Association of Sleep Duration with Mortality from Cardiovascular Disease and Other Causes for Japanese Men and Women: the JACC Study

Satoyo Ikehara, MSc[1]; Hiroyasu Iso, MD, PhD[1]; Chigusa Date, PhD[2]; Shogo Kikuchi, MD, PhD[3]; Yoshiyuki Watanabe, MD, PhD[4]; Yasuhiko Wada, MD, PhD[5]; Yutaka Inaba, PhD[6]; Akiko Tamakoshi, MD, PhD[3]; and the JACC Study Group

[1]Public Health, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Osaka, Japan; [2]Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Women's University, Nara, Japan; [3]Department of Public Health, Aichi Medical University School of Medicine, Nagakute, Aichi, Japan; [4]Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Sciences, Kyoto, Japan; [5]Department of Medical Informatics, Japan Labour Health and Welfare Organization Kansai Rosai Hospital, Amagasaki, Hyogo, Japan; [6]Division of Public Health, Department of Food & Health Sciences, Faculty of Human Life Sciences, Jissen Women's University, Tokyo, Japan

Study Objectives: To examine sex-specific associations between sleep duration and mortality from cardiovascular disease and other causes.

Design: Cohort study.

Setting: Community-based study.

Participants: A total of 98,634 subjects (41,489 men and 57,145 women) aged 40 to 79 years from 1988 to 1990 and were followed until 2003.

Interventions: N/A.

Measurements and Results: During a median follow-up of 14.3 years, there were 1964 deaths (men and women: 1038 and 926) from stroke, 881 (508 and 373) from coronary heart disease, 4287 (2297 and 1990) from cardiovascular disease, 5465 (3432 and 2033) from cancer, and 14,540 (8548 and 5992) from all causes. Compared with a sleep duration of 7 hours, sleep duration of 4 hours or less was associated with increased mortality from coronary heart disease for women and noncardiovascular disease/noncancer and all causes in both sexes. The respective multivariable hazard ratios were 2.32 (1.19-4.50) for coronary heart disease in women, 1.49 (1.02-2.18) and 1.47 (1.01-2.15) for noncardiovascular disease/noncancer, and 1.29 (1.02-1.64) and 1.28 (1.03-1.60) for all causes in men and women, respectively. Long sleep duration of 10 hours or longer was associated with 1.5- to 2-fold increased mortality from total and ischemic stroke, total cardiovascular disease, noncardiovascular disease/noncancer, and all causes for men and women, compared with 7 hours of sleep in both sexes. There was no association between sleep duration and cancer mortality in either sex.

Conclusions: Both short and long sleep duration were associated with increased mortality from cardiovascular disease, noncardiovascular disease/noncancer, and all causes for both sexes, yielding a U-shaped relationship with total mortality with a nadir at 7 hours of sleep.

Keywords: Sleep duration, coronary heart disease, mortality, prospective study

Citation: Ikehara S; Iso H; Date C; Kikuchi S; Watanabe Y; Wada Y; Inaba Y; Tamakoshi A. Association of sleep duration with mortality from cardiovascular disease and other causes for Japanese men and women: the JACC study. *SLEEP* 2009;32(3):259-301.

PREVIOUS COHORT STUDIES HAVE DEMONSTRATED THAT SHORT OR LONG SLEEP DURATION IS ASSOCIATED WITH THE INCIDENCE OF OR MORTALITY FROM cardiovascular disease,[1-4] as well as total mortality.[1,3-6] The National Health and Nutrition Examination Survey I showed a 1.5-fold increase in the risk of stroke for persons with more than 8 hours of sleep, compared with those with 6 to 8 hours of sleep.[1] The Nurse's Health Study also reported that, compared with 8 hours of sleep, short or long sleep duration of 5 or more hours or 9 or more hours was associated with an increased incidence of coronary heart disease for women aged 40 to 65 years,[2] and, compared with 7 hours of sleep, long sleep duration of 9 or more hours was associated with mortality from cardiovascular disease, noncardiovascular disease/noncancer, and all causes, whereas short sleep duration of 5 hours or less was associated with mortality from all causes and noncardiovascular disease for women aged 40 to 65 years.[3] The Whitehall II cohort study found a U-shaped association between sleep duration and mortality from cardiovascular disease and noncardiovascular disease and between sleep duration and all causes for men and women aged 35 to 55 years.[4] An earlier report of our Japanese cohort study[5] also showed a U-shaped relationship between sleep duration and total mortality, but cause-specific analyses were not carried out. Thus, the association between short or long sleep duration and mortality from cardiovascular disease and other causes for Japanese men and women has remained unclear.

To examine the sex-specific associations of sleep duration and mortality from stroke, coronary heart disease, and other causes, as well as total mortality, we analyzed the extended follow-up data from a large-scale prospective study of approximately 98,000 Japanese men and women.

METHODS

Study Population

The Japan Collaborative Cohort Study for Evaluation of Cancer Risk sponsored by Monbusho (JACC study) was conducted from 1988 to 1990, when 110,792 subjects (46,465 men and 64,327 women) aged 40 to 79 years and living in 45 communities across Japan participated in municipal health-screening examinations and completed self-administered questionnaires, including lifestyle data and medical histories of previous cardio-

Submitted for publication June, 2008
Submitted in final revised form November, 2008
Accepted for publication November, 2008
Address correspondence to: Prof. Hiroyasu Iso, MD, PhD, MPH, Public Health, Department of Social and Environmental Medicine, Graduate School of Medicine, Osaka University, Suita-shi, Osaka 565-0871 Japan; Tel: 81 6 6879 3911; Fax: 81 6 6879 3919; E-mail: iso@pbhel.med.osaka-u.ac.jp

vascular disease and cancer at baseline. The details of the study procedure have been described previously.[7] In most communities, informed consent was obtained individually and directly from members of the cohort, whereas, in several communities, informed consent was obtained at the community level after the purpose of the study and confidentiality of the data had been explained to community leaders and mayors. Of the 110,792 cohort participants, data from 6782 (2613 men and 4169 women) were excluded because of missing information on sleep duration, as were data from 5376 subjects (2363 men and 3013 women) who reported a history of cancer, stroke, or coronary heart disease. Finally, a total of 41,489 men and 57,145 women were included in the study.

Mortality Surveillance

For mortality surveillance in each of the communities, investigators conducted a systematic review of death certificates, all of which had been forwarded to the public health center in the area of residency. Mortality data were then centralized at the Ministry of Health and Welfare, and the underlying causes of death were coded for the National Vital Statistics according to the *International Classification of Diseases*, 10th revision (ICD-10). Therefore, all deaths that occurred in the cohort were ascertained by death certificates from a public health center, except for subjects who died after they had moved from their original community, in which case the subject was treated as withdrawals from observation when they moved out. Cause-specific mortality was determined separately in terms of cancer (C00-C97), total cardiovascular disease (I01-I99), coronary heart disease (I20-I25), and total stroke (I60-I69); noncardiovascular disease/noncancer was listed as the cause of death when cardiovascular disease and cancer were excluded. Stroke deaths were further subdivided into intraparenchymal hemorrhage (I61), subarachnoid hemorrhage (I60), and ischemic stroke (I63 and I693). The follow-up is believed to be complete and accurate as a result of systematic examination of death certificates and residency status. By December 31, 2003, except for 4 communities in which follow-up was terminated at the end of 1999, 14,540 subjects were treated as withdrawals from observation when they died, and 4188 subjects were treated as withdrawals from observation when they moved out of the study community. The median follow-up period for the participants was 14.3 years. This study was approved by the ethics committees of the Nagoya University School of Medicine and the University of Tsukuba.

Baseline Survey

The baseline data were collected by means of a self-administered questionnaire, which included sleep duration; demographic characteristics; and histories of hypertension, diabetes mellitus, and other chronic diseases, as well as habits related to smoking, alcohol consumption, diet, and exercise. We obtained information about the average sleep duration on weekdays during the preceding year. The average sleep duration per day was classified into 7 categories: less than 4.5 hours (≤ 4 hours); 5, 6, 7, 8, and 9 hours; and equal to or longer than 9.5 hours (≥ 10 hours). Fractions hours were rounded off (eg, 7 hours represented responses from 6.5 to 7.4 hours). Depressive symptom was assessed by using 4 psychological or behavior items[5]: (1) Do you think that your life is meaningful? (2) Do you think that you make decisions quickly? (3) Are you enjoying your life? (4) Do you feel that others rely very much on you? These 4 items were then combined into an overall index of depressive symptoms. Questions with positive/neutral or negative responses were scored as 0 or 1, respectively. Thus, the overall index of depressive symptoms had a possible range from 0 to 4 (Cronbach α coefficient of 0.52), and subjects were grouped according to whether they had no symptoms, 1 symptom, or 2 or more symptoms. The reproducibility and validity for dietary intake have been reported elsewhere.[8]

Statistical Analysis

Statistical analyses were based on sex-specific mortality rates of disease outcomes and all cause during the follow-up period from 1988-1990 to 2003 (to 1999 for 4 communities). The person-years of follow-up were calculated from the date of filling out the baseline questionnaire to death, moving out of the community, or the end of follow-up, whichever came first. Sex-specific age-adjusted mean values and prevalence of cardiovascular risk factors were calculated. The sex-specific hazard ratios with 95% confidence interval (CI) of mortality from disease outcomes and all causes were calculated with reference to the risk for 7 hours of sleep. These estimates were adjusted for age and other potential confounding factors by means of the Cox proportional hazards model. The other potential confounding factors were history of hypertension, history of diabetes, body mass index (sex-specific quintiles), smoking status (never, exsmoker, current smoker of 1-19, and current smoker of ≥ 20 cigarettes per day), alcohol consumption (nondrinker, exdrinker, current drinker of 0.1-22.9, 23.0-45.9, 46.0-68.9, and ≥ 69.0 g ethanol per day), hours of exercise (almost never and 1-2, 3-4, and ≥ 5 hours per week), hours of walking (almost never and 0.5, 0.6-0.9, and ≥ 1 hours per day), perceived mental stress (low, moderate, and high), depressive symptoms (0, 1, and ≥ 2 symptoms), education level (< 13, 13-15, 16-18, and ≥ 19 years), regular employment or not, fresh fish intake (almost never, 1 to 2 days a month, 1 to 2 days a week, 3 to 4 days a week, and almost every day). SAS (SAS, Inc., Cary, NC)(version 9.13) was used for all statistical analyses.

RESULTS

After a follow-up of 1,270,585 person-years, the deaths of 1964 (men and women: 1038 and 926) from stroke, 881 (508 and 373) from coronary heart disease, 4287 (2297 and 1990) from total cardiovascular disease, 5465 (3432 and 2033) from cancer, and 14,540 (8548 and 5992) from all causes had been documented.

Table 1 shows sex-specific age-adjusted mean values or prevalence of risk characteristics at baseline by sleep-duration category. The respective percentages of ≤ 4, 5, 6, 7, 8, 9, and ≥ 10 hours of sleep were 1%, 3%, 13%, 32%, 39%, 8%, and 4%, respectively, for men and 1%, 5%, 20%, 38%, 29%, 5%, and 2%, respectively, for women. Compared with 7 hours of sleep, short or long sleep duration tended to be associated with older age and more depressive symptoms for both men and women.

