ANTI-AGING MEDICINE

Received: Sep 17, 2007
Accepted: Nov 20, 2007
Published online: Dec 25, 2007

Original Article

DNA polymorphism of obese people in Saku Control Obesity Program (SCOP)

Kouichi Yamada, Jun Takezawa, Akemi Morita, Yasuhiro Matsumura, Shaw Watanabe

Program of Educational Nutrition, The National Institute of Health and Nutrition

Abstract

BACKGROUND: Various genes related to fat-, sugar- or energy-metabolism are suspected that their polymorphisms (SNPs) are susceptible to development of obesity and other metabolic syndromes. Although many lines of evidences are accumulated, inspections as to their relation are still insufficient. Here, we assembled 235 obese people in Saku Control Obesity Program (SCOP), and are going to investigate the association between those SNPs, body mass index (BMI) and other clinical parameters.

METHODS: 235 subjects, whose BMI belonged in upper quintile (over 28.3), are recruited from examinees for periodic medical checkup in the Health Dock of Saku Central Hospital (Nagano, Japan). Genotyping for the SNPs were conducted using the PCR-PFLP method from peripheral venous blood. The frequencies of the SNPs in UCP1 (-3826A/G), UCP2 (Ala55Val), UCP3 (-55C/T), PPARγ2 (Pro12Ala), adiponectin (Ile164Thr), leptin receptor (LEPR, Arg109Lys), calpain 10 (SNP43), β2AR (Arg16Gly, Gln27Glu), β3AR (Trp64Arg) were analyzed and associations between those SNPs, body mass index (BMI) and other clinical parameters were investigated. The frequency was compared with those in HapMap Project or in Nansei Cohort..

RESULTS: Frequencies of above SNPs in SCOP were not different from those of healthy people in Nansei Cohort or HapMapProject, except for adiponectin Ile164Thr. SNPs of UCPs or β2, β3AR genes, often reported their association with BMI, were not confirmed in obese subjects in SCOP. We found significant association in the LEPR Arg109Lys in woman; Lys/Lys minor homozygotes had higher levels of leptin, TNF-α, C-peptide, insulin, triglyceride and fasting serum glucose, and also, the genotype showed a trend of higher values of HbA1c, BMI, body fat mass and waist circumference.

CONCLUSION: Adipocytokines, such as leptin and adiponectin, may play an important role in the development of metabolic syndrome, and they would be influenced by polymorphism of UCP or β2, β3AR genes.

KEY WORDS: SNPs, lipokine, Obesity, Epidemiology, Intervention study

Introduction

Metabolic syndrome is defined as a combination of disorders that increase one's risk for cardiovascular disease and diabetes mellitus type 2. Particularly, visceral obesity is shown to predispose to these syndromes. Although lifestyle is important, there is considerable evidence that genetic factors also have a significant role in its pathogenesis.[1]

Various genes related to fat-, sugar- or energy-metabolism are suspected that their polymorphisms (SNPs) are susceptible to develop obesity and other metabolic syndrome. Uncoupling protein (UCP) 1, 2 or 3, adrenergic receptor (AR) β2 or β3, peroxisome proliferator-activated receptor (PPAR) γ2, adiponectin, leptin receptor (LEPR), calpain 10 are comprised in these genes. Although many lines of evidences are accumulating, inspections as to their relation are still insufficient.

Here, we assembled 235 obesity people in Saku Contorol Obestity Program (SCOP), and are going to investigate the association between those SNPs, body mass index (BMI) and other clinical parameters. Genotyping for their SNPs were conducted using the PCR-PFLP method from peripheral blood after taking written informed consent. The frequencies of the SNPs in UCP1 (-3826A/G), UCP2 (Ala55Val), UCP3 (-55C/T), PPARγ2 (Pro12Ala), adiponectin (Ile164Thr), leptin receptor (LEPR, Arg 109 Lys), calpain 10 (SNP43), β2AR (Arg16Gly, Gln27Glu), β3AR (Trp64Arg) were compared with those of healthy people published in HapMap Project or those previously analyzed in Nansei Cohort (n=422, BMI=23.3).

HapMap is a public database of common variation in the human genome exhibited by The International HapMap Consortium.[2] The map includes information about more than one million SNPs obtained in 269 DNA samples from four populations; Yoruba in Ibadan, Nigeria; Utah, USA; Beijing, China; Tokyo, Japan. Some SNPs were also analyzed in Nansei Cohort. Total 422 healthy people were recruited from the group in 2001 and 2002, who received periodic medical checkup in the Nansei-cho Hospital (Mie Prefecture, Japan).

Kouichi Yamada, PhD. / Program of Education Nutrition,
The National Institute of Health and Nutrition
1-23-1 Toyama, Shinjuku, Tokyo 162-8636, Japan
Tel: +81-3-3203-5723 / Fax: +81-3-3203-0335 / E-mail: kouichi@nih.go.jp

Method

· Subjects

Japanese obese subjects aged 40-64 years old with a BMI greater than 28.3 were selected from people who had undergone a medical checkup in the Saku Central Hospital. They were asked to participate in the intervention program for weight loss named Saku Control Obesity Program (SCOP). The participants had an anthropometric and clinical examination (height, weight, body fat percentage, waist circumference, visceral fat area, and biochemical markers of blood and urine) and were assessed for present illness, physical activity and dietary habit at the start of this program. Details are described in elsewhere.[3,4]

· Genotyping and Statistical Analysis

DNA was purified from subjects' blood using QIAamp DNA blood 96 kit (Qiagen). All the SNPs were genotyped by a polymerase chain reaction (PCR) followed by digestion by restriction enzyme (PCR-RFLP method) ***(Table 1)***. 80 ~ 200 bp fragments containing objective SNP were amplified in a 20 μl reaction mixture including 50 mM Tris-HCl, pH9.0, 20 mM ammonium sulfate, 0.7 mM $MgCl_2$, 0.2 mM 4dNTPs, 1.25 units Taq Pol (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 as initial denature, 35 cycles of 2 min at 94°C, 2 min at 52°C, 1 min at 72°C, and 10 min at 72°C as final extension. PCR product was digested with each restriction enzyme (NEB or Fermentus) and subjected to electrophoresis in Spreadex EL300 gel (Elchrom Scientific) at 55°C.

The database was made in EXCEL file and converted to SPSS database. Genotype frequencies were compared using chi-square test, and analysis of variance (ANOVA) was conducted for detection of association between genotype frequencies and clinical parameters. These statistical analyses were done by SPSS ver14.0. If ANOVA was significant, Bonferroni test was also performed.

Results

Frequencies of SNPs in SCOP were listed in ***Table 2***. There was no statistically significant difference among SNP frequencies in those of Nansei Cohort or HapMapProject. Adiponectin Ile164Thr heterozygotes, however, were significantly more frequent in obese subjects in SCOP than those reported by Kondo et al. As for the SNP frequencies between in men and women showed that UCP1-3826 G/G minor homozygotes were more frequent in woman than that in man (P=0.070), suggesting that the homozygotes were susceptible to obesity in woman ***(Table 3)***.

We investigated the association among the SNPs, contents of adipocytokines, clinical parameters and plasma biochemical markers. Comparison of adipocytokine contents sorted out for every SNPs revealed that, in PPARγ2 12Pro/Ala heterozygotes (woman), plasma C-peptide (P=0.004) and insulin level (P=0.008) were higher than Pro/Pro major homozygotes ***(Table 4)***. Adiponectin 164Ile/Thr heterozygotes had lower adiponectin blood concentration than the major Ile/Ile homozygotes both in men (P=0.072) and women (P=0.016). These results coincide with the report of Kondo et al.[9], in which subjects with 164Thr allele had lower adiponectin concentration in blood.

Leptin receptor (LEPR) 109Lys/Lys minor homozygotes had significantly higher leptin (P=0.002), TNF-α (P=0.046), C-peptide (P=0.018) and insulin level (P=0.064) than Arg/Lys hetero or Arg/Arg major homozygotes only in woman. In calpain10 SNP43G/A heterozygotes (woman), free fatty acid was higher than the G/G major homozygotes (P=0.040). β3AR Trp64 allele had trend in higher C-peptide (P=0.009) and insulin contents (P=0.053) than Arg allele in man, which was contrary to results supposed from the accepted hypothesis. Heterozygotes (Trp/Arg) had the intermediate contents.

Comparison of BMI and other biochemical parameters sorted out for every SNPs revealed that UCP1 -3826G/G homozygotes had elevated total- (P=0.046) and LDL- choesterol level (P=0.063) than other genotypes in woman ***(Table 5)***. In PPARγ2 12Pro/Ala heterozygotes (woman), triglyceride level were higher than Pro/Pro major homozygotes (P=0.052), which is converse with general hypothesis. Adiponectin 164Ile/Thr heterozygotes

Table 1 Genotyping of metabolic syndrome-related genes by PCR-RFLP

gene	SNP	dbSNP	amplified region (Bold letters indicate polymorphysm.)	restriction enz.	generated frag (bp)
UCP1	-3826A/G	rs1800592	5'- CACAAAGAAGAAGCAGAGAGG ... ... **T/C** GATCA ... ... CTCTCATTAGCCACCACTGG -3' (Shihara et al.)[5]	BclI	277 151+126(A)
UCP2	Ala55Val (C/T)	rs660339	5'- TCAAGGGCCAGTGTTTGGAG ... ... **C/T** CAGCGCCCAGTACCGCGGT -3'	BstN I	146→128+18(C)
UCP3	-55C>T	rs1800849	5'- ACCCCAAGTCAAGAGGACTG ... ... **C/T** GTGTGTATAAGACCAGTGCAA -3'	Sma I Dde I	153→131+22(C) 153→129+24(T)
PPARγ2	Pro12Ala (C→G)	rs1801282	5'- TCTGGGAGATTCTCCTATTGAC **C/G** ... ... CTCCTGTAGTTGTCTTCCAG -3'	Hae III Hha I	154→20+134C) 154→22+132(G)
adiponectine	Ile164Thr (T→C)	Not Found	5'- GCTGTACTACTTTGCCTACCACA **T/C** ... ... CTATGCTCTTCACCTATGATCA -3'	Dpn II	96→21+75(T)
LEPR	Arg 109 Lys (G>A)	rs1137100	5'- TTTCCACTGTTGCTTTCGGAG ... ... **A/G** GACATTTGTTTCAACAGTAAATTCTT -3'	Ava II	95→26+69(G)
calpain10	SNP43 (G→A)	rs3792267	5'- ACGCTTGCTGCGAAGTAAGGC **G/A** ... ... GACCATGGGAATCAGAGAGG -3'	Mlu I Nsi I	146→18+128(G) 146→22+124(A)
β2AR	Arg16Gly (A/G) Gln27Glu (C→G)	rs1042713 rs1042714	5'- GCCTTCTTGCTGGCACCCAAT **A/G** ... ... GTTTGGCAATGTGCTGGTCAT -3' 5'- GCCTTCTTGCTGGCACCCAAT ... ... GCAG **C/G** ... ... GTTTGGCAATGTGCTGGTCAT -3'	Nco I Fnu4H I	120→18+102(G) 120→52+68(C)
β3AR	Trp64Arg (T→C)	rs4994	5'- GCAGCTGCCCCTTTAAGCG ... ... CC **T/C** GG ... ... GGTGATGGGACTCCTGG -3'	Nci I	213,125, 148→69+79(C)

