準の平均値と等しい。男児の摂取量は2005年推奨値から10%程度低めになるが、男児の肥満傾向が顕著になっているので10%程度低めの目標は望ましいといえる。現在のエネルギー摂取基準は年齢区分になっているが、同一年齢でも20・以上のばらつきがあるので体重による区割りの方がのぞましい。学校給食へも応用しやすい。一律給食からテイラーメイド給食への道が開け、無駄な残食も減らせると思われる。

社会全体で必要なサポート

私たちは健康日本21で肥満者を減らすという目的が2005年の中間評価で逆に増加してしまったことから、個々人の努力目標として減量を強いるだけでなく、社会全体として長寿社会を目指す枠組みが重要であると思うようになった。たばこの禁煙運動が広がってきたのは社会全体が喫煙と健康の関係を理解し、公共の場が禁煙になったことが大きい。それと同じようにメタボリックシンドロームに連なる内臓肥満の克服には、食品へのエネルギー表示や健康なメニューを選択できる食堂やレストラン、あるいは運動しやすい社会環境つくりが重要と思われる。

私たちはレトルト食品やコンビニ弁当、食堂やレストランのメニューにエネルギー源として単位と栄養素の構成を示すフードアイコンをつけるように提唱している。[3] これはたんぱく質が多いとか、脂肪が多いとか全部わかるので栄養素のバランスも直観的に把握でき、きわめて実用性が高い。また、野菜、果物のサロゲートマーカーとして抗酸化価（AOU）を示すことにより商品の差別化も可能になる。

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糖尿病性腎症を防ぐ食事療法

渡邊 昌

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糖尿病患者の10～15%が糖尿病性腎症に移行し、慢性腎不全（CKD）の大きな要因となっている。透析学会の試算では毎年、腎透析に入る糖尿病患者は1.5万人から2万人と予測されている。2008年から2012年までの累積患者数は21万人を超え、1兆円以上の医療費を使うことになる。糖尿病患者はエネルギーコントロールを強いられることが多く、たんぱく尿が現れると0.8 g/kg体重当たりのたんぱく食を勧められるようになる。一方で低たんぱく食を成功させるには必要十分なエネルギー摂取が必要となるので、おそらく患者も栄養士も戸惑うことが多いと思われる。糖尿病腎症の進行阻止に必要な栄養療法について考えてみたい。

変わるたんぱく質摂取推奨量

日本人の肥満や生活習慣病の増加に、戦後の食生活の変化、特に動物性脂肪の摂取増が原因とされることが多いが、私は動物性たんぱく質摂取の増加も寄与していると思うようになった[1]。日本ではたんぱく質の所要量は時代を追うごとに低くなっている[2]。国連傘下のFAOとWHOは栄養問題に関して、1971年と1981年にエネルギーとたんぱく質についてガイドラインを出し、1985年にはWHOテクニカルレポートとして広く世間に周知させた。

たんぱく質は1g 4 kcalと計算されるが、エネルギーが十分摂れていれば、たんぱく質が脱アミノ反応によってTCA回路で燃焼されることはない。そこで、たんぱく質の摂取量については科学的見地から見直され、2002年にたんぱく質アミノ酸の摂取量について専門家によるワーキンググループが組まれ、2007年にWHOテクニカルレポートとして報告された[3]。

食事摂取基準2010ではWHOのたんぱく質平衡維持量を検討し、平均すると0.65 g/kg体重/日となるので、この値をもって窒素平衡維持量としている。WHOではこの量をもとに標準偏差の2倍を足して体重当たり0.83 g/kgをたんぱく質摂取の安全量としている。日本の摂取基準では、これに基づき成人の1日たんぱく質摂取量を男性60 g、女性50 gとしてある。

これに基づいて人体の食餌必要量が算出された[4]。1日の標準食餌という概念を提唱したのはミュンヘン大学の生理学者フォイトで、彼は労働者の消費する食品の燃焼熱や成分を測定して得た平均値を基に1日に熱量は2,976kcal、たんぱく質118 g、脂肪56 g、炭水化物500 gを摂ることが必要と提案した。当時は摂取量を測定し、それを必要量と考えたのである。この値は明治時代にドイツ医学を導入した際に日本へもたらされた。

それに対し、エール大学のチッテンデンは1902年11月から1日にたんぱく質を30 gないし35 gの食事を7ヶ月間続け、これだけ摂れば自分自身の窒素平衡を保てることを証明した。チッテンデンの食事は体格から計算するとエ

表1　1985年基準と今回のアミノ酸摂取基準比較

アミノ酸	2007WHO		1985FAO/WHO/UN	
	mg/kg per day	mg/kg protein	mg/kg per day	mg/g protein
Histidine	10	15	8～12	15
Isoleucine	20	30	10	15
Leucine	39	59	14	21
Lysine	30	45	12	16
Methionine + cystaine	15	22	13	20
Methionine	10	16	–	–
Cystaine	4	6	–	–
Phenylalanine + tyrosine	25	38	14	21
Threonine	15	23	7	11
Tryptophan	4	6	3.5	5
Valine	26	39	10	15
必須アミノ酸総量	184	277	93.5	141

1体重1kg当たり0.66gの摂取たんぱく質量としての血漿量106mgから換算