Table1—Sex-Specific, Age-Adjusted Mean Values or Prevalence of Cardiovascular Risk Factors at Baseline by Sleep Duration

	Sleep duration, h/day						
	≤4	5	6	7	8	9	≥10
Men							
No. at risk	215	1142	5513	13423	16042	3491	1663
Age, y	60.1	58.6	56.1	55.1	57.3	60.2	64.4
BMI, kg/m^2	22.3	22.7	22.9	22.6	22.6	22.4	22.5
Overweight	18.9	21.9	22.0	17.8	17.5	17.2	18.6
History of hypertension	27.0	24.8	20.4	19.4	19.9	21.5	22.9
History of diabetes	13.4	7.8	7.5	6.5	6.3	6.1	7.1
Ethanol intake, g/day	41.9	36.7	33.4	32.1	34.9	37.4	40.1
Current smoker	47.3	45.8	50.3	53.5	55.5	56.8	58.7
College or higher education	12.1	17.5	20.1	19.8	16.2	12.4	11.1
High perceived mental stress	37.9	37.7	31.4	23.6	19.3	20.3	20.8
2 or more depressive symptoms	14.5	9.2	6.1	4.5	5.2	7.3	10.0
Exercise ≥ 5 h/wk	9.5	7.5	6.8	6.7	7.2	8.8	7.8
Walking ≥ 1 h/day	42.4	45.8	46.5	49.3	51.3	53.0	51.3
Regular employment	60.7	73.1	76.7	77.8	76.6	72.9	68.8
Fresh fish intake, no./wk	6.4	6.8	6.7	6.8	7.1	7.1	7.4
Women							
No. at risk	430	2699	11668	21501	16643	2935	1269
Age, y	62.8	58.5	55.6	55.4	59.0	63.0	67.5
BMI, kg/m^2	22.8	22.8	22.9	22.8	23.0	23.1	23.2
Overweight	21.0	23.1	22.4	21.5	23.6	25.2	26.7
History of hypertension	22.9	23.6	22.1	22.2	22.8	22.0	23.9
History of diabetes	2.8	5.0	3.9	3.5	3.9	4.0	4.4
Ethanol intake, g/day	12.9	13.0	10.1	9.5	11.1	12.2	13.4
Current smoker	7.8	8.5	5.7	4.7	5.3	4.9	7.9
College or higher education	9.7	11.4	11.5	10.4	9.0	7.0	7.1
High perceived mental stress	36.8	30.7	24.6	19.2	16.7	16.6	19.4
2 or more depressive symptoms	17.1	10.1	7.4	7.0	7.8	10.6	19.1
Exercise ≥ 5 h/wk	4.3	5.2	4.4	4.3	5.1	4.4	4.3
Walking ≥ 1 h/day	50.4	49.7	51.7	51.9	52.0	53.0	44.6
Regular employment	32.5	33.7	34.8	34.1	31.6	29.4	31.1
Fresh fish intake, no./wk	6.0	6.7	6.9	7.1	7.3	7.2	7.2

Data are presented as percentage, except age, body mass index (BMI), ethanol intake, and fresh fish intake, which are presented as mean.

Men and women with short sleep duration were more likely to have high perceived mental stress, whereas those with long sleep duration were less educated.

Tables 2 show sex-specific, age-adjusted, and multivariable hazard ratios of total stroke, stroke subtypes, coronary heart disease, total cardiovascular disease, cancer, noncardiovascular disease/noncancer, and all causes by sleep duration. Increased risks of age-adjusted mortality from total and ischemic strokes and total cardiovascular disease, cancer, noncardiovascular disease/noncancer, and all causes were observed among men and women with 10 or more hours of sleep, compared with those with 7 hours of sleep. These associations, except for mortality from cancer, were slightly weaker but remained statistically significant after adjustment for cardiovascular risk factors and depressive symptoms. The respective multivariable hazard ratios (95% CI) of mortality from total and ischemic strokes, total cardiovascular disease, noncardiovascular disease/noncancer, and all causes for long sleepers were 1.66 (1.31-2.08), 1.58 (1.19-2.12), 1.56 (1.33-1.83), 1.66 (1.44-1.91), and 1.41 (1.29-1.54), respectively, for men, and 1.69 (1.29-2.20), 2.37 (1.70-3.32), 1.54 (1.28-1.86), 1.99 (1.65-2.39), and 1.56 (1.40-1.75), respectively, for women.

There was an increased risk of mortality from coronary heart disease for women with 4 or fewer hours and 5 hours of sleep, compared with those with 7 hours of sleep. The respective multivariable hazard ratios (95% CI) for those with 4 or fewer hours and 5 hours of sleep were 2.32 (1.19-4.50) and 1.64 (1.07-2.53). Short sleep of 4 or fewer hours for men tended to be associated with an increased risk of mortality from hemorrhagic stroke, although this association did not reach statistical significance (hazard ratio = 2.15, 95% CI: 0.78-5.89, P = 0.14). When stratified by alcohol-consumption status, the multivariable hazard ratios of mortality from hemorrhagic stroke for male short sleepers (≤ 4 and 5 hours of sleep) were 2.03 (1.01-4.08) for current drinkers and 1.33 (0.38-4.66) for exdrinkers or never drinkers. After further adjustment for individual quantity of alcohol consumption as a continuous variable, the multivariable hazard ratio for male short sleepers was 1.92 (95% CI: 0.96-3.86, P = 0.07). Also, short sleep duration or 4 or fewer hours was associated with increased risk of mortality from noncardiovascular disease/noncancer for both men and women; the multivariable hazard ratios were 1.49 (1.02-2.18) for men and 1.47 (1.01-2.15) for women.

Table 2—Sex-Specific Hazard Ratios and 95% Confidence Intervals for Mortality from Cardiovascular Disease and Other Causes by Sleep Duration

	Sleep duration (h/day)						
	≤ 4	5	6	7	8	9	≥ 10
Men							
Person-years	**2501**	**14176**	**69125**	**173026**	**204761**	**43152**	**18724**
Total stroke							
No.	10	28	105	244	413	126	112
Age-adjusted HR (95%CI)	1.62 (0.86-3.06)	0.95 (0.64-1.40)	0.96 (0.76-1.21)	1.00	1.15 (0.98-1.35)	1.25 (1.00-1.55)	1.90 (1.51-2.38)
Multivariable HR (95%CI)	1.56 (0.82-2.94)	0.85 (0.58-1.26)	0.95 (0.76-1.20)	1.00	1.11 (0.95-1.30)	1.14 (0.92-1.42)	1.66 (1.31-2.08)
Hemorrhagic stroke							
No.	4	11	40	82	145	31	26
Age-adjusted HR (95%CI)	2.45 (0.90-6.70)	1.29 (0.69-2.43)	1.14 (0.78-1.67)	1.00	1.30 (0.99-1.71)	1.10 (0.73-1.67)	1.73 (1.10-2.70)
Multivariable HR (95%CI)	2.15 (0.78-5.89)	1.20 (0.64-2.26)	1.13 (0.77-1.65)	1.00	1.27 (0.97-1.66)	1.01 (0.66-1.53)	1.56 (0.99-2.45)
Ischemic stroke							
No.	5	15	50	143	235	85	74
Age-adjusted HR (95%CI)	1.18 (0.48-2.87)	0.78 (0.46-1.34)	0.75 (0.55-1.04)	1.00	1.07 (0.87-1.31)	1.29 (0.98-1.69)	1.84 (1.39-2.45)
Multivariable HR (95%CI)	1.28 (0.52-3.15)	0.70 (0.41-1.20)	0.76 (0.55-1.04)	1.00	1.02 (0.83-1.26)	1.18 (0.90-1.55)	1.58 (1.19-2.12)
Coronary heart disease							
No.	1	17	53	140	206	54	37
Age-adjusted HR (95%CI)	0.31 (0.04-2.18)	1.05 (0.64-1.75)	0.86 (0.63-1.18)	1.00	1.02 (0.83-1.27)	0.99 (0.72-1.35)	1.19 (0.82-1.72)
Multivariable HR (95%CI)	0.29 (0.04-2.05)	1.02 (0.62-1.70)	0.86 (0.63-1.19)	1.00	1.02 (0.82-1.27)	0.96 (0.70-1.31)	1.12 (0.77-1.63)
Total cardiovascular disease							
No.	16	70	248	548	913	274	228
Age-adjusted HR (95%CI)	1.17 (0.71-1.92)	1.06 (0.83-1.36)	1.01 (0.87-1.18)	1.00	1.14 (1.02-1.26)	1.22 (1.05-1.41)	1.74 (1.48-2.03)
Multivariable HR (95%CI)	1.11 (0.67-1.83)	0.99 (0.77-1.27)	1.01 (0.87-1.18)	1.00	1.11 (1.00-1.24)	1.14 (0.99-1.32)	1.56 (1.33-1.83)
Cancer							
No.	26	91	413	940	1361	385	216
Age-adjusted HR (95%CI)	1.31 (0.89-1.94)	0.90 (0.72-1.12)	1.02 (0.91-1.14)	1.00	1.04 (0.96-1.13)	1.13 (1.01-1.28)	1.17 (1.00-1.35)
Multivariable HR (95%CI)	1.24 (0.84-1.83)	0.90 (0.72-1.12)	1.03 (0.92-1.16)	1.00	1.02 (0.94-1.11)	1.07 (0.95-1.21)	1.10 (0.94-1.27)
Noncardiovascular/noncancer							
No.	28	104	359	660	1040	342	286
Age-adjusted HR (95%CI)	1.76 (1.20-2.57)	1.34 (1.09-1.65)	1.23 (1.08-1.39)	1.00	1.09 (0.99-1.20)	1.30 (1.14-1.48)	1.89 (1.64-2.17)
Multivariable HR (95%CI)	1.49 (1.02-2.18)	1.20 (0.97-1.48)	1.20 (1.06-1.37)	1.00	1.06 (0.96-1.17)	1.20 (1.05-1.37)	1.66 (1.44-1.91)
All causes							
No.	70	265	1020	2148	3314	1001	730
Age-adjusted HR (95%CI)	1.42 (1.12-1.80)	1.08 (0.95-1.23)	1.08 (1.00-1.16)	1.00	1.08 (1.02-1.14)	1.21 (1.12-1.30)	1.56 (1.43-1.69)
Multivariable HR (95%CI)	1.29 (1.02-1.64)	1.02 (0.90-1.16)	1.08 (1.00-1.16)	1.00	1.06 (1.00-1.12)	1.13 (1.05-1.22)	1.41 (1.29-1.54)
Women							
Person-years	5183	34039	151458	284289	217774	37576	14801
Total stroke							
No.	12	46	125	228	339	96	80
Age-adjusted HR (95%CI)	1.15 (0.64-2.05)	1.05 (0.77-1.45)	0.95 (0.76-1.18)	1.00	1.28 (1.08-1.51)	1.35 (1.06-1.71)	1.87 (1.44-2.43)
Multivariable HR (95%CI)	1.07 (0.59-1.91)	0.99 (0.72-1.37)	0.93 (0.75-1.16)	1.00	1.24 (1.05-1.47)	1.29 (1.01-1.64)	1.69 (1.29-2.20)
Hemorrhagic stroke							
No.	3	19	54	115	142	33	13
Age-adjusted HR (95%CI)	0.74 (0.24-2.35)	1.01 (0.62-1.64)	0.84 (0.61-1.17)	1.00	1.19 (0.93-1.52)	1.16 (0.78-1.71)	0.84 (0.47-1.50)
Multivariable HR (95%CI)	0.68 (0.22-2.15)	0.93 (0.57-1.52)	0.82 (0.60-1.14)	1.00	1.17 (0.91-1.51)	1.16 (0.78-1.72)	0.78 (0.43-1.40)
Ischemic stroke							
No.	9	27	62	94	159	52	61
Age-adjusted HR (95%CI)	1.67 (0.84-3.32)	1.31 (0.86-2.02)	1.10 (0.80-1.51)	1.00	1.33 (1.03-1.72)	1.48 (1.05-2.09)	2.68 (1.92-3.73)
Multivariable HR (95%CI)	1.57 (0.79-3.13)	1.26 (0.82-1.94)	1.10 (0.79-1.51)	1.00	1.29 (1.00-1.67)	1.38 (0.98-1.95)	2.37 (1.70-3.32)
Coronary heart disease							
No.	10	28	60	83	127	45	20
Age-adjusted HR (95%CI)	2.40 (1.24-4.64)	1.68 (1.09-2.58)	1.24 (0.89-1.72)	1.00	1.27 (0.96-1.67)	1.61 (1.11-2.32)	1.16 (0.70-1.90)
Multivariable HR (95%CI)	2.32 (1.19-4.50)	1.64 (1.07-2.53)	1.23 (0.88-1.72)	1.00	1.24 (0.94-1.64)	1.52 (1.05-2.19)	1.04 (0.63-1.72)
Total cardiovascular disease							
No.	30	117	275	470	725	217	156
Age-adjusted HR (95%CI)	1.34 (0.93-1.95)	1.28 (1.04-1.56)	1.01 (0.87-1.17)	1.00	1.31 (1.16-1.47)	1.43 (1.22-1.69)	1.70 (1.41-2.04)
Multivariable HR (95%CI)	1.28 (0.88-1.86)	1.22 (1.00-1.50)	1.00 (0.86-1.16)	1.00	1.28 (1.14-1.44)	1.37 (1.17-1.62)	1.54 (1.28-1.86)
Cancer							
No.	24	113	333	672	638	156	97
Age-adjusted HR (95%CI)	1.16 (0.77-1.75)	1.10 (0.90-1.34)	0.90 (0.79-1.03)	1.00	0.97 (0.87-1.08)	1.05 (0.88-1.25)	1.28 (1.03-1.59)
Multivariable HR (95%CI)	1.14 (0.76-1.72)	1.07 (0.87-1.31)	0.90 (0.79-1.03)	1.00	0.95 (0.85-1.06)	1.01 (0.85-1.21)	1.20 (0.97-1.50)
Noncardiovascular/noncancer							
No.	29	92	314	446	676	224	161
Age-adjusted HR (95%CI)	1.56 (1.07-2.28)	1.15 (0.91-1.43)	1.35 (1.17-1.56)	1.00	1.35 (1.20-1.52)	1.72 (1.46-2.02)	2.16 (1.79-2.60)
Multivariable HR (95%CI)	1.47 (1.01-2.15)	1.07 (0.85-1.34)	1.34 (1.16-1.54)	1.00	1.33 (1.18-1.50)	1.65 (1.40-1.94)	1.99 (1.65-2.39)
All causes							
No.	83	322	949	1588	2039	597	414
Age-adjusted HR (95%CI)	1.34 (1.08-1.67)	1.17 (1.03-1.32)	1.06 (0.98-1.15)	1.00	1.18 (1.10-1.26)	1.37 (1.25-1.51)	1.70 (1.52-1.90)
Multivariable HR (95%CI)	1.28 (1.03-1.60)	1.11 (0.98-1.25)	1.05 (0.97-1.14)	1.00	1.16 (1.08-1.24)	1.32 (1.20-1.45)	1.56 (1.40-1.75)