Table 2 Genotype frequencies of metabolic syndrome-related genes

gene	SNP	cohort	n	genotype major homo	genotype hetero	genotype minor homo	HWP	allele frequency	P-value of SCOP compared with
UCP1	-3826A/G	SCOP	233	52 (22.3)	127 (54.5)	54 (23.2)	0.17	0.496 : 0.504	
		Nansei	422	117 (27.7)	206 (48.8)	99 (23.5)	0.65	0.521 : 0.479	0.264
		HapMap-JPT		Not Found	(Not Analyzed)				
		Shihara et al. (Japanese men) [5]	349	87 (24.9)	191 (54.7)	71 (20.3)	0.07	0.523 : 0.477	0.631
UCP2	Ala55Val (C/T)	SCOP	232	61 (26.3)	108 (46.6)	63 (27.2)	0.29	0.496 : 0.504	
		Nansei	422	116 (27.5)	204 (48.3)	102 (24.2)	0.51	0.517 : 0.483	0.702
		HapMap-JPT	44	11 (25.0)	26 (59.1)	7 (15.9)	0.20	0.545 : 0.455	0.216
		JBIC	1500					0.500 : 0.500	0.611
		Shiinoki et al. (Japanese) [6]	120	28 (23.3)	71 (59.2)	21 (17.5)	0.04	0.529 : 0.471	0.054
UCP3	-55C>T	SCOP	232	108 (46.6)	104 (44.8)	20 (8.6)	0.47	0.690 : 0.310	
		Nansei	422	194 (46.0)	179 (42.4)	49 (11.6)	0.43	0.672 : 0.328	0.477
		HapMap-JPT	44	21 (47.7)	21 (47.7)	2 (4.5)	0.25	0.716 : 0.284	0.654
		JBIC	1478	Not Applicable				0.675 : 0.325	0.664
		Liu et al (Caucasian) [7]	1873	Not Applicable				0.735 : 0.265	0.096
PPARγ2	Pro12Ala (C→G)	SCOP	232	215 (92.7)	17 (7.3)	0	0.56	0.963 : 0.037	
		Nansei	422	392 (92.9)	30 (7.1)	0	0.45	0.964 : 0.036	0.918
		HapMap-JPT	44	39 (88.6)	5 (11.4)	0	0.69	0.943 : 0.057	0.365
		Mori et al. (Japanese men) [8]	215	203 (94.4)	11 (5.1)	1 (0.5)	0.06	0.970 : 0.030	0.370
adiponectine	Ile164Thr (T→C)	SCOP	232	225 (97.0)	7 (3.0)	0	0.82	0.985 : 0.015	
		Kondo et al. (Japanese) [9]	452	450 (99.6)	2 (0.4)	0	0.96	0.998 : 0.002	0.005
LEPR	Arg109Lys (G>A)	SCOP	232	142 (61.2)	77 (33.2)	13 (5.6)	0.55	0.778 : 0.222	
		HapMap-JPT	44	27 (61.4)	16 (36.4)	1 (2.3)	0.44	0.795 : 0.205	0.631
		Park et al. (Korean) [10]	680	458 (67.4)	200 (29.4)	22 (3.2)	0.92	0.821 : 0.179	0.115
		Matsuoka et al.(Japanese) [11]	68	Not Applicable				0.765 : 0.235	0.913
calpain10	SNP43 (G→A)	SCOP	232	212 (91.4)	20 (8.6)	0	0.49	0.957 : 0.043	
		Nansei	247	226 (91.5)	21 (8.5)	0	0.49	0.957 : 0.043	0.963
		HapMap-JPT	45	37 (82.2)	8 (17.8)	0	0.51	0.911 : 0.089	0.062
		Horikawa et al. (Japanese) [12]	172	Not Applicable				0.950 : 0.05	0.712
β2AR	Arg16Gly (A/G)	SCOP	233	51 (21.9)	115 (49.4)	67 (28.8)	0.90	0.466 : 0.534	
		HapMap-JPT	44	6 (13.6)	22 (50.0)	16 (36.4)	1.00	0.386 : 0.614	0.379
		Yamada et al. (Japanese) [13]	450	113 (25.1)	224 (49.8)	113 (25.1)	0.92	0.500 : 0.500	0.486
		Hayakawa et al.(Japanese men) [14]	210	57 (27.1)	104 (495)	49 (23.3)	0.91	0.519 : 0.481	0.288
β2AR	Gln27Glu (C→G)	SCOP	233	195 (83.7)	37 (15.9)	1 (0.4)	0.59	0.916 : 0.084	
		HapMap-JPT	44	38 (86.4)	5 (11.4)	10 (2.3)	0.15	0.921 : 0.079	0.321
		Yamada et al. (Japanese) [13]	450	389 (86.4)	59 (131)	2 (0.4)	0.88	0.930 : 0.070	0.614
		Hayakawa et al. (Japanese men) [14]	210	188 (895)	22 (10.5)	0	0.42	0.948 : 0.052	0.153
β3-AR	Trp64Arg (T→C)	SCOP	233	165 (70.8)	61 (26.2)	7 (3.0)	0.64	0.839 : 0.161	
		Nansei	422	290 (68.7)	115 (27.3)	17 (4.0)	0.20	0.823 : 0.177	0.745
		JBIC	1064	Not Applicable				0.821 : 0.179	0.596
		Shihara et al. (Japanese men) [5]	349	262 (75.1)	84 (24.1)	3 (0.9)	0.18	0.871 : 0.129	0.114

HWP : Hardy Weinberg Plot
JBIC : Japanese Biological Informatics Consortium's data
P-values are given by the chi-square test.

Table 3 Genotype frequencies of metabolic syndrome-related genes (male/female)

gene	genotype	Male n	Male %	Female n	Female %	p
UCP1	A/A	29	25.2	23	19.7	0.070
(-3826A/G)	A/G	67	58.3	60	51.3	
	G/G	19	16.5	34	29.1	
UCP2	Ala/Ala	34	29.6	27	23.1	0.355
(Ala55Val)	Ala/Val	54	47.0	54	46.2	
	Val/Val	27	23.5	36	30.8	
UCP3	C/C	58	50.4	50	42.7	0.464
(-55C/T)	C/T	47	40.9	57	48.7	
	T/T	10	8.7	10	8.5	
PPARγ2	Pro/Pro	105	91.3	110	94.0	0.428
(Pro12Ala)	Pro/Ala	10	8.7	7	6.0	
adiponectin	Ile/Ile	111	96.5	114	97.4	0.684
(Ile164Thr)	Ile/Thr	4	3.5	3	2.6	
LEPR	Arg/Arg	65	56.5	77	65.8	0.345
(Arg109Lys)	Lys/Arg	43	37.4	34	29.1	
	Lys/Lys	7	6.1	6	5.1	
calpain10	G/G	102	88.7	110	94.0	0.149
(SNP43 G→A)	G/A	13	11.3	7	6.0	
β2AR	Arg/Arg	23	20.0	28	23.9	0.687
(Arg16Gly)	Arg/Gly	60	52.2	55	47.0	
	Gly/Gly	32	27.8	34	29.1	
β2AR	Gln/Gln	97	84.3	97	82.9	0.604
(Gln27Glu)	Gln/Glu	18	15.7	19	16.2	
	Glu/Glu	0	0.0	1	0.9	
β3AR	Trp/Trp	78	67.8	87	74.4	0.363
(Trp64Arg)	Trp/Arg	32	27.8	28	23.9	
	Arg/Arg	5	4.3	2	1.7	

P-values are given by the chi-square test.

had higher total- (P=0.015) and LDL-cholesterol (P=0.040) than the major Ile/Ile homozygotes in man (***Table 5***), although in woman, these association were insignificant. LEPR 109Lys/Lys minor homozygotes had significantly higher triglycerid level in woman (P=0.088). Also, their fasting blood sugar level was higher than that of Arg/Arg major homozygotes (P=0.054). To the contrary, in man, Lys/Lys homozygotes had the reduced waist circumference than the other (P=0.033). In calpain10 SNP43G/G major homozygotes (woman), triglyceride was significantly higher level than the G/A heterozygotes (P=0.042), in agreement with negative association in the level of HDL cholesterol (P=0.012). In some genotypes which is reportedly preposition to obesity or diabetes (PPARγ2 Pro/Pro in both sexes, adiponectin 164Ile/Thr in both sexes, LEPR 109Lys/Lys in woman, β3AR Arg/Arg in woman), C-reactive protein (CRP) showed higher trend than other genotypes.

The difference among LEPR Arg109Lys SNP was further tested with Bonferroni adjustment (***Table 6***). Acutally, leptin level, C-peptide level and BMI were significantly higher in the Lys/Lys homozygotes than in other genotypes in women.

Table 4 Genotypes of metabolic syndrome-related genes and adipocytokines

gene	genotype	Male	fFA	leptin	TNF-α	adiponectin	C-peptide	insulin	Female	fFA	leptin	TNF-α	adiponectin	C-peptide	insulin
UCP1	A/A	29	0.50±0.20	7.68±4.97	1.19±0.40	2.88±2.09	2.79±1.36	11.95±12.55	23	0.50±0.21	21.55±10.72	1.11±0.34	4.88±2.99	2.85±1.46	11.52±7.99
	A/G	67	0.51±0.18	8.62±6.26	1.34±0.55	2.87±1.72	2.83±1.16	11.70±8.43	60	0.56±0.21	22.53±10.92	1.25±0.43	5.68±2.89	2.45±0.72	10.94±5.54
	G/G	19	0.50±0.18	7.56±4.16	1.25±0.46	2.35±1.44	3.04±1.44	13.79±11.21	34	0.62±0.22	19.11±11.84	1.24±0.48	5.43±3.43	2.50±0.76	11.25±5.07
UCP2	Ala/Ala	34	0.54±0.20	8.21±5.63	1.21±0.51	2.59±1.33	2.84±1.09	11.05±7.13	27	0.60±0.23	22.91±14.12	1.16±0.47	6.11±3.56	2.42±0.64	9.68±3.80
	Ala/Val	54	0.48±0.18	7.93±6.17	1.30±0.50	2.73±1.95	2.77±1.33	12.40±11.20	54	0.55±0.20	21.06±10.47	1.24±0.42	5.48±3.00	2.51±0.72	10.79±4.75
	Val/Val	27	0.50±0.16	8.75±4.54	1.36±0.51	3.17±1.91	3.04±1.32	12.87±10.82	36	0.57±0.23	19.92±9.55	1.19±0.42	5.02±2.88	2.63±1.32	12.49±8.19
UCP3	C/C	58	0.51±0.20	8.16±5.13	1.28±0.52	2.56±1.54	2.96±1.35	12.78±11.49	50	0.57±0.19	20.88±10.99	1.22±0.43	5.88±3.43	2.42±0.75	10.43±4.97
	C/T	47	0.51±0.17	7.94±6.13	1.27±0.46	2.93±2.05	2.75±1.16	11.42±8.49	57	0.57±0.24	21.37±11.67	1.19±0.42	5.33±2.93	2.64±1.07	11.51±6.65
	T/T	10	0.48±0.20	9.74±6.30	1.39±0.66	3.52±1.50	2.71±1.15	11.46±7.67	10	0.55±0.20	21.10±8.79	1.26±0.50	4.44±2.02	2.34±0.78	11.36±5.42
PPARγ2	Pro/Pro	105	0.51±0.18	8.26±5.74	1.28±0.51	2.81±1.83	2.87±1.26	12.28±10.22	110	0.57±0.21	21.14±11.18	1.21±0.43	5.52±3.16	2.46±0.78	10.69±5.16
	Pro/Ala	10	0.45±0.19	7.64±4.56	1.33±0.48	2.58±1.10	2.71±1.29	10.34±7.58	7	0.57±0.24	21.16±10.61	1.14±0.35	4.89±1.90	3.50±2.08 p=0.004	16.86±12.14 p=0.008
adiponectin	Ile/Ile	111	0.50±0.19	8.09±5.49	1.28±0.50	2.84±1.78	2.85±1.27	12.10±10.17	114	0.57±0.22	21.32±11.18	1.21±0.43	5.59±3.06	2.52±0.93	11.09±5.95
	Ile/Thr	4	0.53±0.13	11.45±9.25	1.40±0.81	1.23±0.26 p=0.072	2.86±0.63	12.45±4.02	3	0.61±0.13	14.37±3.47	0.97±0.21	1.27±0.25 p=0.016	2.44±0.34	9.50±1.66
LEPR	Arg/Arg	65	0.50±0.19	7.74±4.60	1.37±0.53	2.74±1.71	2.87±1.29	12.40±11.13	77	0.56±0.21	20.47±9.75	1.14±0.42	5.37±3.32	2.48±0.81	11.04±5.35
	Lys/Arg	43	0.51±0.18	9.20±7.18	1.17±0.45	2.90±1.95	2.87±1.28	12.04±8.91	34	0.61±0.22	19.93±10.80	1.32±0.41	5.84±2.82	2.43±0.75	10.15±4.87
	Lys/Lys	7	0.54±0.18	6.44±1.60	1.21±0.49	2.57±1.20	2.61±0.72	9.85±4.48	6	0.51±0.20	36.93±18.59 p=0.002	1.48±0.44 p=0.046	4.92±0.66	3.57±2.21 p=0.018	16.28±13.25 p=0.064
caipain10	G/G	102	0.51±0.19	8.20±5.74	1.28±0.49	2.82±1.82	2.86±1.27	12.06±10.19	110	0.56±0.21	21.41±11.18	1.21±0.43	5.37±3.03	2.55±0.94	11.14±5.91
	G/A	13	0.46±0.16	8.28±4.90	1.36±0.63	2.51±1.35	2.84±1.17	12.51±8.81	7	0.72±0.21 p=0.040	17.90±10.14	1.17±0.50	6.86±3.72	2.20±0.73	9.96±5.68
β2AR Arg16Gly	Arg/Arg	23	0.48±0.14	7.24±4.52	1.34±0.61	2.87±1.78	2.78±0.98	10.81±7.08	28	0.57±0.26	21.12±11.99	1.16±0.42	6.09±3.88	2.72±1.32	12.46±8.02
	Arg/Gly	60	0.50±0.19	8.83±6.85	1.33±0.52	2.83±1.78	2.89±1.36	12.51±11.47	55	0.57±0.20	21.42±9.87	1.18±0.38	5.54±2.71	2.53±0.84	11.17±5.57
	Gly/Gly	32	0.54±0.19	7.73±3.30	1.17±0.36	2.65±1.80	2.85±1.26	12.30±9.00	34	0.56±0.19	21.40±12.67	1.32±0.51	4.78±2.78	2.43±0.61	10.02±4.13
β2AR Gln27Glu	Gln/Gln	97	0.51±0.19	8.11±5.88	1.25±0.50	2.91±1.82	2.83±1.26	12.28±10.42	97	0.56±0.22	21.19±11.26	1.19±0.40	5.50±3.14	2.57±0.96	11.22±6.10
	Gln/Glu	18	0.46±0.13	8.70±4.11	1.46±0.49	2.14±1.36 p=0.093	3.01±1.22	11.22±7.62	19	0.60±0.17	22.32±11.16	1.33±0.57	5.05±2.66	2.45±0.71	10.83±5.21
	Glu/Glu	0							1	0.49	17.90	1.30	8.20	2.06	10.20
β3AR	Trp/Trp	78	0.48±0.17	8.88±6.28	1.33±0.55	2.61±1.69	3.10±1.40	13.65±11.55	87	0.57±0.22	20.40±9.98	1.22±0.42	5.34±3.13	2.57±0.99	11.44±6.36
	Trp/Arg	32	0.56±0.20	6.96±3.71	1.19±0.37	3.24±1.99	2.35±0.65	9.08±4.13	28	0.57±0.20	23.23±13.43	1.19±0.48	5.82±2.99	2.50±0.70	10.44±4.36
	Arg/Arg	5	0.51±0.22	5.68±2.75	1.18±0.54	2.66±1.17	2.28±0.55 p=0.009	7.54±3.35 p=0.053	2	0.37±0.01	35.85±20.29 p=0.090	1.20±0.42	5.00±0.14	2.31±1.25	8.10±5.80

Table 5a Genotypes of metabolic syndrome-related genes and plasma biochemical markers (Male)

gene	genotype	Male	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
UCP1	A/A	29	204.2 ± 34.2	51.38 ± 10.06	119.8 ± 32.7	165.2 ± 77.9	5.65 ± 0.78	108.0 ± 18.3	0.15 ± 0.18	29.57 ± 2.12	27.51 ± 3.83	100.9 ± 6.6
	A/G	67	205.2 ± 27.2	49.13 ± 9.51	118.7 ± 33.1	187.0 ± 142.9	5.91 ± 1.10	113.5 ± 28.7	0.20 ± 0.35	30.82 ± 4.18	29.80 ± 4.50	102.1 ± 10.1
	G/G	19	200.6 ± 19.9	49.74 ± 9.94	121.5 ± 24.1	147.0 ± 76.5	5.72 ± 0.73	111.4 ± 18.7	0.14 ± 0.08	30.45 ± 2.63	28.75 ± 4.64 p=0.065	100.3 ± 6.1
UCP2	Ala/Ala	34	203.4 ± 23.6	49.74 ± 10.27	114.0 ± 33.1	198.4 ± 169.5	6.02 ± 1.02	115.1 ± 29.1	0.14 ± 0.15	30.93 ± 4.14	29.05 ± 4.11	102.0 ± 10.3
	Ala/Val	54	203.9 ± 32.3	49.94 ± 9.06	122.9 ± 32.3	155.5 ± 84.3	5.74 ± 1.06	111.0 ± 24.6	0.19 ± 0.37	29.87 ± 3.04	28.34 ± 4.41	99.9 ± 7.8
	Val/Val	27	205.6 ± 24.3	49.59 ± 10.48	119.3 ± 27.4	184.1 ± 104.5	5.70 ± 0.68	109.0 ± 19.6	0.18 ± 0.20	30.99 ± 3.66	30.44 ± 4.70	104.0 ± 8.0
UCP3	C/C	58	203.8 ± 27.6	49.64 ± 9.66	118.4 ± 34.6	179.4 ± 142.9	5.95 ± 0.96	114.8 ± 26.5	0.15 ± 0.15	30.62 ± 3.50	29.08 ± 4.59	101.5 ± 8.4
	C/T	47	205.1 ± 29.4	48.91 ± 9.66	120.4 ± 30.3	179.1 ± 97.2	5.58 ± 0.65	107.0 ± 16.7	0.19 ± 0.39	30.16 ± 3.23	29.15 ± 3.83	101.1 ± 8.8
	T/T	10	201.6 ± 25.1	54.90 ± 9.26	121.0 ± 15.3	129.1 ± 55.8	6.09 ± 1.87 p=0.097	116.0 ± 42.2	0.25 ± 0.28	30.75 ± 5.31	28.35 ± 6.38	103.6 ± 10.5
PPARγ2	Pro/Pro	105	203.7 ± 29.0	49.80 ± 9.53	118.9 ± 32.5	175.6 ± 124.7	5.82 ± 1.01	112.2 ± 25.5	0.18 ± 0.29	30.51 ± 3.67	29.13 ± 4.48	101.6 ± 9.0
	Pro/Ala	10	208.9 ± 12.6	49.80 ± 11.76	125.4 ± 16.4	167.7 ± 59.9	5.67 ± 0.43	107.2 ± 17.0	0.12 ± 0.05	29.76 ± 1.88	28.09 ± 4.09	101.0 ± 5.8
adiponectin	Ile/Ile	111	203.0 ± 26.6	50.06 ± 9.68	118.3 ± 30.7	173.3 ± 121.1	5.83 ± 0.98	111.9 ± 25.2	0.15 ± 0.15	30.39 ± 3.50	28.94 ± 4.46	101.3 ± 8.6
	Ile/Thr	4	237.3 ± 46.5 p=0.015	42.50 ± 7.00	151.0 ± 38.7 p=0.040	218.0 ± 96.9	5.35 ± 0.45	107.8 ± 11.9	0.80 ± 1.27 p<0.001	31.78 ± 5.15	31.88 ± 2.56	107.2 ± 10.3