Multivariable adjustment: age, body mass index (quintiles), history of hypertension, history of diabetes, alcohol consumption, smoking, education level, hours of exercise, hours of walking, regular employment, perceived mental stress, depressive symptoms and frequency of fresh fish intake. HR refers to hazard ratio; CI, confidence interval.

As for total cardiovascular disease for women and noncardiovascular disease and all causes for men and women, there was a U-shaped relationship between sleep duration and mortality, with a nadir at 7 hours of sleep. These associations were essentially unchanged when we excluded subjects whose events occurred within 5 years after baseline. Compared with women

who slept for 7 hours, the multivariable hazard ratios (95% CI) of mortality from total cardiovascular disease were 1.41 (0.94-2.12) for 4 hours or less, 1.24 (0.99-1.57) for 5 hours, 1.04 (0.88-1.23) for 6 hours, 1.29 (1.13-1.47) for 8 hours, 1.35 (1.12-1.62) for 9 hours, and 1.51 (1.21-1.87) for 10 hours or longer. The respective multivariable hazard ratios of mortality from noncardiovascular disease/noncancer were 1.65 (1.08-2.52), 1.32 (1.05-1.66), 1.26 (1.09-1.45), 1.08 (0.97-1.21), 1.19 (1.03-1.39) and 1.66 (1.40-1.95), respectively, for men, and 1.43 (0.94-2.18), 1.03 (0.80-1.33), 1.35 (1.16-1.58), 1.34 (1.18-1.53), 1.58 (1.32-1.90), and 1.83 (1.48-2.26), respectively, for women. Furthermore, the respective hazard ratios of mortality from all causes were 1.27 (0.96-1.68), 1.06 0.91-1.22), 1.07 (0.98-1.17), 1.04 (0.98-1.11), 1.11 (1.01-1.21) and 1.37 (1.24-1.52) for men, and 1.26 (0.98-1.62), 1.08 (0.95-1.24), 1.04 (0.95-1.14), 1.13 (1.05-1.22), 1.27 (1.14-1.42), and 1.46 (1.29-1.67) for women.

DISCUSSION

In this large-scale prospective study of Japanese men and women aged 40 to 79 years, we confirmed that, compared with 7 hours of sleep, short sleep duration of 4 hours or less was associated with a 2-fold increase in mortality from coronary heart disease for women and a 1.5-fold increase in mortality from noncardiovascular disease/noncancer and a 1.3-fold increase in total mortality for both men and women, whereas long sleep duration ($\geq$ 10 hours) was associated with a 1.5- to 2-fold increase in mortality from total stroke, ischemic stroke, total cardiovascular disease, noncardiovascular disease/noncancer and all causes for both men and women. There was a robust U-shaped relationship between sleep duration and mortality from all causes, with a nadir at 7 hours of sleep in both sexes, which extended the evidence of the earlier report,[5] based on the approximately 30% larger number of deaths.

To the best of our knowledge, ours is the first study to provide evidence of the association of short sleep duration with an increase in mortality from coronary heart disease for Asian women. Previous studies of Americans or Europeans support our findings. The Nurses' Health Study of 71,617 women aged 40 to 65 years reported that, compared with 8 hours of sleep, short sleep duration of 5 hours or less was associated with a 1.4-fold increase in risk of coronary heart disease.[2] The MONICA/KORA Augsburg Cohort Study of 3508 men and 3388 women aged 45 to 74 years showed that the risk of myocardial infarction was approximately 3 times higher for women with 5 or fewer hours of sleep, compared with 8 hours of sleep, but such an increase in risk was not observed for men.[9] We observed an increased mortality from coronary heart disease associated with short sleep only for women, and the mortality among female short sleepers did not differ significantly from that among male short sleepers. The hazard ratio (95% CI) of coronary heart disease for short sleepers in women versus those in men was 4.60 (0.58-36.2). This finding contrasts with the result that risk of mortality from cardiovascular disease, other causes, and all causes were approximately half among women than among men. The age-adjusted hazard ratios for women versus men were 0.56 (0.51-0.61) for total stroke, 0.46 (0.40-0.53) for coronary heart disease, 0.55 (0.51-0.58) for total cardiovascular disease, 0.39 (0.37-0.41) for cancer, 0.44 (0.42-0.47) for noncardiovascular disease/noncancer, and 0.45 (0.44-0.47) for all causes.

Short sleep of 4 or fewer hours was found to be associated with increased risk of mortality from hemorrhagic stroke for men, although this association was not statistically significant. However, when stratified by alcohol consumption habits, an increased risk of mortality from hemorrhagic stroke was observed among male drinkers with 4 or fewer hours and 5 hours of sleep. A cross-national study on sleep habits of approximately 35,000 men and women of 10 countries, including Japan,[10] showed that the prevalence of the use of alcohol as a sleep aid was the highest in Japan (30.3%). A recent cross-sectional survey conducted in Japan[11] also reported that the prevalence of alcohol consumption as a sleep aid at least once a week was 48% for men aged 20 years or older. It is possible that the habit of using alcohol as a sleep aid enhances the risk of mortality from hemorrhagic stroke associated with short sleep duration.

There is some evidence that may explain why short sleep duration is associated with an increase in mortality from coronary heart disease and total cardiovascular disease. Previous studies showed that short-term sleep deprivation leads to increased sympathetic nervous system activity,[12,13] elevated blood pressure,[12,14] elevated cortisol levels,[13] impaired glucose tolerance,[13] and increased inflammatory markers,[15] which may reflect and increase the risk of cardiovascular disease. Furthermore, recent epidemiologic studies have demonstrated that short sleep duration is associated with higher levels of hemoglobin A (1c),[16] total cholesterol,[17] and triglycerides,[17] higher blood pressure,[17] and increased incidence of hypertension.[18] Short sleep was also associated with increased mortality from noncardiovascular disease/noncancer for both men and women. This finding suggests other mechanisms for increasing nonspecific mortality, which need to be explored in further studies.

The association of long sleep duration with higher risks of mortality from total stroke, total cardiovascular disease, noncardiovascular disease/noncancer, and all causes observed in our study was consistent with the results of previous cohort studies.[1-3] A 10-year follow-up of the National Health and Nutrition Examination Survey I cohort comprising 7844 men and women aged 32 years and older showed a 1.5-fold increase in risk of stroke for persons with more than 8 hours of sleep, compared with those with 6 to 8 hours of sleep.[1] The Nurses' Health Study of 82,969 women aged 40 to 65 years showed that, compared with 7 hours of sleep, long sleep of 9 or more hours was associated with a 1.6-fold increase in mortality from cardiovascular disease, a 1.5-fold for noncardiovascular disease/noncancer, and a 1.4-fold for all causes.[3] Another report from the Nurses' Health Study of 71,617 women aged 40 to 65 years showed that, compared with 8 hours of sleep, long sleep of 9 or more hours was associated with 1.4-fold increased risk of coronary heart disease.[2] Although the mechanisms for the association between long sleep duration and increased mortality from cardiovascular disease and other causes were not clear, long sleep duration may be an early symptom of disease and may precede clinical diagnoses. However, the association of long sleep duration with excess mortality from total cardiovascular disease, noncardiovascular disease/noncancer, and all causes did not change substantially after exclusion of the subjects whose events occurred within 5 years from baseline.

The following limitations of our study need to be addressed. First, we could not obtain information about the quality of sleep, such as the presence or absence of sleep apnea, which is associated with increased risk of cardiovascular disease.[19] A previous cohort study of 1024 volunteers showed that short sleep duration was associated with an increased body mass index along with a reduction in leptin and elevated levels of ghrelin.[20] Since being overweight is a strong risk factor for sleep apnea, this disorder can be a confounder for the association between short sleep duration and increased risk of mortality from cardiovascular disease. However, men and women with short sleep duration enrolled in our study did not have a higher mean body mass index, and we did not have a higher percentage of overweight subjects among short sleepers than long sleepers, so that the potential confounding effect of sleep apnea may be minor. Second, data on sleep duration were obtained by self-administrated questionnaire and, thus, may include misclassification. However, self-assessed sleep duration was shown in a previous study to yield valid results in comparison with quantitative sleep assessment with actigraphy.[21] Another study has suggested, however, that depressed mood is associated with both underextimation and overestimation of habitual sleep duration.[22] We therefore conducted a statistical analysis including these psychological factors as covariates, which showed that the association between sleep duration and coronary heart disease remained substantially unchanged. Finally, we used the mortality data, rather than incidence data, as endpoints, which may lead to misclassification in the diagnosis of discuss outcomes, especially stroke, stroke subtypes, and coronary heart disease. However, the widespread use of computed tomography in local hospitals since the 1980s has probably made the diagnosis of stroke and its subtypes reported on the death certificates sufficiently accurate.[23,24] For coronary heart disease, approximately one fourth to one third of deaths attributed to ischemic heart disease on the death certificate were misdiagnosed, according to the validation studies.[25,26] Therefore, the contamination of other cardiovascular diseases in the diagnosis of coronary heart disease would probably underestimate the excess mortality from coronary heart disease for female short sleepers, and the real association may be stronger.