Table 5a

gene	genotype	Male	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
LEPR	Arg/Arg	65	203.1 ± 29.1	48.86 ± 8.91	117.8 ± 32.1	182.1 ± 131.3	5.88 ± 1.09	112.0 ± 28.9	0.18 ± 0.35	29.99 ± 2.67	29.08 ± 4.00	100.2 ± 6.9
	Lys/Arg	43	205.3 ± 27.9	51.60 ± 10.57	121.7 ± 30.7	160.0 ± 93.6	5.71 ± 0.82	111.6 ± 19.4	0.17 ± 0.16	31.14 ± 4.69	29.26 ± 5.22	104.2 ± 10.9
	Lys/Lys	7	207.7 ± 19.1	47.43 ± 10.56	120.4 ± 33.5	199.6 ± 163.8	5.83 ± 0.62	110.6 ± 14.2	0.16 ± 0.11	30.33 ± 2.02	27.39 ± 2.78	97.9 ± 4.8 p= 0.033
caipain10	G/G	102	205.0 ± 27.4	50.61 ± 9.77	120.0 ± 31.4	172.2 ± 124.8	5.78 ± 0.92	111.4 ± 21.8	0.18 ± 0.30	30.38 ± 3.58	28.84 ± 4.26	101.4 ± 8.9
	G/A	13	198.0 ± 32.4	43.46 ± 6.19 p= 0.012	115.3 ± 32.9	195.8 ± 76.8	6.05 ± 1.34	114.5 ± 43.2	0.13 ± 0.11	30.90 ± 3.43	30.60 ± 5.57	102.5 ± 7.3
β2AR Arg16Gly	Arg/Arg	23	200.6 ± 27.3	46.04 ± 10.26	114.7 ± 43.2	199.3 ± 207.7	5.73 ± 0.89	110.1 ± 18.7	0.20 ± 0.21	30.41 ± 4.37	29.12 ± 5.17	100.7 ± 9.5
	Arg/Gly	60	203.5 ± 24.8	50.40 ± 9.26	120.0 ± 27.2	166.1 ± 86.3	5.81 ± 0.93	111.0 ± 25.2	0.18 ± 0.35	30.49 ± 3.91	29.22 ± 4.52	101.4 ± 9.8
	Gly/Gly	32	207.9 ± 33.9	51.38 ± 9.64	121.9 ± 29.5	173.8 ± 88.5	5.88 ± 1.12	114.3 ± 28.4	0.15 ± 0.13	30.38 ± 1.92	28.66 ± 3.77	102.2 ± 5.6
β2AR Gln27Glu	Gln/Gln	97	203.6 ± 28.2	49.44 ± 9.48	119.2 ± 32.5	174.9 ± 124.7	5.74 ± 0.81	110.4 ± 22.2	0.18 ± 0.30	30.48 ± 3.80	28.97 ± 4.60	101.5 ± 9.3
	Gln/Glu	18	207.3 ± 27.1	51.72 ± 10.79	120.8 ± 26.0	174.6 ± 96.0	6.20 ± 1.57 p= 0.064	119.1 ± 36.0	0.15 ± 0.10	30.24 ± 1.68	29.48 ± 3.40	101.5 ± 4.5
	Glu/Glu	0										
β3AR	Trp/Trp	78	204.6 ± 28.7	48.31 ± 9.94	121.2 ± 30.7	175.6 ± 96.3	5.86 ± 1.06	113.0 ± 26.0	0.20 ± 0.33	30.71 ± 3.72	29.30 ± 4.49	102.1 ± 9.2
	Trp/Arg	32	203.9 ± 26.9	52.94 ± 7.91	114.5 ± 34.9	182.2 ± 171.7	5.67 ± 0.77	109.1 ± 23.4	0.11 ± 0.07	30.10 ± 3.21	28.73 ± 4.27	101.3 ± 7.1
	Arg/Arg	5	199.8 ± 28.4	53.00 ± 12.17 p= 0.055	123.6 ± 17.7	116.6 ± 26.6	5.94 ± 0.68	109.8 ± 16.7	0.15 ± 0.09	28.40 ± 2.18	26.92 ± 4.80	94.0 ± 7.9

Table 5b Genotypes of metabolic syndrome-related genes and plasma biochemical markers (Female)

gene	genotype	Female	Total-Cho	HDL-Cho	LDL-Cho	TG	HbA1c	Fasting Glc	CRP	BMI	body fat %	Waist Circumf.
UCP1	A/A	23	209.3 ± 36.2	57.48 ± 10.72	122.8 ± 33.0	145.4 ± 75.2	5.96 ± 1.64	110.6 ± 19.4	0.17 ± 0.15	31.49 ± 3.82	41.92 ± 6.14	104.7 ± 9.7
	A/G	60	211.2 ± 35.8	55.63 ± 11.85	127.8 ± 30.2	139.1 ± 82.1	5.81 ± 0.92	111.6 ± 28.8	0.19 ± 0.20	31.24 ± 2.98	40.43 ± 5.07	104.0 ± 8.2
	G/G	34	230.6 ± 45.7 p= 0.046	55.59 ± 11.90	142.1 ± 39.2 p= 0.063	164.8 ± 73.0	6.01 ± 1.13	114.6 ± 27.7	0.17 ± 0.15	30.63 ± 2.97	40.39 ± 5.66	102.7 ± 7.8
UCP2	Ala/Ala	27	216.1 ± 40.1	58.19 ± 11.86	133.5 ± 36.0	122.0 ± 50.2	5.97 ± 1.41	116.8 ± 32.1	0.14 ± 0.13	31.77 ± 2.93	41.01 ± 4.38	103.8 ± 8.1
	Ala/Val	54	211.9 ± 36.0	54.44 ± 10.69	127.7 ± 28.4	148.9 ± 77.9	5.91 ± 1.13	111.2 ± 23.1	0.18 ± 0.19	30.64 ± 2.90	40.12 ± 4.90	103.3 ± 7.8
	Val/Val	36	223.8 ± 44.6	56.64 ± 12.60	134.1 ± 40.5	165.5 ± 92.0 p= 0.090	5.81 ± 0.96	110.5 ± 27.7	0.20 ± 0.18	31.32 ± 3.59	41.38 ± 6.83	104.4 ± 9.5
UCP3	C/C	50	213.4 ± 44.0	55.50 ± 11.77	128.1 ± 36.5	149.0 ± 74.5	5.91 ± 1.35	110.4 ± 23.3	0.17 ± 0.16	31.07 ± 2.71	40.75 ± 5.17	103.3 ± 8.0
	C/T	57	220.5 ± 36.8	56.35 ± 11.77	134.7 ± 33.6	146.9 ± 82.6	5.89 ± 0.92	113.8 ± 25.0	0.18 ± 0.18	31.19 ± 3.59	40.83 ± 5.75	104.0 ± 8.8
	T/T	10	209.7 ± 34.6	56.30 ± 10.49	124.1 ± 23.0	147.0 ± 80.7	5.90 ± 1.29	113.2 ± 47.8	0.21 ± 0.23	30.87 ± 2.67	39.82 ± 5.52	104.4 ± 8.2
PPARγ2	Pro/Pro	110	215.2 ± 40.4	55.87 ± 11.55	130.5 ± 34.6	144.3 ± 76.0	5.88 ± 1.16	112.3 ± 27.0	0.18 ± 0.18	31.13 ± 3.07	40.64 ± 5.15	103.7 ± 8.0
	Pro/Ala	7	236.7 ± 20.7	57.71 ± 12.75	138.6 ± 24.7	203.4 ± 99.3 p= 0.052	6.09 ± 0.96	112.6 ± 22.8	0.14 ± 0.13	30.87 ± 4.47	41.79 ± 9.50	104.9 ± 13.6
adiponectin	Ile/Ile	114	216.7 ± 40.0	56.19 ± 11.63	130.9 ± 34.4	147.8 ± 79.2	5.89 ± 1.16	112.4 ± 27.0	0.17 ± 0.18	31.11 ± 3.18	40.67 ± 5.46	103.8 ± 8.3
	Ile/Thr	3	209.7 ± 30.9	48.00 ± 6.24	132.3 ± 22.6	147.3 ± 41.2	6.20 ± 0.20	107.3 ± 6.0	0.31 ± 0.04	31.00 ± 1.15	42.17 ± 5.56	103.9 ± 11.3
LEPR	Arg/Arg	77	215.6 ± 34.8	55.53 ± 11.74	131.7 ± 31.7	142.1 ± 72.5	5.81 ± 1.02	108.1 ± 18.9	0.16 ± 0.15	31.17 ± 3.17	40.60 ± 5.38	103.6 ± 8.7
	Lys/Arg	34	219.5 ± 50.7	57.12 ± 11.59	132.7 ± 41.3	148.9 ± 70.0	6.03 ± 1.30	119.6 ± 34.9	0.19 ± 0.17	30.47 ± 2.63	40.51 ± 4.69	102.9 ± 6.3
	Lys/Lys	6	210.7 ± 34.6	55.33 ± 10.84	112.3 ± 5.8	215.2 ± 155.8 p= 0.088	6.32 ± 1.74	125.0 ± 47.5 p= 0.054	0.31 ± 0.40	33.95 ± 4.34 p= 0.041	43.25 ± 9.79	110.4 ± 11.9
caipain10	G/G	110	215.7 ± 40.5	55.31 ± 11.23	130.1 ± 34.3	151.5 ± 79.1	5.90 ± 1.16	112.6 ± 27.2	0.18 ± 0.18	31.06 ± 3.20	40.63 ± 5.56	103.6 ± 8.4
	G/A	7	229.1 ± 22.5	66.57 ± 12.78 p= 0.012	144.7 ± 29.7	89.6 ± 30.7 p= 0.042	5.89 ± 0.90	107.6 ± 17.5	0.13 ± 0.09	31.86 ± 2.15	41.96 ± 3.20	107.1 ± 7.5
β2AR Arg16Gly	Arg/Arg	28	218.1 ± 33.5	57.79 ± 12.44	134.5 ± 30.4	128.9 ± 59.4	5.59 ± 0.62	108.8 ± 27.1	0.15 ± 0.11	31.03 ± 3.25	40.27 ± 6.18	102.9 ± 9.7
	Arg/Gly	55	223.4 ± 44.3	56.95 ± 11.99	135.6 ± 37.5	154.5 ± 81.5	5.87 ± 1.05	109.3 ± 17.1	0.19 ± 0.17	30.96 ± 3.25	41.22 ± 5.67	103.3 ± 8.5
	Gly/Gly	34	204.1 ± 34.3 p= 0.081	52.94 ± 9.79	120.6 ± 29.7	152.6 ± 86.1	6.20 ± 1.52	120.1 ± 36.6	0.19 ± 0.22	31.42 ± 2.95	40.25 ± 4.43	105.3 ± 6.8
β2AR Gln27Glu	Gln/Gln	97	218.0 ± 40.8	56.45 ± 11.96	132.4 ± 35.7	146.0 ± 75.3	5.87 ± 1.13	112.1 ± 27.4	0.18 ± 0.19	30.99 ± 3.23	40.66 ± 5.52	103.6 ± 8.6
	Gln/Glu	19	209.8 ± 34.6	54.16 ± 9.45	124.7 ± 25.3	154.4 ± 95.3	6.05 ± 1.28	113.5 ± 24.3	0.15 ± 0.10	31.77 ± 2.78	40.84 ± 5.35	105.0 ± 7.2
	Glu/Glu	1	196.0	45.00	111.0	198.0	5.50	112.0	0.30	30.80	42.90	97.6
β3AR	Trp/Trp	87	218.3 ± 42.4	55.76 ± 10.49	133.0 ± 35.8	147.6 ± 74.4	5.92 ± 1.22	111.5 ± 26.9	0.16 ± 0.15	31.08 ± 3.34	40.53 ± 5.64	103.7 ± 8.7
	Trp/Arg	28	213.7 ± 30.6	56.61 ± 14.99	126.4 ± 28.8	153.3 ± 91.4	5.87 ± 0.95	115.9 ± 26.9	0.19 ± 0.16	31.20 ± 2.65	41.54 ± 4.89	103.9 ± 7.5
	Arg/Arg	2	179.0 ± 4.2	57.00 ± 2.83	106.0 ± 4.2	80.0 ± 15.6	5.35 ± 0.07	98.0 ± 11.3	0.57± 0.77 p= 0.005	31.10 ± 0.71	37.15 ± 3.89	103.8 ± 7.4

Table 6 Significance in LEPR Arg109Lys SNP with ANOVA or Bonferroni adjustment

LEPR(Female)		leptin	TNF-α	C-peptid	insulin	TG	Fasting Glc	BMI
ANOVA		**0.002**	**0.046**	**0.018**	0.064	0.088	0.054	**0.041**
Bonferroni								
Arg/Arg	Lys/Arg	1.000	0.164	1.000	1.000	1.000	0.108	0.812
Arg/Arg	Lys/Lys	**0.001**	0.192	**0.021**	0.124	0.084	0.394	0.108
Lys/Arg	Lys/Lys	**0.001**	1.000	**0.015**	0.058	0.166	1.000	**0.037**

P-values less than 0.05 are shown in bold.