The strengths of our study are its prospective design and high statistical power to detect sex-specific associations of short and long sleep duration with cause-specific mortality, as well as with total mortality.

In conclusion, short sleep duration was associated with increased mortality from coronary heart disease for women and from noncardiovascular disease/noncancer and all causes for both sexes, whereas long sleep duration was associated with increased mortality from stroke, total cardiovascular disease, noncardiovascular disease/noncancer, and all causes for both sexes, yielding a U-shaped relationship with total mortality, with a nadir at 7 hours of sleep.

ACKNOWLEDGMENTS

The JACC Study (Japan Collaborative Cohort Study) was supported by grants in aid for scientific research from the Ministry of Education, Science, Sports and Culture of Japan (Monbusho); 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, and 18014011. The authors sincerely express their appreciation to Dr. Kunio Aoki, Professor Emeritus, Nagoya University School of Medicine and the former chairman of the JACC Study, and Dr. Haruo Sugano, the former Director, Cancer Institute, Tokyo, who greatly contributed to the initiation of the JACC Study, as well as Dr. Yoshiyuki Ohno, Professor Emeritus, Nagoya University School of Medicine, who was the past chairman of the study. The authors also wish to thank Dr. Tomoyuki Kitagawa, Cancer Institute of the Japanese Foundation for Cancer Research and the former chairman of Grant-in-Aid for Scientific Research on Priority Area 'Cancer,' and Dr. Kazao Tajima, Aichi Cancer Center Research Institute and the former chairman of Grant-in Aid for Scientific Research on Priority Area of Cancer Epidemiology, for their full support of this study.

Study Investigators

Dr. Akiko Tamakoshi (present chairperson of the study group), Aichi Medical University School of Medicine; Mitsuru Mori and Fumio Sakauchi, Sapporo Medical University School of Medicine, Japan; Yutaka Motohashi, Akita University School of Medicine, Japan; Ichiro Tsuji, Tohoku University Graduate School of Medicine, Japan; Yosikazu Nakamura, Jichi Medical School, Japan; Hiroyasu Iso, Osaka University School of Medicine, Japan; Haruo Mikami, Chiba Cancer Center, Japan; Michiko Kurosawa, Juntendo University School of Medicine, Japan; Yoshiharu Hoshiyama, University of Human Arts and Sciences, Japan; Hiroshi Suzuki, Niigata University School of Medicine, Japan; Koji Tamakoshi, Nagoya University School of Medicine, Japan; Kenji Wakai, Nagoya University Graduate School of Medicine, Japan; Shinkan Tokudome, Nagoya City University Graduate School of Medical Sciences, Japan; Koji Suzuki, Fujita Health University School of Health Sciences, Japan; Shuji Hashimoto, Fujita Health University School of Medicine, Japan; Shogo Kikuchi, Aichi Medical University School of Medicine, Japan; Yasuhiko Wada, Kansai Rosai Hospital, Japan; Takashi Kawamura, Kyoto University Center for Student Health, Japan; Yoshiyuki Watanabe and Kotaro Ozasa, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Japan; Tsuneharu Miki, Kyoto Prefectural University of Medicine Graduate School of Medical Science, Japan; Chigusa Date, Faculty of Human Environmental Sciences, Nara Women's University, Japan; Kiyomi Sakata, Iwate Medical University, Japan; Yoichi Kurozawa, Tottori University Faculty of Medicine, Japan; Takesumi Yoshimura, Fukuoka Institute of Health and Environmental Sciences, Japan; Yoshihisa Fujino, University of Occupational and Environmental Health, Japan; Akira Shibata, Kurume University School of Medicine, Japan; Naoyuki Okamoto, Kanagawa Cancer Center, Japan; Hideo Shio, Moriyama Municipal Hospital, Japan.

Funding: The JACC Study (Japan Collaborative Cohort Study) was supported by grants in aid for scientific research from the Ministry of Education, Science, Sports, and Culture of Japan (Monbusho); 61010076, 62010074, 63010074, 1010068, 2151065, 3151064, 4151063, 5151069, 6279102, 11181101, 17015022, and 18014011.

DISCLOSURE STATEMENT

This was not an industry supported study. The authors have indicated no financial conflicts of interest.

REFERENCES

1. Qureshi AI, Giles WH, Croft JB, Bliwise DL. Habitual sleep patterns and risk for stroke and coronary heart disease: a 10-year follow-up from NHANES I. Neurology 1997;48:904-11.
2. Ayas NT, White DP, Manson JE, et al. A prospective study of sleep duration and coronary heart disease in women. Arch Intern Med 2003;163:205-9.
3. Patel SR, Ayas NT, Malhotra MR, et al. A prospective study of sleep duration and mortality risk in women. Sleep 2004;27:440-4.
4. Ferrie JE, Shipley MJ, Cappuccio FP, et al. A prospective study of change in sleep duration: associations with mortality in the Whitehall II cohort. Sleep 2007;30:1659-66.
5. Tamakoshi A, Ohno Y; JACC Study Group. Self-reported sleep duration as a predictor of all-cause mortality: results from the JACC study, Japan. Sleep 2004;27:51-4.
6. Kripke DF, Garfinkel L, Wingard DL, Klauber MR, Marler MR. Mortality associated with sleep duration and insomnia. Arch Gen Psychiatry 2002;59:131-6.
7. Ohno Y, Tamakoshi A, JACC Study Group. Japan Collaborative Cohort Study for Evaluation of Cancer Risk Sponsored by Monbusho (JACC Study). J Epidemiol 2001;11:144-50.
8. Date C, Fukui M, Yamamoto A, Wakai K, at al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC study. J Epidemiol 2005;15 (Suppl):9-23.
9. Meisinger C, Heier M, Löwel H, Schneider A, Döring A. Sleep duration and sleep complaints and risk of myocardial infarction in middle-aged men and women from the general population: the MONICA/KORA Augsburg cohort study. Sleep 2007;30:1121-7.
10. Soldatos CR, Allaert FA, Ohta T, Dikeos DG. How do individuals sleep around the world? Results from a single-day survey in ten countries. Sleep Med 2005;6:5-13.
11. Kaneita Y, Uchiyama M, Takemura S, at al. Use of alcohol and hypnotic medication as aids to sleep among the Japanese general population. Sleep Med 2007;8:723-32.
12. Tochikubo O, Ikeda A, Miyajima E, Ishii M. Effects of insufficient sleep on blood pressure monitored by a new multibiomedical recorder. Hypertension 1996;27:1318-24.
13. Spiegel K, Leproult R, Van Cauter E. Impact of sleep debt on metabolic and endocrine function. Lancet 1999;354:1435-9.
14. Kato M, Phillips BG, Sigurdsson G, Narkiewicz K, Pesek CA, Somers VK. Effects of sleep deprivation on neural circulatory control. Hypertension 2000;35:1173-5.
15. Meier-Ewert HK, Ridker PM, Rifai N, et al. Effect of sleep loss on C-reactive protein, an inflammatory marker of cardiovascular risk. J Am Coll Cardiol 2004;43:678-83.
16. Nakajima H, Kaneita Y, Yokoyama E, et al. Association between sleep duration and hemoglobin A (1c) level. Sleep Med 2008;9:745-752.
17. Bjorvatn B, Sagen IM, Øyane N, et al. The association between sleep duration, body mass index and metabolic measures in the Hordaland Health Study. J Sleep Res 2007;16:66-76.
18. Gangwisch JE, Heymsfield SB, Boden-Albala B, et al. Short sleep duration as a risk factor for hypertension: analyses of the first National Health and Nutrition Examination Survey. Hypertension 2006;47:833-9.
19. Yaggi HK, Concato J, Kernan WN, Lichtman JH, Brass LM, Mohsenin V. Obstructive sleep apnea as a risk factor for stroke and death. N Engl J Med 2005;353:2034-41.
20. Taheri S, Lin L, Austin D, Young T, Mignot E. Short sleep duration is associated with reduced leptin, elevated ghrelin, and increased body mass index. PLoS Med 2004;1:210-7.
21. Lockley SW, Skene DJ, Arendt J. Comparison between subjective and actigraphic measurement of sleep and sleep rhythms. J Sleep Res 1999;8:175-83.
22. Bliwise DL, Friedman L, Yesavage JA. Depression as a confounding variable in the estimation of habitual sleep time. J Clin Psychol 1993;49:471-7.
23. Iso H, Jacobs DR Jr, Goldman L. Accuracy of death certificate diagnosis of intracranial hemorrhage and nonhemorrhagic stroke. The Minnesota Heart Survey. Am J Epidemiol 1990;132:993-8.
24. Sankai T, Miyagaki T, Iso H et al. A population-based study of the proportion by type of stroke determined by computed tomography scan. Nippon Koshu Eisei Zassi 1991;38:901-9.
25. Yamashita T, Ozawa H, Aono H, Hosokawa H, Saito I, Ikebe T. Heart disease deaths on death certificates re-evaluated by clinical records in a Japanese city. Jpn Circ J 1997;61:331-8.
26. Baba S, Ozawa H, Sakai Y, Terao A, Konishi M, Tatara K. Heart disease deaths in a Japanese urban area evaluated by clinical and police records. Circulation 1994;89:109-15.

ORIGINAL

Advance Publication
doi: 10.1507/endocrj. K09E-339

Phenotypical variety of insulin resistance in a family with a novel mutation of the insulin receptor gene

Ikuko Takahashi[1], Yuichiro Yamada[2], Hiroko Kadowaki[3,4], Momoko Horikoshi[4], Takashi Kadowaki[4], Takuma Narita[2], Satoko Tsuchida[1], Atsuko Noguchi[1], Akio Koizumi[5] and Tsutomu Takahashi[1]

[1] *Department of Pediatrics, Akita University Graduate School of Medicine, Akita, Japan*
[2] *Department of Endocrinology, Diabetes and Geriatric Medicine, Akita University Graduate School of Medicine, Akita, Japan*
[3] *Department of Child Studies, Kasei-Gakuin University, Tokyo, Japan*
[4] *Department of Metabolic Diseases, Graduate School of Medicine, University of Tokyo, Tokyo, Japan*
[5] *Department of Health and Environmental Sciences, Graduate School of Medicine, Kyoto University, Kyoto, Japan*

Abstract. A novel mutation of insulin receptor gene (INSR gene) was identified in a three generation family with phenotypical variety. Proband was a 12-year-old Japanese girl with type A insulin resistance. She showed diabetes mellitus with severe acanthosis nigricans and hyperinsulinemia without obesity. Using direct sequencing, a heterozygous nonsense mutation causing premature termination at amino acid 331 in the α subunit of INSR gene (R331X) was identified. Her father, 40 years old, was not obese but showed impaired glucose tolerance. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus for 15 years. Interestingly, they had the same mutation. One case of leprechaunism bearing homozygous mutation at codon 331 was identified. These findings led to the hypothesis that R331X may contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population. In addition, the proband showed low level C-peptide/insulin molar ratio, indicating that this ratio is considered to be a useful index for identifying patients with genetic insulin resistance. In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α subunit of the insulin receptor was identified in Japanese diabetes patients. Further investigations are called for to address the molecular mechanism.

Key words: Insulin receptor, Insulin resistance, Type 2 diabetes, Leprechaunism, C-peptide/insulin molar ratio

THE INTERACTION of insulin with its cell surface receptor is the first step in insulin action and the first identified target of insulin resistance. Mutations in the insulin receptor gene lead to the insulin resistance in several syndromic forms. The human insulin receptor is encoded by a single gene with 22 exons and is an assembly of a disulfide bond-linked tetramer composed of two α and two β subunits [1-5]. After binding of insulin to the extracellular α subunit, the tyrosine kinase of the membrane spanning β subunit is activated and the receptor is autophosphorylated [6]. Insulin receptor kinase regulates the action of insulin on metabolism and growth through signal transduction pathways and is therefore thought to be central to insulin action [7].