Discussion

Association of BMI and other clinical parameters with genotypes of UCPs or β2, β3AR genes are reported in many studies. In obese subjects in SCOP, however, only a few of these associations were confirmed. On the contrary, some converse results were obtained; for example, Arg/Arg minor homozygote of β3AR showed lower C-peptide (P=0.009) and insulin (P=0.053), and higher HDL cholesterol level (P=0.055) in men. Plausibly, comparison with non-obese people in Saku cohort (case-control study) may explain these disagreements.

Leptin is an adipocyte-specific hormone, and regulates adipose-tissue mass via hypothalamus. The leptin receptor is found in many tissues including the hypothalamus, and has a single transmembrane domain, which is common with the cytokine receptor family. It is easily inferred from the important role of LEPR that the dysfunction resulted from SNPs may predispose to obesity and other metabolic syndromes. Several reports suggest the association between their SNPs and these diseases, however definite statement cannot be made as yet.

We showed significant association in the LEPR Arg109Lys, which is resided in exon 4 of the gene; Lys/Lys minor homozygotes (woman) had higher levels of leptin, TNF-α, C-peptide or insulin, compared with other genotypes; and in the homozygotes, triglyceride and fasting serum glucose were significantly higher, and also, the genotype showed a trend of higher values of HbA1c, BMI, body fat mass and waist circumference.

Associations described above were observed only in woman *(Table 4)*. Similarly, Rosmond et al.[15] reported that Arg/Arg homozygotes had lower leptin concentration, and probably as a result, lower BMI and abdominal sagittal diameter, as well as lower blood pressure, but that was in men. Wauters et al.[16] found associations of Lys109Arg with fasting glucose and oral glucose tolerance test in post- and pre-menopausal woman with impaired glucose tolerance. The fact that in females leptin levels are higher than males suggests that estrogens have effect on leptin secretion. Probably, combination of increased secretion of leptin by estrogens with dysfunction of leptin receptor predispose to obesity and other metabolic syndromes. However, the mechanism as to the development in detail remains to be elucidated.

A (Lys) allele of Lys109Arg is the major type in European (A:G=0.658:0.342, HapMap-CEU) or Sub-Saharan African (A:G=0.883:0.117, HapMap-YRI). On the contrary, the SNP frequencies are reversed in Asian; G (Arg) allele is the major type in Asian (A:G=0.205:0.795, HapMap-JPT; A:G=0.144:0.856, HapMap-HCB). The frequency in SCOP was A:G=0.222:0.778, and also in Korean, Arg was a major allele.[10] This racial difference may influence their distinct susceptibility to obesity and other metabolic diseases.

Also, we demonstrated that adiponectin 164Ile/Thr heterozygotes had truly lower plasma adiponectin level than the major Ile/Ile homozygotes both in men and women. These results are consistent with the report by Kondo et al.[9] The heterozygotes had higher total cholesterol (P=0.015) and LDL-cholesterol (P=0.040) than the major Ile/Ile homozygotes in man, although in woman, these association were insignificant. Besides, C-reactive protein (CRP) showed higher trend in the heterozygotes than another genotype (P<0.001). The results coincided with the report by Mita et al. [17]; higher CRP is the marker of early-stage type 2 diabetes mellitus in Japanese patients.

Imbalance between the adiponectin and leptin would influence and lead to insulin resistance, which cause type 2 diabetes mellitus. [18] Accordingly, adipocytokines, such as leptin and adiponectin, may play an important role in the development of diabetes mellitus. Here, we showed that SNPs of leptin receptor and adiponectin genes are remarkable for the development of metabolic syndromes as well as UCP or β2, β3AR genes.

Acknowledgments

This work was supported by the Grants in aid for scientific Research from the Ministry of Health, Labor and Welfare, Japan. We thank Dr. Hitoshi Kakimoto and Dr. Seiji Sekioka (Nansei-cho Hospital) for their kind cooperation.

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Received: Oct 19, 2007
Accepted: Dec 17, 2007
Published online: 2008

Original Article

Regional Characteristics of Secular Changes in Obesity-Related Lifestyle Behavior in Japan

Miki Miyoshi [1], Fumi Hayashi [1], Yusuke Arai [2], Miho Nozue [2], Katsushi Yoshita [2], Nobuo Yoshiike [1]

1) Center for Collaboration and Partnership, National Institute of Health and Nutrition
2) Project for the National Health and Nutrition Survey, Nutritional Epidemiology Program, National Institute of Health and Nutrition

Abstract

BACKGROUND: In 2000 the Japanese Ministry of Health, Labour, and Welfare initiated a new health promotion program, Health Japan 21, in which obesity control is one of the prioritized objectives. For the program's effective implementation, it is important to enhance individual nutritional education as well as to identify the socioeconomic and environmental factors affecting obesity-related lifestyle habits. This study aimed to investigate the secular changes in dietary and exercise habits between sexes and among four types of residential areas in Japan.

METHODS: The datasets of annual national nutrition surveys (1976–2003) were used for the secondary analyses. Data on the dietary and exercise habits of 385,559 persons aged 15 years and older were analyzed. Residential areas were divided into four groups: metropolitan areas, large cities, small cities, and small towns.

RESULTS: Similar trends were observed in the proportions of people skipping breakfast and eating out lunch/dinner, with those living in metropolitans having the highest proportions. The secular trend showed that the proportions of people eating dinner out were highest in the early 1990s, which corresponds to the "bubble period" in Japan.

CONCLUSIONS: Our findings suggest that dietary habits could be greatly influenced by the country's economic situation, especially in metropolitan areas. Thus, area-specific population approaches should be enhanced to promote appropriate lifestyle habits, especially for the young to middle-aged.

KEY WORDS: overweight, lifestyle behavior, residential area, national nutrition survey, Japan

Introduction

Overweight and obesity, which are defined as abnormal or excessive fat accumulation that may impair health, is now one of the major public health problems in Japan.[1] The nation suffered from a serious food shortage during and after World War II. As Japan achieved economic development, however, the population's diet and physical activity patterns have greatly changed. Consequently, the nutrition problem gradually shifted from malnutrition to overweight. According to a study that examined the changes in the prevalence of overweight using the datasets of the National Nutrition Survey of Japan from 1976 through 1995, there was an increasing trend in overweight among males of all age groups and females over 60 years of age.[2]

Therefore, in 2000 the Ministry of Health, Labour and Welfare started a new program of health promotion policies and strategies, Health Japan 21, which has 70 goals to be achieved by 2010 in nine areas: nutrition and diet, physical activity and exercise, rest and mental health, tobacco, alcohol, dental health, diabetes, cardiovascular diseases, and cancer.[3] As one of the prioritized objectives in Health Japan 21, various efforts have been made toward obesity control, including enhancement of individual dietary guidance and nutritional education. Yet, the progress has not been sufficient to reach the goals. The midterm evaluation in 2005 showed that the prevalence of being overweight in males aged 20–60 years was 29.0% and that in females aged 40–60 years was 24.6%, whereas the 2010 target values are less than 15% and 20%, respectively.[4] Studies have also indicated, however, that the rapid development of an obesity epidemic in genetically stable populations can be attributed to environmental factors affecting diet or physical activity level.[5] Thus, in order to effectively control obesity, in addition to considering energy intake, other obesity-related lifestyle habits like eating behaviors and physical activity also must be taken into account. Although one of the goals in the area of nutrition and diet of Health Japan 21 is "environment-building to support behavioral change", few studies have examined how this may be achieved. Thus, the aims of this study was to investigate the secular changes in obesity-related lifestyle habits from 1976 to 2003 between sexes and among types of residential areas in Japan.

Miki Miyoshi / Center for Collaboration and Partnership,
National Institute of Health and Nutrition,
1-23-1, Toyama, Shinjuku, Tokyo 162-8636, Japan
Tel: +81-3-3202-5721 / Fax: +81-3-3202-3278 / E-mail: mikimiy@nih.go.jp

Methods

Data Collection in the National Nutrition Survey

The first National Nutrition Survey, Japan (J-NNS) was carried out in Tokyo Metropolitan area in 1945. The Nutrition Improvement Law of 1952 stipulated that the J-NNS be conducted annually. Following the enactment of the Health Promotion Law in 2003, the J-NNS was renamed the National Health and Nutrition Survey. In the annual survey, target populations are selected from the entire national population aged 1 year and over by stratified random sampling; approximately 6000 households in 300 areas are randomly selected from enumerated districts based on the population census. The main components of data collection are household dietary surveys, physical examination (e.g., anthropometry, blood test, physical activity), and a questionnaire on lifestyle habits of the households' individuals.[6] Of these data, the following variables were included in this study: age, sex, residential area, skipping breakfast, eating lunch out, eating dinner out, alcohol consumption, exercise habits, and physical activity levels.

To consider the differences in socioeconomic background, residential areas are divided into four types, according to the size of municipality of residence: metropolitan areas (≥ 1,000,000 people), large cities (≥ 150,000 people), small cities (≥ 50,000 people), and small towns/villages (< 50,000 people).

Data for skipping breakfast, eating lunch out, and eating dinner out were derived from the dietary survey, as it was regarded that these responses would reflect their usual dietary habits as well. The definitions of "skipping" and "eating out" changed in 2001, after which the former included consuming supplements only or drinks only and the latter included takeaway/delivered meals.

Questions regarding alcohol consumption and exercise habits began to be included in the annual surveys in 1986, and these were asked of subjects aged 20 years and over. If a person drinks more than a bottle of beer (633 mL, or other alcohol equivalent to 23 g ethanol) three times a week, s/he is categorized as a drinker. As for exercise habits, if a person exercises more than twice a week for 30 min or longer over a 1-year period, s/he is regarded as a regular exerciser. The assessment of physical activity started in 1991, by measuring the number of steps per day with pedometer (AS-200, Yamasa Tokei Keiki Co., Ltd.).

Data Sources and Analyses

The datasets of the annual national nutrition surveys (1976–2003) were used, with the permission of the Ministry of Health, Labour, and Welfare. Data for 385,559 persons aged 15 years and over were included in the secondary analyses. As described in the previous section, the numbers of subjects analyzed differ across the variables. ***Table 1*** shows the numbers of study subjects by sex for each variable.

We calculated Body Mass Index (BMI) (kg/m^2) using the recorded body weight and height. A cutoff of 25.0 was used to classify overweight individuals (BMI ≥ 25.0 kg/m^2) and others, according to the international classification by the World Health Organization (WHO).[7]

All the data were tabulated/analyzed using SPSS® for Windows version 11.5.1J (SPSS Inc.). To avoid strong year-to-year fluctuations in the survey results, we calculated overweight prevalence and proportions of each lifestyle habit for six time periods (1976–1980, 1981–1985, 1986–1990, 1991–1995, 1996–2000, and 2001–2003) by sex. These data were further classified by seven age groups and the four types of residential areas. For t-test, $p < 0.05$ was used as the level of statistical significance.