Some dozens of mutations in the human insulin receptor gene have already been identified to date [8-11]. Homozygous or compound-heterozygous mutations in the insulin receptor gene are found in patients with syndromes of severe insulin resistance [12]. More severe Donohue syndrome ("Leprechaunism" OMIM 246200) and the milder Rabson-Mendenhall syndrome (OMIM 262190) are characterized by intrauterine and postnatal growth retardation, facial dysmorphism, lack of subcutaneous fat and altered glucose homeostasis with hyperinsulinemia, acanthosis nigricans and reduced life expectancy [13-15]. Cells from most patients with Donohue syndrome show absent or

Received Nov. 25, 2009; Accepted Feb. 18, 2010 as K09E-339
Released online in J-STAGE as advance publication Mar. 25, 2010

Correspondence to: Ikuko Takahashi, M.D., Department of Pediatrics, Akita University Graduate School of Medicine, Hondo 1-1-1, Akita-shi, Akita, 010-8543, Japan.
E-mail takaiku@doc.med.akita-u.ac.jp

severely reduced insulin binding, whereas those with Rabson-Mendenhall retain some insulin binding capacity. Therefore, it has been proposed that severity of the phenotype is determined by the degree of insulin resistance and that residual insulin binding capacity correlates with survival. Heterozygous mutations in the insulin receptor gene have been demonstrated in type A insulin resistance with the triad of insulin resistance, acanthosis nigricans, and hyperandrogenism (OMIM147670) [16].

In this study, we identified a heterozygous mutation causing premature termination at amino acid 331 substituting a termination codon for arginine in the L2 domain in α subunit of the insulin receptor gene in a Japanese patient with diabetes mellitus and hyperinsulinemia. Interestingly, her family members shared the same mutation but showed different clinical course.

Materials and Methods

Subjects

The proband, a girl of 12 years old, was referred to our hospital because of glucosuria detected by school urinary screening. She presented with mild symptoms of polydipsia and polyuria. She was born to unrelated Japanese parents at 37 weeks of gestation (birth weight 2495 g, birth length 48 cm). At birth, she did not have the dysmorphic features characteristic of leprechaunism or Rabson-Mendenhall syndrome, including intrauterine growth retardation, fasting hypoglycemia. Sensorineural hearing loss in right side was diagnosed when she was infant, but did not deteriorate.

At presentation, she was not obese, but showed severe acanthosis nigricans with scratching scar of her neck. It also mildly existed at the axilla and elbow. Hirsutism was not observed. Body mass index (BMI) was 21.6 (height 148.6 cm, weight 47.7 kg). Blood pressure was 110/70 mmHg. Pubertal stage was B2 and PH1. Laboratory tests revealed the following; HbA1c, 9.2 %; FPG, 124 mg/dL; IRI, 65.7 μU/mL; C-peptide, 3.18 ng/mL; AST, 20 IU/L; ALT, 18 IU/L; total cholesterol, 194 mg/dL; HDL cholesterol, 43.7 mg/dL; testosterone, 0.33 ng/mL. Islet associated autoantibodies were absent. Urine testing showed no ketonuria but proteinuria (microalbumin 64.4mg/g cr) and glucosuria. Ocular complication and retinopathy was not detected. Abdominal CT revealed no fatty liver and area of visceral fat on umbilical level was 41.8 cm^2 (normal: 60>). Although she showed diabetes mellitus with severe insulin resistance, her data of body composition was not suggested risk for obesity or metabolic syndrome. Self monitored blood glucose levels were 120-140 mg/dL at premeal time and 170-200 mg/dL at postprandial time. Her father, 40 years old, was healthy and no obesity (BMI 21.8) from a clinical point of view at the time of investigation. Her paternal grandmother, 66 years old, has been suffered from diabetes mellitus. She was also not obese (BMI 21.6) and has been treated with sulfonylureas for 15-years. She already developed retinopathy and presented vitreous hemorrhage 10 years ago. Her younger brother, seven years of age, had mild mental retardation and supported by special education. He showed mild obesity but normal response to oral glucose tolerance test without hyperinsulinemia (FPG, 86 mg/dL; IRI, 8.6 μU/mL; C-peptide, 1.53 ng/mL).

Measurements

The standard 75 g oral glucose tolerance test (OGTT) was performed, after overnight fast. Levels of glucose, insulin and C-peptide were measured at 0, 30, 60, 90 and 120 min. Insulin was measured using an enzyme immunoassay (E test TOSOH II; TOSOH Corporation, Tokyo, Japan). Cross-reactivity with proinsulin was 2 %. C-peptide was measured using a chemiluminescent enzyme immunoassay (LUMIPULSE Presto C-peptide; FUJIREBIO Inc., Tokyo, Japan). Proinsulin was measured using a RIA2 antibody method (HUMAN PROINSULIN RIA KIT; Linco Research Inc., St. Charles, MO).

We calculated C-peptide/insulin molar ratio from each molecular weight and international unit of insulin i.e. 26 IU/mg. We estimated molecular weight of insulin at 5800 and C-peptide at 3600. Consequently, 1 μU/mL of insulin is 6.09 pmol/L and 1 ng/mL of C-peptide is 0.278 nmol/L.

Sequence analysis

Informed consent was obtained from her family. Genomic DNA was extracted from peripheral blood lymphocytes using a DNA isolation kit for mammalian blood. Exon 1-2 of the insulin gene and Exons 1-22 of the insulin receptor gene were individually amplified using primer sets as described [17, 18]. PCR products were purified for direct sequence analysis on an ABI gene analyzer 310 or 3100 system according to the manufacturer's instructions (Applied Biosystems).

Analysis for prevalence of R331X mutant in population

We tested the frequency of R331X in type 2 DM or by chance hyperglycemia in adult people, living in the Akita prefecture located in northern Japan. We studied 272 participants of a group medical examination, comprised 92 healthy cases checked normoglycemia and 180 cases already diagnosed type 2 DM or detected hyperglycemia. These included 47 cases with family history of DM and 14 cases diagnosed before third decade. All participants gave informed consent, and the Ethics Committee of Kyoto University School of Medicine approved the study.

Genotyping of R331X was assayed with PCR restriction fragment length polymorphism. PCR reactions were conducted in a reaction volume of 7.5 μL with 20 ng genomic DNA, 2× GC buffer, 200 μM dNTPs, 10 pmol of each primer and 1 unit of LA Taq polymerase (Takara, Tokyo, Japan). The PCR primers used were 5′-AGATGTCTGAAGGACCTTGGA-3′ as a forward primer and 5′-ACAGCTCAGAGGGACATGGA-3′ as a reverse primer. PCR was performed with 39 cycles of the following 94°C for 45 s, 54°C for 45 s and 74°C for 1 min in a thermocycler. Obtained PCR products showed a single fragment at 285 bp. Six μL of 285-bp product were then digested with 2 units of BspCNI restriction enzyme at 25 °C for 2 h. Digestion products were visualized on a 3 % agarose gel. Wild-type allele produced double band at 269 and 16 bp and mutant allele produced three bands at 165, 104 and 16 bp.

Table 1. C-peptide/insulin molar ratio in family members

Patient

OGTT (1) on admission

Time (min)	0	30	60	90	120
PG (mg/dL)	124	224	263	280	262
IRI (μU/mL)	65.7	114.8	191.5	279.1	290.1
CPR (ng/mL)	3.18	4.62	7.01	9.80	10.00
CPR/IRI molar ratio	2.21	1.84	1.67	1.60	1.57

OGTT (2) 2 weeks after admission

Time (min)	0	30	60	90	120
PG (mg/dL)	85	190	224	220	202
IRI (μU/mL)	52.7	136.9	187.4	239.2	313.6
CPR (ng/mL)	2.68	5.80	8.00	8.99	10.50
CPR/IRI molar ratio	2.32	1.93	1.95	1.72	1.53

Father

OGTT

Time (min)	0	30	60	90	120
PG (mg/dL)	88	169	256	214	172
IRI (μU/mL)	10.1	37.0	100.5	108.1	110.5
CPR (ng/mL)	1.24	3.14	6.96	8.05	8.22
CPR/IRI molar ratio	5.60	3.87	3.16	3.40	3.40

Grandmother

Fasting time	
PG (mg/dL)	146
IRI (μU/mL)	30.1
CPR (ng/mL)	2.51
CPR/IRI molar ratio	3.84

Results

An OGTT revealed a diabetic pattern with hyperinsulinemia (Table 1). The homeostasis model assessment of insulin resistance (HOMA-IR), an index of insulin resistance, was 20.1. The C-peptide/insulin molar ratio was extremely low. The fasting and 120 min levels were 2.21 and 1.57, respectively (normal level of fasting is 4.0<). An insulin tolerance test (0.1U/kg insulin i.v.) showed insulin resistance with only 37 % reduction in plasma glucose levels. Metformin was started from 250 mg/day and increased up to 500 mg/day. HbA1c levels improved to 5-6 % six months later. At that point in time, her fasting proinsulin level was 71.7 pmol/L when the IRI level was 49.1 μg/mL. Proinsulin/insulin molar ratio was 0.24 (normal 0.1-0.2). Her insulin levels were still high; however, the acanthosis nigricans had disappeared after she had regained diabetic control.

Her clinical course suggested two genetic diseases of glucose metabolism. One was the insulin gene mutation, as characterized by a low level C-peptide/insulin molar ratio, and sometimes presents as type 2 DM. The other was the insulin receptor gene mutation, which clinically demonstrated type A insulin resistance.

A sequencing analysis of the 22 exons as well as the intron-exon junctions identified a heterozygous mutation at nucleotide position 1072 substituting a termination codon for arginine 331, a conserved amino acid in the insulin-like growth factor I receptor and insulin receptor-related receptor, in the putative receptor L2 domain of the patient's insulin receptor (Fig. 1) [19]. No other mutations were found in any of the insulin receptor genes analyzed in this study.

Her father and grandmother also had the same

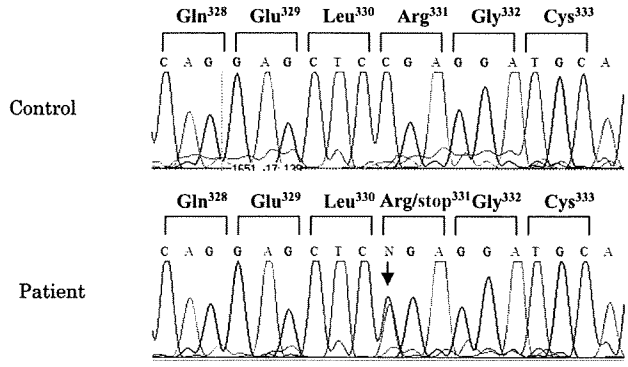

Fig. 1. Partial nucleotide sequence of the insulin receptor gene in the patient. Sequence from the patient is shown in comparison with that from the control. The patient is heterozygous for a mutation at the nucleotide position 1072, converting Arg 331(CGA) to a termination codon (TGA). An arrow indicates the position of mutation.

heterozygous mutation (data not shown). The fasting C-peptide/insulin molar ratio of her grandmother was relatively low under the treatment of sulfonylureas (3.84, IRI; 30.1 μU/mL, CPR 2.51 ng/mL). The HbA1c level of her father was 4.7 %, but OGTT showed impaired glucose tolerance (Table1). Although the fasting insulin level was 10.1 μU/mL, it increased up to 100 μU/mL in 60 -120 min. The C-peptide/insulin molar ratio was 5.60 in fasting and 3.40 in 120 min. They showed milder insulin resistance in comparison to the proband. The heterozygous mutation seemed to significantly affect the insulin resistance of the three subjects, even if no typical skin lesions were observed in either the father or grandmother.