Table 1 Number of subjects included in the analyses

Variable (survey year)	Males	Females	Total
Residential area (1976–)			
Metropolitan	32856	37273	70129
Large cities	50055	56927	106982
Small cities	48869	55656	104525
Small towns	48761	55198	103959
Total	180541	205018	385559
Height, weight (1976–)	134248	174727	308975
Skipping breakfast (1976–)	180541	205018	385559
Eating lunch out (1976–)	180175	204904	385079
Eating dinner out (1976–)	180463	204970	385433
Drinking habit (1986–) *	67193	88228	155421
Exercise habit (1986–) *	67194	88237	155431
Physical activity level (1991–)	57774	67866	125640

* Study subjects aged 20 years or over

Results

Data Sources and Analyses

Figures 1a and 1b show the changes in prevalence of overweight by 20-year age group and the residential areas in the six time periods for males and females, respectively. For males, the prevalence of being overweight consistently increased regardless of age group and type of residential area. For those aged 20–59 years, the proportions were highest in small towns and lowest in metropolitan areas. In contrast, there was a decreasing trend in the prevalence of being overweight among females aged 20–59 years, except for those living in small towns. For females aged over 60 years, however, overweight prevalence increased across all types of residential areas.

Changes in Dietary and Exercise Habits

The secular changes in dietary and exercise habits were examined for each lifestyle factor, first by 10-year age group, followed by further analyses by type of residential area focusing on the 20-year age groups who were at risk for each factor.

Dietary Habit (Skipping Breakfast, Eating out for Lunch/Dinner)

As was mentioned in the Methods, the definitions of "skipping" and "eating out" were expanded in 2001 and thereafter. Thus, the secular trends of dietary habits were examined for 1976–2000 only.

Figure 2a shows the secular changes in the proportion of subjects who skipped breakfast according to age group. The proportion was highest in both male and female subjects aged 20–29 years throughout the study period, followed by males aged 30–39 years and females aged 15–19 years. For both males and females less than 50 years old, the proportions were highest in 1991–1995. When the analysis was performed focusing on the age group 20–39 years, there was a large gap between the proportions in metropolitan areas and small towns during 1975–1995, after which this regional difference became less obvious (***Figure 2b***).

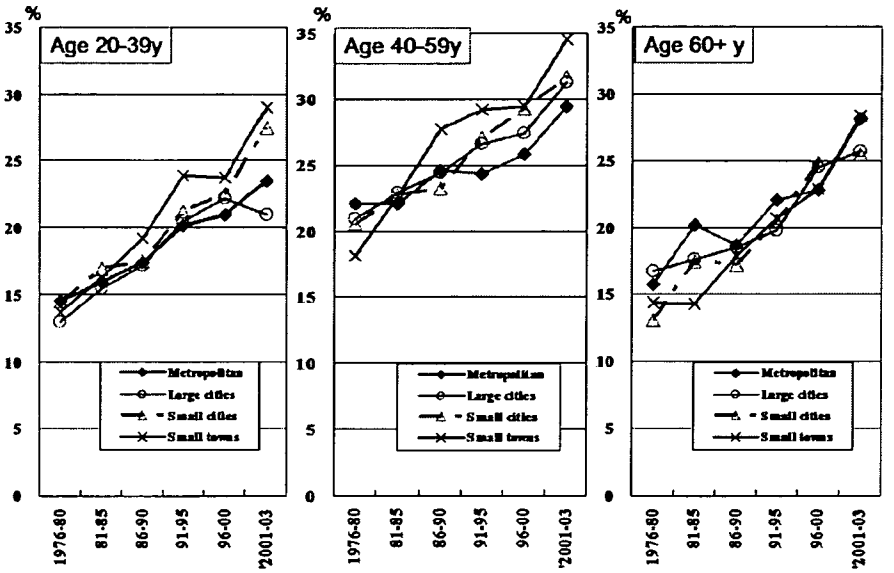

Fig. 1a.

Changes in overweight prevalence among types of residential areas (males)

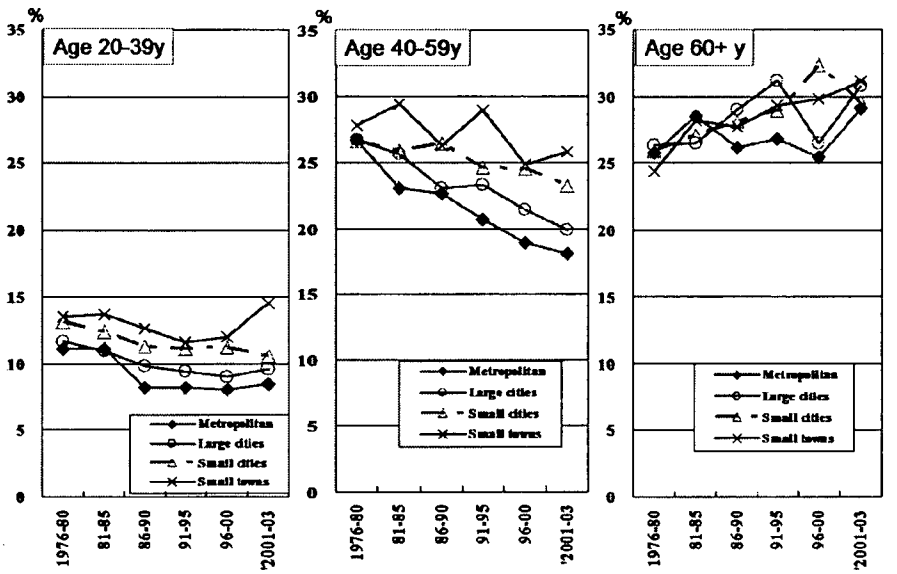

Fig. 1b.

Changes in overweight prevalence among types of residential areas (females)

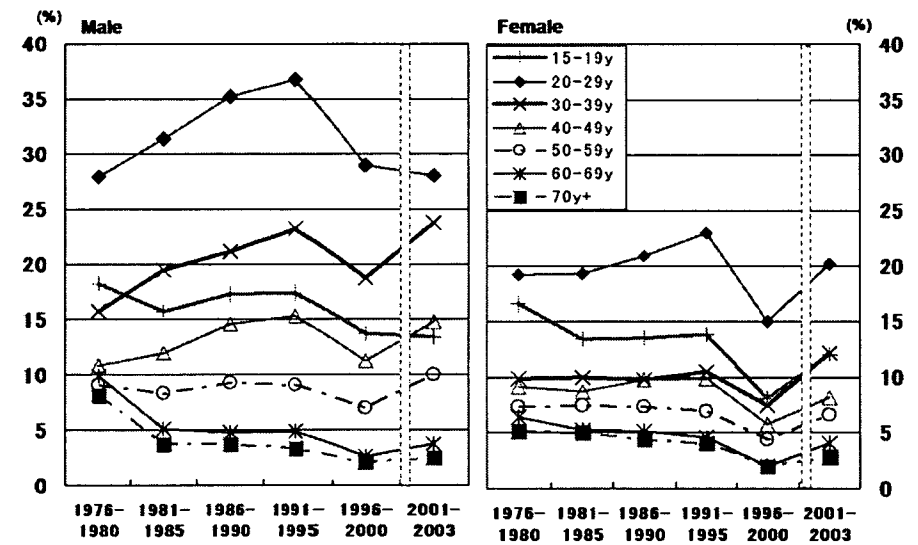

Fig. 2a.

Proportions of the subjects skipping breakfast by age group

Note: Definition of "Skipping" changed from 2001

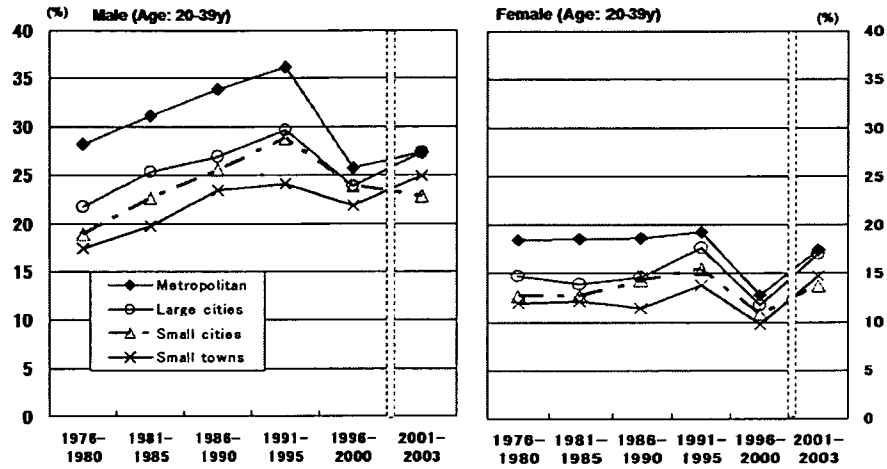

Fig. 2b.

Proportions of the subjects skipping breakfast by residential area
Note: Definition of "Skipping" changed from 2001

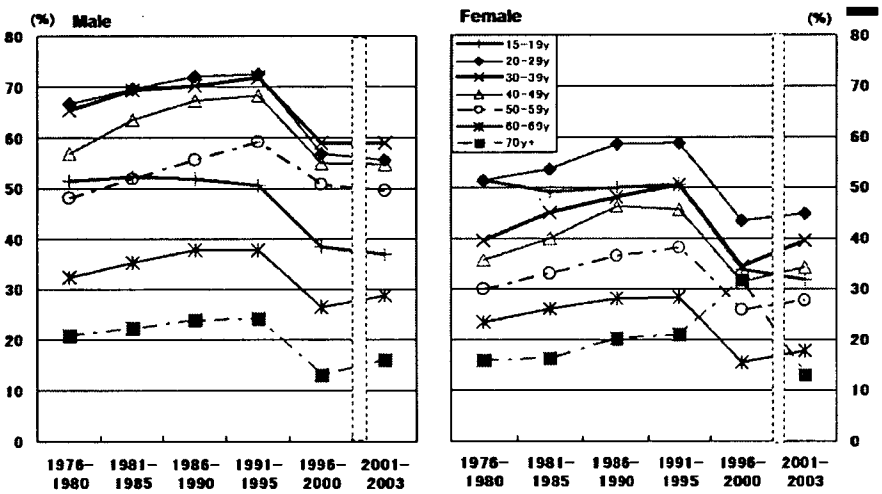

Fig. 3a.