One unrelated case of leprechaunism with R331X homozygous mutation was identified in Tokyo, Japan. The patient was born to unrelated parents at 39 weeks of gestation with a birth weight of 1743 g. She showed an extreme degree of insulin resistance (FPG, 200 mg/dL<; IRI 10,000 μU/mL<). She thereafter started to receive subcutaneous injections of recombinant human IGF-I. After treatment, her glucose metabolic abnormality was improved. Informed consent was obtained from her parents for sequence analysis. Her parents had R331X heterozygous mutation. They did not demonstrate any symptoms of diabetes mellitus. Information on the glucose tolerance including OGTT was unavailable.

These findings led to the hypothesis that insulin receptor genetic variants contribute to the variation of DM in the general population in Japan. An extensive search was done in 272 participants in a group medical examination that included 92 healthy cases of normoglycemia and 180 cases already diagnosed as type 2 DM or detected hyperglycemia. The search, however, failed to detect any R331X mutation in this local population.

Discussion

Type A insulin resistance was initially characterized in young female patients with acanthosis nigricans, ovarian hyperandrogenism and virilization [20]. Over 30 mutations have so far been described in these patients, which are mainly clustered in the tyrosine kinase domain of the insulin receptor [21, 22].

A nonsense mutation was identified in one allele of a patient substituting the termination codon (TGA) for the CGA codon normally encoding Arg^{331} located in a putative L2 domain, which is a single stranded right-hand beta-helix and is suggested to make up the bilobal ligand binding site [23]. The nonsense mutation at codon 331 truncated the C-terminal half of the receptor α subunit as well as the entire β subunit including the transmembrane anchor and the tyrosine kinase domain. Therefore, it is unlikely that this truncated receptor, translated from the mutant allele, would be either functional or located on the cell surface. In fact, extreme insulin resistance was observed in a female leprechaunism patient with homozygous R331X alleles.

Hyperinsulinemia is usually considered to be the result of resistance to the physiological effects of insulin and consequent compensatory increased insulin secretion. Recently, the C-peptide/insulin ratio is widely used as a surrogate of hepatic insulin clearance for the evaluation in type 2 DM or glucose intolerance [24, 25]. This index, should clarify whether impaired hepatic insulin clearance or increased insu-

lin secretion has a dominant effect on such patients. Insulin and C-peptide are secreted into the portal vein in a 1:1 molar ratio after β-cell stimulation by carbohydrate or other secretagogues. A large fraction of endogenous insulin is cleared by the liver, whereas C-peptide, which is cleared primarily by the kidney and has a lower metabolic clearance rate than insulin, and traverses the liver with essentially no extraction by hepatocytes [26, 27]. Diminished insulin clearance has been demonstrated to be an important underlying mechanism for the hyperinsulinemia found in various insulin-resistant conditions [28-30]. For example, to evaluate hyperinsulinemia in African Americans, at risk for type 2 DM, several studies used C-peptide/insulin molar ratio as an index of hepatic insulin clearance. African American children and adults showed lower C-peptide/insulin ratio than White Americans, thus suggesting that high insulin levels could be partly attributed to lower clearance [31, 32].

Therefore, the use of the C-peptide/insulin molar ratio reflects of hepatic insulin clearance [33]. A low C-peptide/insulin molar ratio of our patients suggests impaired hepatic insulin clearance because of, not only DM, but also abnormal insulin receptor expression in the liver. To this day, a low C-peptide/insulin molar ratio has not been substantially observed among individuals with type A insulin resistance. Two family cases with an insulin receptor gene mutation reported the presence of a low C-peptide/insulin molar ratio [34, 35]. They showed hyperinsulinemic hypoglycemia, severe insulin resistance and the C-peptide/insulin molar ratio ranged from 1.1 to 3.8.

As well as this reported cases, the molar ratio of the proband of our family was very low similar to that observed in subjects with insulin gene mutation. Previously, low C-peptide/insulin ratio was well reported to be a clinical feature of mutations in the human insulin gene causing either familial hyperinsulinemia or familial hyperproinsulinemia. The elevated circulating IRI consisted mainly of the unprocessed mutated proinsulin, which had accumulated because of proinsulin's relatively low clearance compared with insulin. In these subjects, proinsulin levels were tends to be extremely high, namely over three hundred pmol/L [36, 37]. Due to dramatic improvements in the assay techniques of IRI, cross-reactivity with proinsulin is normally seen at very low levels. Consequently, there have been no new reports regarding hyperproinsulinemia with insulin gene mutations for the last decade.

Recently, the fasting proinsulin/insulin ratio is used as a marker of β-cell dysfunction. In peripheral blood, fasting proinsulin accounts for 10-20% of insulin but it may reach values as high as 50 % in type 2 DM. Taura *et al.* evaluated the basal and dynamic proinsulin-insulin relationship to assess the β-cell function during OGTT in type 2 DM [38]. The proinsulin/insulin molar ratio was higher in type 2 DM (0.39 ± 0.05) subjects than normal (0.14 ± 0.01) and impaired glucose-tolerant (0.13 ± 0.02) subjects. In comparison to this study, the fasting proinsulin/insulin ratio of the proband, 0.20 was slightly higher than normal. It is difficult to consider that her low C-peptide/insulin molar ratio is derived from structural abnormalities in the proinsulin molecule.

We calculated the C-peptide/insulin molar ratio of several previous cases with insulin receptor gene mutation from data measured simultaneously. Severe cases, Rabson-Mendenhall syndrome or Donohue's syndrome, showed very low level (0.69 to 1.83) [14, 39-41]. Milder cases, type A insulin resistance or DM, also showed relatively low molar ratio (1.47 to 4.26) [35, 42]. However, most previous case reports only recorded the IRI data, more investigations are needed to discuss these clinical characteristics.

Interestingly, the patient's father did not show hyperinsulinemia while demonstrating a normal C-peptide/insulin molar ratio after fasting. However, after oral glucose ingestion, the insulin level increased 100.5 μU/mL at 60 min and the molar ratio gradually decreased from 5.60 to 3.40. Meier *et al.* studied the C-peptide/insulin molar ratio as calculated at singular time points after oral glucose administration in non-diabetic subjects [37]. They reported that the molar ratio decreased to half level at 30 minutes and then it gradually increased up to the initial level through 120 min. In contrast to their data, the proband and her father showed a gradually decreasing pattern from 0 to 120 minutes. Receptor-mediated insulin endocytosis and degradation in hepatocyte underlie the basic mechanism of insulin clearance. Insulin is targeted for degradation after internalization, whereas the receptor recycles back to the cell surface [43]. CEACAM1, a transmembrane glycoprotein, plays a significant role in receptor-mediated insulin endocytosis [44]. *In vitro* studies suggest that upon its phosphorylation by the insulin receptor kinase, CEACAM1 binds indirectly to the receptor to undergo internalization in clathrin-coated vesicles as part of endocytosis complex [45].

CEACAM1 is considered to interact with two separate domains of the insulin receptor: a C-terminal for its phosphorylation, and cytoplasmic juxtamembrane domain required for internalization [46]. R331X mutant defects these important domains for endocytosis of insulin-insulin receptor complex. A reduction of endocytosis may also affect recycle of insulin receptor and may cause prolonged low hepatic extraction after glucose oral load observed in subjects having R331X mutation. Although her father showed normal data in fasting period, the oral glucose test may be a supplementary means for evaluating of insulin receptor mutant subjects.

As stated above, C-peptide is believed to be a better index of the pancreatic β-cell function than insulin because C-peptide levels are unaffected by hepatic clearance. When comparing the father's C-peptide levels of OGTT with proband, only a slight difference was observed. This result indicates that the insulin secreting function of β-cell is not substantially different and the cause of hyperinsulinemia in the proband is dominantly affected by impaired hepatic insulin clearance. The evaluation of the C-peptide/insulin molar ratio is thus considered to be a useful index for identifying genetic insulin resistance patients. On the other hand, a mild phenotype such as that observed in her father may not be effectively evaluated by the fasting data alone.

Unrelated Japanese patients with another mutation of the insulin receptor gene have been previously reported. They showed different phenotypes: one was detected as a heterozygous mutation in type A insulin resistance, while the other was detected as a compound heterozygous mutation in leprechaunism, thus indicating that the severity of such mutations will determine the phenotype [47]. The phenotype of heterozygous R331X differed substantially among the current family members. Although the proband and her grandmother showed diabetes mellitus with insulin resistance, the difference in the age of onset was around forty years. In addition, her father did not show insulin resistance after fasting. The reason for this difference may be conditioned by heredity and environment. The lifestyle for children has changed over the last few decades in Japan. The proband often consumed high caloric foods before detecting glucosuria. Numerous genetic factors related to diabetes mellitus have also been investigated. The insulin receptor pathway plays an important role in the glucose metabolism. The phenotype of a homozygous mutation, leprechaunism, revealed this important function in humans. However, a heterozygous mutation including Type A insulin resistance shows a mild phenotype. Variance in the current family case suggests that various genetic factors may therefore have played a role in their glucose metabolism. Contrary to expectations, the hypothesis that R331X determines the phenotype for glucose tolerance in Japanese people was ruled out. In addition, the influence of other reported mutations was unclear.

In conclusion, a nonsense mutation causing premature termination after amino acid 331 in the α subunit of the insulin receptor was identified in Japanese diabetes patients. The phenotype of R331X showed variety, and therefore further investigations, including determination of the mRNA level as well as ligand binding and receptor autophosphorylation, are thus called for to address the molecular mechanism by which this mutation leads to the occurrence of diabetes, as was observed in the current patient. In addition, the C-peptide/insulin molar ratio is considered to be a useful index for identifying genetic insulin resistance patients.

Acknowledgement

The study was supported in part by the Grants-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology and the Global Center of Excellence (COE) program of Japan.

References

1. Massague J, Pilch PE, Czech MP (1980) Electrophoretic resolution of three major insulin receptor structures with unique subunit stoichiometries. *Proc Natl Acad Sci USA* 77: 7137-7141.
2. Kasuga M, Hedo JA, Yamada KM, Kahn CR (1982) The structure of insulin receptor and its subunits. Evidence for multiple nonreduced forms and a 210,000 possible proreceptor. *J Biol Chem 257*: 10392-10399.
3. Christiansen K, Tranum-Jensen J, Carlsen J, Vinten J (1991) A model for quaternary structure of human pla-