Proportions of the subjects eating lunch out by age group
Note: Definition of "eating out" changed from 2001

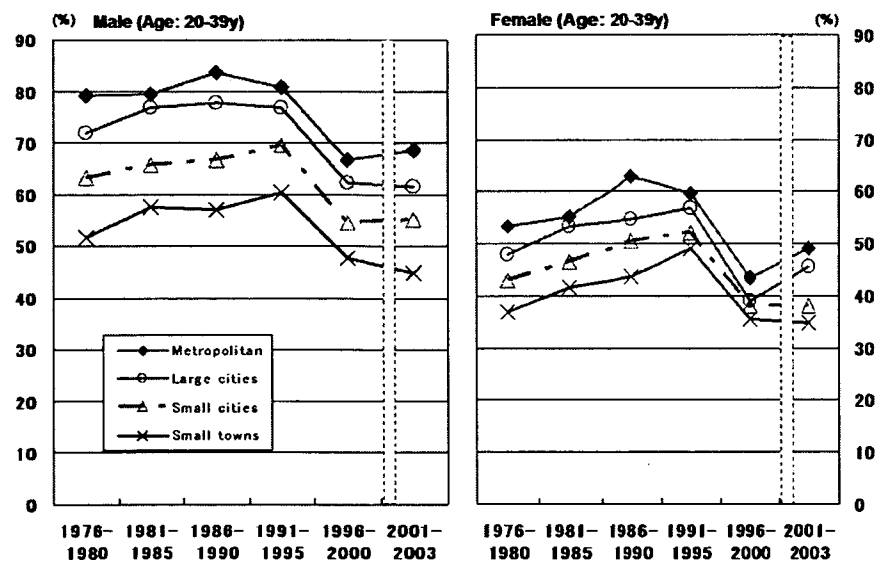

Fig. 3b.

Proportions of the subjects eating lunch out by residential area
Note: Definition of "eating out" changed from 2001

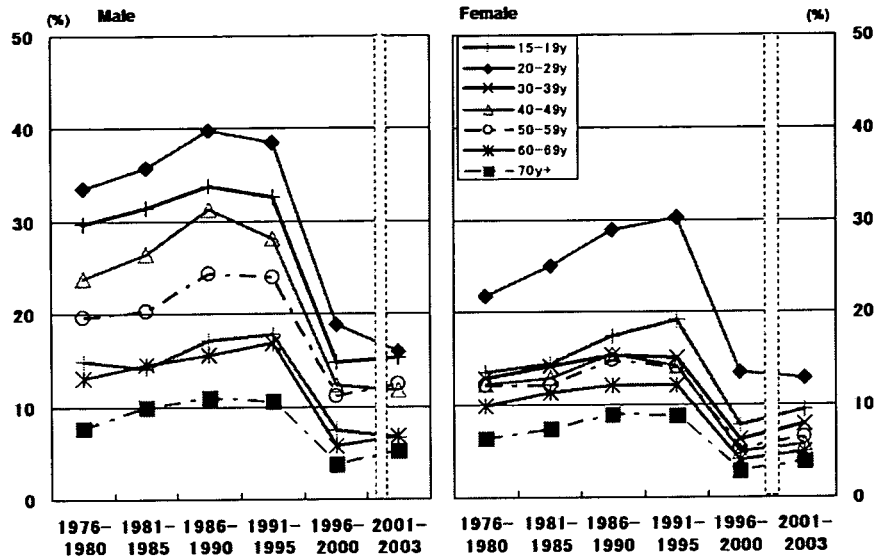

Fig. 4a.

Proportions of the subjects eating dinner out by age group
Note: Definition of "eating out" changed from 2001

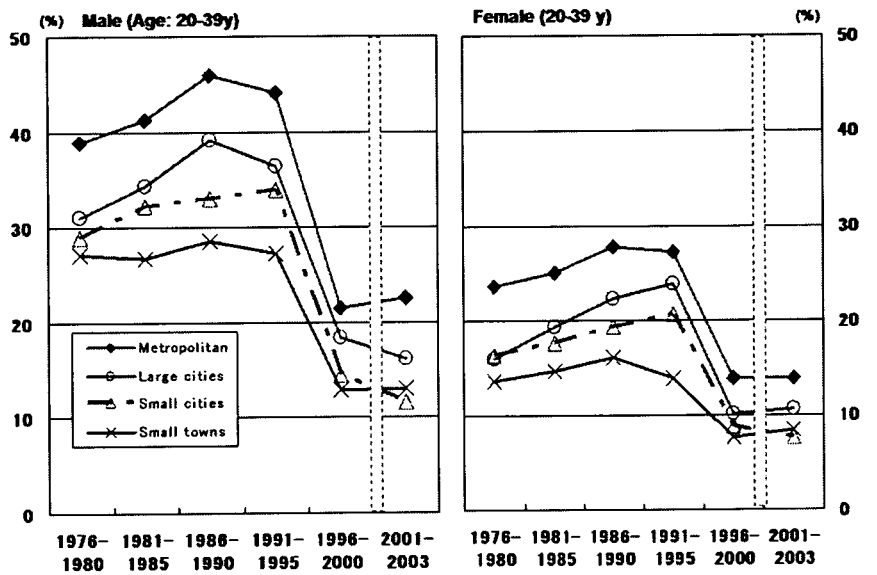

Fig. 4b.

Proportions of the subjects eating dinner out by residential area
Note: Definition of "eating out" changed from 2001

Secular changes in the proportion of subjects who ate lunch and dinner out are shown in ***Figures 3a and 4a***, respectively, according to age group. There were increasing trends for both lunch and dinner, with the highest proportion in those aged 20–29 years, although the proportions dramatically decreased after 1995. Similar trends were observed in the distributions by type of residential area in the subjects aged 20–39 years: the proportions were highest between 1986 and 1995, with those living in metropolitan areas having the highest proportions (***Figure 3b*** for lunch, ***Figure 4b*** for dinner).

Drinking and Exercise Habits

Figure 5a shows that the proportions of subjects classified as drinkers remained essentially stable during the study periods, regardless of sex and age group, with the highest proportion in males aged 50–59 years and in females aged 40–49 years. Similarly, when looking at the secular trend in the subjects aged 40–59 years, the proportions remained stable across all types of residential areas (***Figure 5b***). Among males the proportions of drinkers were highest in small towns and lowest in metropolitan areas, whereas the reverse trend was observed among females.

Among males and females aged 20–49 years, the proportions of those classified as regular exercisers remained relatively low and stable from 1986 to 2003, in contrast to the older subjects (≥ 50 years; ***Figure 6a***). When the data were analyzed by type of residential area focusing on the subjects aged 40–59 years, there was an increasing trend in the proportions of regular exercisers, especially in larger cities (***Figure 6b***).

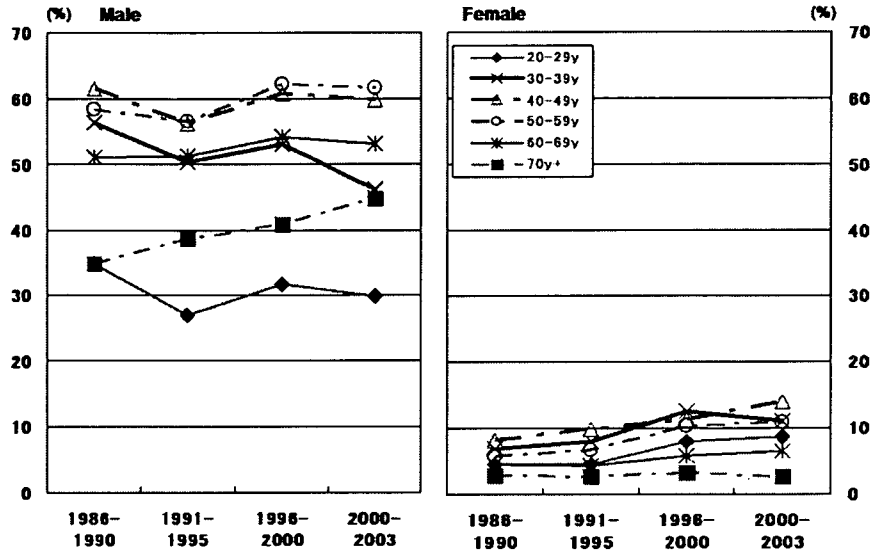

Fig. 5a.

Proportions of the subjects classified as "drinker" by age group

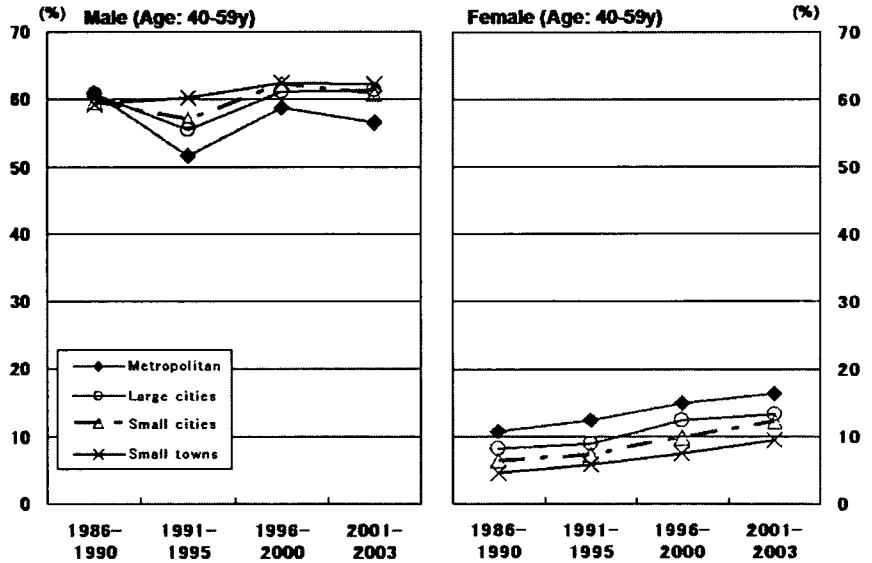

Fig. 5b.

Proportions of the subjects classified as "drinker" by residential area

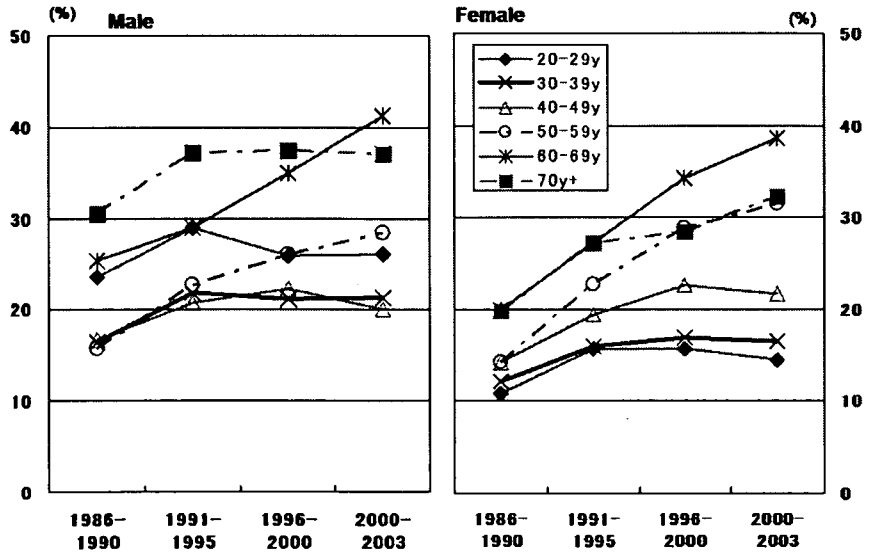

Fig. 6a.