cental insulin receptor deduced from electron microscopy. *Proc Natl Acad Sci USA* 88: 249-252.
4. Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, TDull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M, *et al.* (1985) Human insulin receptor and its relationship to tyrosine kinase family of oncogenes. *Nature* 313: 756-761.
5. Ebina Y, Ellis L, Jarnagin K, Edery M, Graf L, Clauser E, Ou JH, Masiarz F, Kan YW, Goldfine ID, *et al.* (1985) The human insulin receptor cDNA: the structural basis for hormone-activated transmembrane signaling. *Cell* 40: 747-758.
6. Kasuga M, Fujita-Yamaguchi Y, Blithe DL, Kahn CR (1983) Tyrosine-specific protein kinase activity is associated with the purified insulin receptor. *Proc Natl Acad Sci USA* 80: 2137-2141.
7. Rosen OM (1987) After insulin binds. *Science* 237: 1452-1458.
8. Taylor SI, Kadowaki T, Kadowaki H, Accili D, Cama A, McKeon C (1990) Mutations in insulin receptor gene in insulin-resistant patients. *Diabetes Care* 13: 257-279.
9. Taylor SI, Cama A, Accili D, Barbetti F, Quon MJ, de la Luz Sierra M, Suzuki Y, Koller E, Levy-Toledano R, Wertheimer E, *et al.* (1992) Mutation in the insulin receptor gene. *Endocri Rev* 13: 565-595.
10. Taylor SI (1992) Lessons from patients with mutation in the insulin-receptor gene. *Diabetes* 41: 1473-1490.
11. Taira M, Taira M, Hashimoto N, Shimada F, Suzuki Y, Kanatsuka A, Nakamura F, Ebina Y, Tatibana M, Makino H, *et al.* (1989) Human diabetes associated with a deletion of the tyrosine kinase domain of the insulin receptor. *Science* 245: 63-66.
12. Taylor SI, Accili D, Cama A, Kadowaki H, Kadowaki T, Imano E, Sierra ML (1991) Mutations in the insulin receptor gene in patients with genetic syndromes of insulin resistance. *Adv Exp Med Biol* 293: 197-213.
13. Longo N, Langley SD, Griffin LD, Elsas LJ (1992) Reduced mRNA and a nonsense mutation in the insulin receptor gene produce heritable severe insulin resistance. *Am J Hum Genet* 50: 998-1007.
14. Longo N, Wang Y, Smith SA, Langley SD, DiMeglio LA, Giannella-Neto D (2002) Genotype-phenotype correlation in inherited severe insulin resistance. *Hum Mol Genet* 11: 1465-1475.
15. Longo N, Singh R, Griffin LD, Langley SD, Parks JS, Elsas LJ (1994) Impaired growth in Rabson-Mendenhall syndrome: Lack of effect of growth hormone and insulin-like growth factor I. *J Clin Endocrinol Metab* 79: 799-805.
16. Kahn CR, Flier JS, Bar RS, Archer JA, Gorden P, Martin MM, Roth J (1976) The syndromes of insulin resistance and acanthosis nigricans. Insulin-receptor disorders in man. *N Eng J Med* 294: 739-745.
17. Bell GI, Picter RL, Rutter WJ, Cordell B, Tischer E, Goodman HM (1980) Sequence of the human insulin gene. *Nature* 284: 26-32.
18. Barbetti F, Gejiman PV, Taylor SI, Raben N, Cama A, Bonora E, Pizzo P, Moghetti P, Muggeo M, Roth J (1992) Detection of mutations in insulin receptor gene by denaturing gradient gel electrophoresis. *Diabetes* 41: 408-415.
19. Shier P, Watt VM (1983) Primary structure of a putative receptor for a ligand of the insulin family. *J Biol Chem* 264: 359-399.
20. Tritos NA, Mantzoros CS (1998) Syndromes of severe insulin resistance. *J Clin Endocr Metab* 83: 3025-3030.
21. Kadowaki H, Kadowaki T, Cama A, Marcus-Samuel B, Rovira A, Bevins CL, Taylor SI (1990) Mutagenesis of Lysine 460 in the human insulin receptor. *J Biol Chem* 265: 21285-21296.
22. Moller DE, Yokota A, White MF, Pazianos AG, Flier JS (1990) A naturally occurring mutation of insulin receptor alanine 1134 impaires tyrosine kinase function and is associated with dominantly inherited insulin resistance. *J Biol Chem* 265: 14979-14985.
23. Garrett TPJ, McKern NM, Lou M, Frenkel MJ, Bentley JD, Lovrecz GO, Elleman TC, Cosgrove LJ, Ward CW (1998) Crystal structure of the first three domains of the type-1 insulin-like growth factor receptor. *Nature* 394: 395-399.
24. Meier JJ, Holst JJ, Schmid WE, Nauck MA (2007) Reduction of hepatic insulin clearance after oral glucose ingestion is not mediated by glucagon-like peptide 1 or gastric inhibitory polypeptide in humans. *Am J Physiol Endocrinol Metab* 293: E846-E856.
25. Osei K, Rhinesmith S, Gaillard T, Schuster D (2003) Metabolic effects of chronic glipizide gastrointestinal therapeutic system on serum glucose, insulin secretion, insulin sensitivity, and hepatic insulin extraction in glucose-tolerant, first degree relatives of African American patients with type 2 diabetes: new insights on mechanisms of action. *Metabolism* 52: 565-572.
26. Horwitz DL, Starr JI, Mako ME, Blackard WG, Rubenstein AH (1975) Proinsulin, insulin, and C-peptide concentrations in human portal and peripheral blood. *J Clin Invest* 55: 1278-1283.
27. Shapiro ET, Tillil H, Miller MA, Frank BH, Galloway JA, Rubenstein AH, Polonsky KS (1987) Insulin secretion and clearance. Comparison after oral and intravenous glucose. *Diabetes* 36: 1365-1371.
28. Polonsky KS, Given BD, Hirsch L, Shapiro ET, Tillil H, Beebe C, Galloway JA, Frank BH, Karrison T, Van Ceuter E (1988) Quantitative study of insulin secretion and clearance in normal and obese subjects. *J Clin Invest* 81: 435-441.
29. Jones CNO, Pei D, Staris P, Polonsky KS, Ida Chen YD, Reaven GM (1997) Alternations in the glucose-stimulated insulin secretory dose-response curve and in insulin clearance in nondiabetic insulin-resistant indi-

viduals. *J Clin Endocrinol Metab* 82: 1834-1838.
30. Inokuchi T, Watanabe K, Kameyama H, Orita M (1988) Altered basal C-peptide/insulin molar ratio in obese patients with fatty liver. *Jpn J Med* 27: 272-276.
31. Arslanian SA, Saad R, Lewy V, Danadian K, Janosky J (2002) Hyperinsulinemia in African-American Children. *Diabetes* 51: 3014-3019.
32. Osei K, Schster DP, Owusu SK, Amoah AG (1996) Race and ethnicity determine serum insulin and C-peptide concentrations and hepatic insulin extraction and insulin clearance: comparative studies of three populations of West African ancestry and white Americans. *Metabolism* 46: 53-58.
33. Polonsky KS, Rubenstein AH (1984) C-peptide as a measure of the secretion and hepatic extraction of the insulin. Pitfalls and limitations. *Diabetes* 33: 486-494.
34. Højlund K, Hansen T, Lajer M, Henriksen JE, Levin K, Lindholm J, Pedersen O, Beck-Nielsen H (2004) A novel syndrome of autosomal-dominant hyperinsulinemic hypoglycemia linked to a mutation in the human insulin receptor gene. *Diabetes* 53: 1592-1598.
35. Huang Z, Li Y, Tang T, Xu W, Liao Z, Yao B, Hu G, Weng J (2009) Hyperinsulinaemic hypoglycemia associated with a heterozygous missense mutation of R1174W in the insulin receptor (IR) gene. *Clin Endocrinol* 71: 659-665.
36. Røder ME, Vissing H, Nauck MA (1996) Hyperproinsulinemia in a three-generation Caucasian family due to mutant proinsulin (Arg65→His) not associated with impaired glucose tolerance: the contribution of mutant proinsulin to insulin bioactivity. *J Clin Endocrinol Metab* 81: 1634-1640.
37. Meier JJ, Gallwitz B, Siepmann N, Holst JJ, Deacon CF, Schmidt WE, Nauck MA (2003) The reduction in hepatic insulin clearance after oral glucose is not mediated by gastoric inhibitory polypeptide (GIP). *Regul Pept* 113: 95-100.
38. Taura A, Pacini G, Kautzky-Willer A, Ludvik B, Prager R, Thomaseth K (2003) Basal and dynamic proinsulin-insulin relationship to assess β-cell function during OGTT in metabolic disorders. *Am J Physiol Endocrinol Metab* 285: E155-E162.
39. Thiel CT, Knebel B, Knerr I, Sticht H, Müller-Wieland D, Zenker M, Reis Andre, Dörr HG, Rauch A (2008) Two novel mutations in the insulin binding impairment in a patient with Rabson-Mendenhall syndrome. *Mol Genet and Metab* 94: 356-362.
40. Unal S, Aycan Z, Halsall DJ, Kibar AE, Eker S, Ozaydin E (2009) Donohue syndrome in a neonate with homozygous deletion of exon 3 of the insulin receptor gene. *J Pediatr Endocrinol Metab* 22: 669-674.
41. Tuthill A, Semple RK, Day R, Soos MA, Sweeney E, Seymour PJ, Didi M, O'Ragilly S (2007) Functional characterization of a novel insulin receptor mutation contributing to Rabson-Mendenhall syndrome. *Clin Endocrinology* 66: 21-26.
42. Riqué S, Nogués C, Ibáñez L, Marcos MV, Ferragut J, Carrascosa A, Potau N (2000) Identification of three novel mutations in the insulin receptor gene in type A insulin resistant patients. *Clin Genet* 57: 67-69.
43. Carpentier JL (1993) Robert Feulgen Prize Lecture 1993. The journey of the insulin receptor into the cell: from cellular biology to pathophysiology. *Histochemistry* 100: 169-184.
44. Poy MN, Yang Y, Rezaei K, Fernström MA, Lee AD, Kido Y, Erickson SK, Najjar SM (2002) *Nature Genet* 30: 270-276.
45. Choice CV, Howard MJ, Poy MN, Hankin MH, Najjar SM (1998) *J Biol Chem* 273: 22194-22200.
46. Najjar SN (2002) Regulation of insulin action by CEACAM1. *Trends Endocrinol Metab* 13: 240-245.
47. Kadowaki H, Takahashi Y, Ando A, Momomura K, Kaburagi Y, Quin JD, MacCuish AC, Koda N, Fukushima Y, Taylor SI, Akanuma Y, Yazaki Y, Kadowaki T (1997) Four mutant alleles of the insulin receptor gene associated with genetic syndromes of extreme insulin resistance. *Biochem. Biophys. Res. Commun* 237: 516-520.

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

International Diabetes Federation

Case report

A case of insulinoma following total gastrectomy—Effects of an alpha-glucosidase inhibitor on suppressing GIP and GLP-1 elevations☆

Takehiro Sato[a], *Takuma Narita*[a], *Mihoko Hosoba*[a], *Masafumi Kakei*[a], *Hiroshi Nanjo*[b], *Hiroshi Uchinami*[c], *Kohei Satoh*[c], *Yuzo Yamamoto*[c], *Yuichiro Yamada*[a,*]

[a] *Department of Endocrinology, Diabetes and Geriatric Medicine, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan*

[b] *Department of Clinical Pathology, Akita University Hospital, Akita, Japan*

[c] *Department of Gastroenterological Surgery, Akita University Graduate School of Medicine, Akita, Japan*

ARTICLE INFO

Article history:
Received 11 September 2009
Received in revised form
18 December 2009
Accepted 4 January 2010
Published on line 1 February 2010

Keywords:
Incretin
GLP-1
GIP
Insulinoma
Alpha-glucosidase inhibitor
Gastrectomy

ABSTRACT

A 61-year-old woman with fasting hypoglycemia following total gastrectomy was diagnosed as insulinoma. GIP and GLP-1 levels after a mixed meal were extremely increased. Administration of miglitol, an alpha-glucosidase inhibitor, suppressed the GIP and GLP-1 elevations.

1. Introduction

Hypoglycemia following gastric surgery has been reported. Dumping syndrome after gastrectomy was described by Mix in 1922 [1], and nesidioblastosis and insulinoma were described by Service et al. in 2005 [2]. Over-secretion of gut hormones, gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) is at least partly responsible for the hyperinsulinemic hypoglycemia developing after gastric surgery.

Alpha-glucosidase inhibitors (AGIs) limit the metabolism of disaccharide to monosaccharide and have been developed for treating diabetic patients in the clinical setting. It has been reported that administration of AGIs could effectively control dumping syndrome [3]. Subsequent studies revealed that AGIs

☆ The study was supported in part by the Grants-in-Aid from the Japanese Ministry of Education, Culture, Sports, Science and Technology and Global Center of Excellence (COE) program of Japan.

* *Corresponding author.* Tel.: +81 18 884 6768; fax: +81 18 884 6768.
E-mail address: yamada@gipc.akita-u.ac.jp (Y. Yamada).

0168-8227/$ – see front matter
doi:10.1016/j.diabres.2010.01.006

suppress GIP secretion but increase GLP-1 secretion in type 2 diabetic patients [4]. However, to our knowledge, effects of AGIs on GIP and GLP-1 levels in patients following total gastrectomy have not been examined.