Proportions of the subjects classified as "regular exerciser" by age group

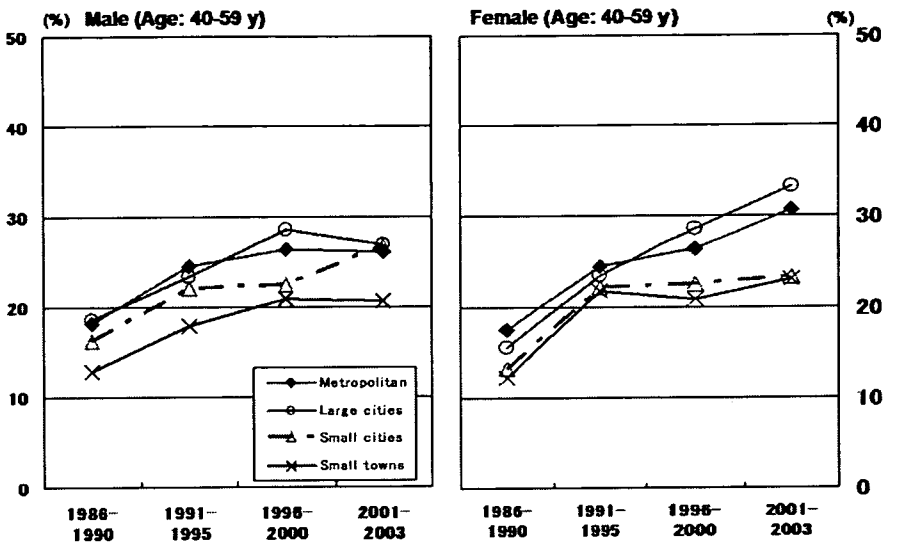

Fig. 6b.

Proportions of the subjects classified as "regular exerciser" by residential area

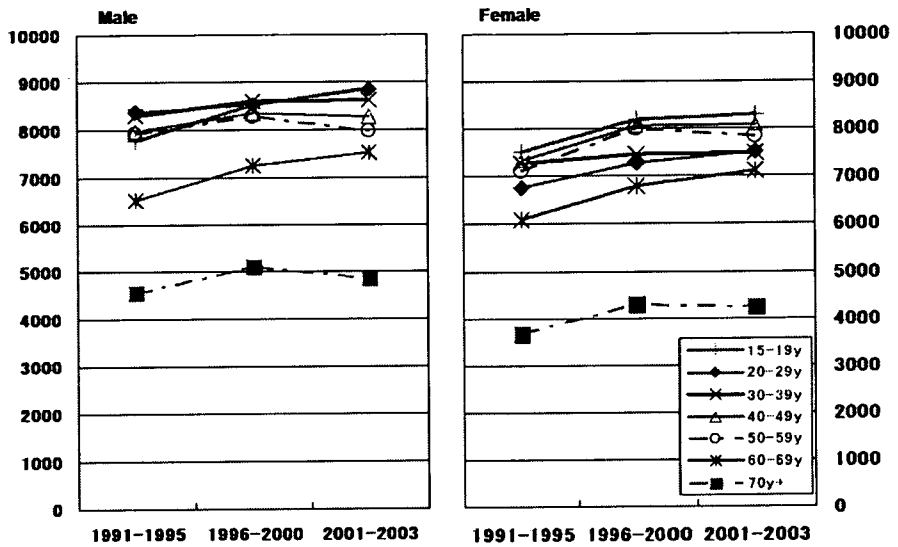

Fig. 7a.

Mean numbers of steps per day measured using pedometer by age group

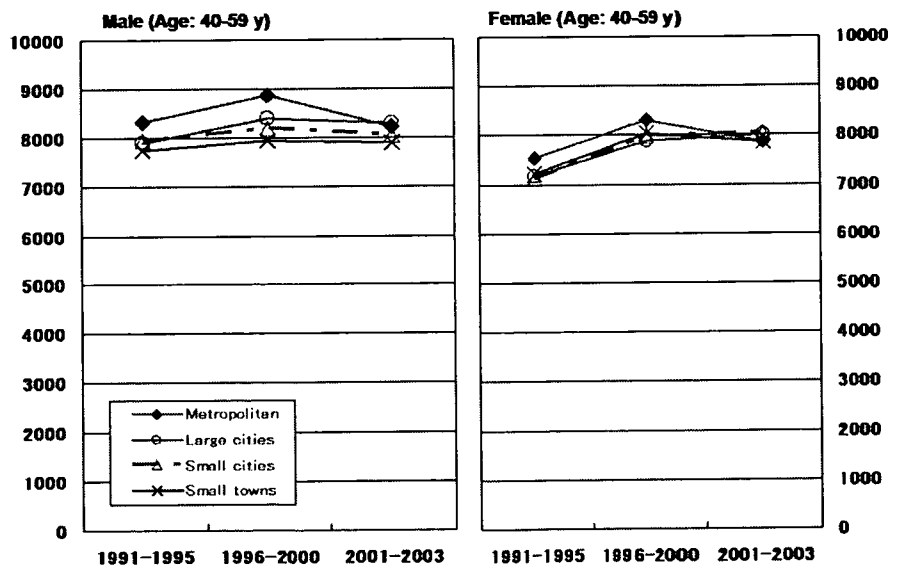

Fig. 7b.

Mean numbers of steps per day by residential area

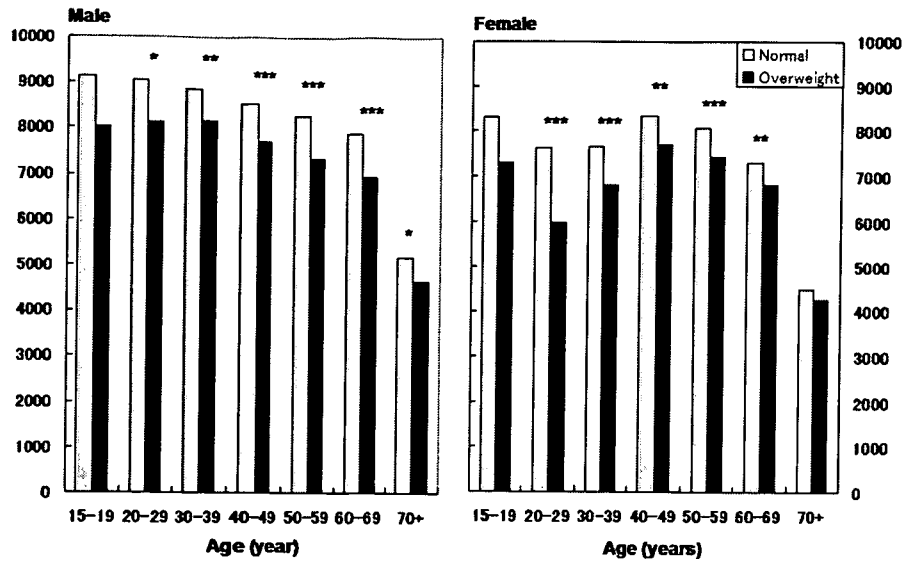

Fig. 8.

Mean numbers of steps: normal vs. overweight (2001-2003)
p value: * < 0.05, ** < 0.01, *** < 0.001

Physical Activity Levels

Mean numbers of daily steps measured using a pedometer, as a proxy of physical activity level, are shown by age group in ***Figure 7a***. Overall, there was an increasing trend in both males and females during the past decade, except for those 70 years or older. When the data were analyzed for subjects aged 40–59 years, the mean values remained essentially stable across all types of residential areas (***Figure 7b***).

Furthermore, our analysis revealed that physical activity had a highly significant association with BMI. ***Figure 8*** compares the mean numbers of steps between overweight subjects (BMI ≥ 25.0) and normal-weight subjects (BMI < 25.0) in 2001–2003. Regardless of age group and sex, the subjects with normal BMI walked more than overweight subjects (*t*-test: $p < 0.05$ except males and females aged 15–19 years and females 70 years or over).

Discussion

Although several studies have reported the epidemiology and secular changes of overweight prevalence in Japan,[2,8] to our knowledge this is the first study to report the secular changes in lifestyle behaviors and the associations between lifestyle behaviors and overweight using nationally representative samples. As was documented in a previous study for the period 1976–1995,[2] there was an increasing trend in overweight prevalence in all male age groups and in elderly women, but a decreasing trend in females less than 50 years old. Our study demonstrated that this trend was consistently observed after 1996 as well, with differences among types of residential areas being expanded.

In this study, similar trends were observed in the proportions of people skipping breakfast and eating out lunch/dinner, with the age group 20–39 years and those living in metropolitan areas having the highest proportion. The secular trend showed that the proportions of people eating out were highest in the early 1990s, and this trend was especially obvious among males in metropolitan areas. Interestingly, this corresponds to the "bubble period" in Japan (1986–1991), which was a time of skyrocketing land and stock prices, leading to the peak of economic boom in the Japanese economy.[9] The number of fast food shops and restaurants in the country has dramatically increased since the 1970s.[10] Our findings suggest that dietary habits could be greatly influenced by the country's economic situation, especially in metropolitan areas. Similarly, prevalence of overweight in male greatly increased around bubble period. On the other hand, the association between exercise habit and economic situation was unclear as the data on exercise habit were available after 1986 only. The increasing trend of regular exercisers after 1990, especially among the middle-aged and the elderly, was possibly due to the enhanced national health promotion program. This study also demonstrated that physical activity level (as measured by number of steps per day) was inversely associated with BMI in both male and female. This finding coincides with those of other studies in Japan.[11,12]

We recognize the limitations of observational studies. Furthermore, the data for skipping breakfast and eating out lunch/dinner were obtained from dietary surveys (not questionnaires), thus we could not distinguish between subjects who actually have these habits and those who happened to exhibit these behaviors on the day of the dietary survey. Nevertheless, because our findings are based on nationally representative samples, they should have useful policy implications with respect to health promotion of the national population.

The Health Japan 21 program is characterized by a two-pronged approach, where a population approach (to reduce risk factors across the entire population) and a high-risk approach (to prevent disease by reducing the risk to those individuals at a higher risk within the population) are combined.[3] To improve one's lifestyle behavior, it is essential to incorporate the population approach in addition to the high-risk individual approach. Following the initiation of Health Japan 21, the Ministry of Health, Labour, and Welfare established a committee to investigate the possible approaches to improve food environment for health promotion.[13] These population approaches are now well incorporated in "Shokuiku," which started in 2005 following the enactment of "Basic Act on Shokuiku."[14] As for exercise and physical activity; our findings showed that the proportions of people with regular exercise habit increased after 60 years old, though the numbers of steps decreased. With a rapid shift to aging society, there is a growing concern on future health of the current young and middle-aged,

who will become the elderly after the decades. More efforts are therefore required to establish the environment, especially in small towns, to promote regular exercise and physical activity, so that the future elderly will be able to acquire habitual exercise and physical activity after retirement without a difficulty.

Our findings of differential patterns in overweight prevalence as well as in lifestyle behaviors across types of residential areas suggest that the area-specific population approaches should be enhanced to promote appropriate lifestyle behavior for the young and middle aged. In particular, the importance of physical activity should be emphasized.

Acknowledgments

This study was financially supported by a grant-in aid to Nobuo Yoshiike from the Ministry of Health, Labour, and Welfare, Japan.

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