In this paper, we have presented a patient, who suffered from insulinoma after total gastrectomy. She showed markedly elevated levels of both GIP and GLP-1 which could be suppressed with an AGI.

2. Case report

A 61-year-old woman was admitted to our hospital because of fasting hypoglycemia. She underwent total gastrectomy 10 years ago due to gastric cancer. She had developed postprandial hypoglycemia thereafter and had been treated as dumping syndrome. Her symptoms of hypoglycemia had been improved by an administration of prednisolone [5]. Prednisolone could be gradually decreased and she was doing well without steroid. However, 3 years after steroid withdrawal, she experienced early morning unconsciousness that was improved after taking sugar.

Serum insulin (IRI) level was 3.7 μU/ml and serum C-peptide (CPR) level was 1.0 ng/ml when her plasma glucose level (PG) was 34 mg/dl. Tuner index (IRI × 100/PG-30) [6] was 92.5, which suggested that she had insulinoma. However, none of computerized tomography, ultrasonography and magnetic resonance imaging demonstrated pancreatic tumors. Therefore, arterial stimulation and venous sampling (ASVS) study was carried out. After injecting calcium gluconate (0.025 mEquiv./kg) into each of the superior mesenteric, common hepatic, gastroduodenal and splenic arteries, blood samples were collected from the right hepatic vein at 30, 60, and 120 s after injection. A remarkable increase of IRI levels was observed following the stimulation of proximal splenic artery. Although none of imaging examinations could reveal a responsible lesion, the ASVS study strongly implied an existence of insulinoma in the body of the pancreas, and therefore she underwent surgery. Intraoperative ultrasonographic examination revealed a 6-mm hypoechoic nodule in the body of the pancreas. Although this small nodule seemed to be a responsible lesion, we performed distal pancreatectomy including this nodule because we could not still rule out a possibility of nesidioblastosis. Histological examination

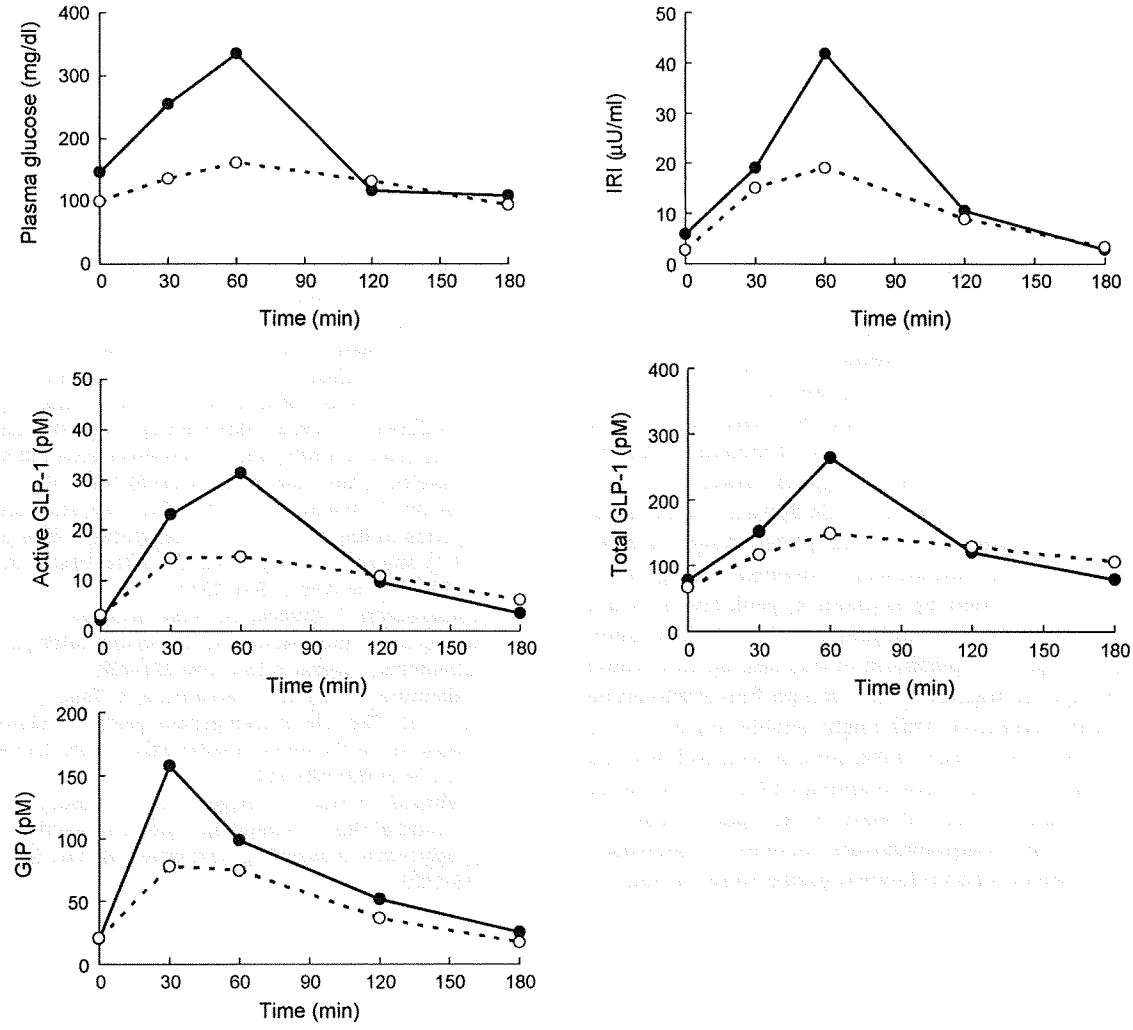

Fig. 1 – Plasma glucose, insulin, GIP, active and total GLP-1 levels after mixed meal test. The patient was ingested a mixed meal before (solid line) and after (broken line) administration of miglitol.

showed a solitary insulinoma and the symptom of hypoglycemia has disappeared thereafter.

After surgery, we measured plasma total GIP and GLP-1 (active and total forms) levels as well as PG and IRI levels, at 0, 30, 60, 120, 180 min after ingestion of a mixed meal (JANEF E460F18 56.5 g of carbohydrates, 18 g of protein, 18 g of fat, Q.P. Corporation, Tokyo, Japan). The patient showed markedly elevated secretion of GIP (peak at 30 min, 158 pM) and GLP-1 (peak at 60 min, active 31.3 pM, total 263 pM). We next examined the effects of miglitol, an alpha-glucosidase inhibitor. Following administration of miglitol three times a day for 14 days, peak levels of both GIP and GLP-1 were decreased (GIP, 77.6 pM; active GLP-1, 14.7 pM; total GLP-1, 148 pM) (Fig. 1).

3. Discussion

Adult hyperinsulinaemic hypoglycemia is usually caused by insulinoma. PG/IRI ratio was 49.1, IRI/PG ratio was 0.02 and Tuner index was 3.8 when she was diagnosed as dumping syndrome [5]. This time, PG/IRI ratio was decreased and both IRI/PG ratio and Turner index were increased, indicating that hyperinsulinemic hypoglycemia had developed for 10 years. The relationship between gastrectomy and either insulinoma or nesidioblastosis was not clear at present, but basic studies demonstrated that both GIP and GLP-1 stimulate cell proliferation and inhibit apoptosis of pancreatic beta cells [7–9]. Clinical studies showed that both GIP and GLP-1 are increased after gastric surgery [10–12]. In addition, it was reported that secretion of GIP and GLP-1 is exaggerated in patients with dumping syndrome [13]. These studies suggested that GIP and GLP-1 are at least partly responsible for the development of insulinoma, nesidioblastosis, or late dumping syndrome and that suppression of GIP and GLP-1 levels might be important for preventing these states after gastric bypass surgery.

We reported that administration of miglitol to type 2 diabetic patients suppressed postprandial GIP levels and increased postprandial GLP-1 levels [4]. Miglitol restrains glucose absorption in the upper portion of intestine, and the amount of glucose that passes through the lower portion of intestine is increased. Therefore, L cells that exist a lot in the lower portion of intestine could be stimulated to secrete more GLP-1. However, in our patient who underwent total gastrectomy with reconstruction by Roux-en-Y, peak levels of not only GIP but also GLP-1 were suppressed after administration of miglitol. A possible explanation to this discrepancy would be rapid passages of miglitol to the lower portion of intenstine without gastric retension. This might enable miglitol to be effective in the lower portion of intestine as well and resulted in the limited secretion of postprandial GLP-1. On the other hand, K cells that secrete GIP exist in the upper portion of intestine. Therefore postprandial GIP secretion is decreased either in the patient who underwent gastrectomy or not.

Conflict of interest

The authors declare that they have no conflict of interest.

REFERENCES

[1] C.L. Mix, "Dumping stomach" following gastrectomy, Clin. North Am. 2 (1922) 617–622.
[2] G.J. Service, G.B. Thompson, F.J. Service, J.C. Andrews, M.L. Collazo-Clavell, R.V. Lloyd, Hyperinsulinemic hypoglycemia with nesidioblastosis after gastric-bypass surgery, N. Engl. J. Med. 353 (2005) 249–254.
[3] A. Imhof, M. Schneemann, A. Schaffner, M. Brändle, Reactive hypoglycaemia due to late dumping syndrome: successful treatment with acarbose, Swiss Med. Wkly 131 (2001) 81–83.
[4] T. Narita, Y. Katsuura, T. Sato, M. Hosoba, H. Fujita, T. Morii, et al., Miglitol induces prolonged and enhanced glucagon-like peptide-1 and reduced gastric inhibitory polypeptide responses after ingestion of a mixed meal in Japanese Type 2 diabetic patients, Diabet. Med. 26 (2009) 87–88.
[5] C. Shibata, Y. Funayama, K. Fukushima, K. Shiiba, I. Sasaki, K. Itoi, et al., Effect of steroid therapy for late dumping syndrome after total gastrectomy: report of a case, Dig. Dis. Sci. 49 (2004) 802–804.
[6] R.C. Turner, N.W. Oakley, J.D. Nabarro, Control of basal insulin secretion, with special reference to the diagnosis of insulinomas, Br. Med. J. 2 (1971) 132–135.
[7] L. Farilla, A. Bulotta, B. Hirshberg, S. Li Calzi, N. Khoury, H. Noushmehr, et al., Glucagon-like peptide 1 inhibits cell apoptosis and improves glucose responsiveness of freshly isolated human islets, Endocrinology 144 (2003) 5149–5158.
[8] J.F. List, J.F. Habener, Glucagon-like peptide 1 agonists and the development and growth of pancreatic beta-cells, Am. J. Physiol. Endocrinol. Metab. 286 (2004) E875–881.
[9] S.J. Kim, C. Nian, S. Widenmaier, C.H. McIntosh, Glucose-dependent insulinotropic polypeptide-mediated up-regulation of beta-cell antiapoptotic Bcl-2 gene expression is coordinated by cyclic AMP (cAMP) response element binding protein (CREB) and cAMP-responsive CREB coactivator 2, Mol. Cell. Biol. 28 (2008) 1644–1656.
[10] J. Miholic, C. Orskov, J.J. Holst, J. Kotzerke, H.J. Meyer, Emptying of the gastric substitute, glucagon-like peptide-1 (GLP-1), and reactive hypoglycemia after total gastrectomy, Dig. Dis. Sci. 36 (1991) 1361–1370.
[11] J.J. Andreasen, C. Orskov, J.J. Holst, Secretion of glucagon-like peptide-1 and reactive hypoglycemia after partial gastrectomy, Digestion 55 (1994) 221–228.
[12] J. Takemura, Y. Seino, T. Yamamura, K. Tsuda, S. Seino, N. Itoh, et al., The role of endogenous gastric inhibitory polypeptide in the enteroinsular axis, J. Clin. Endocrinol. Metab. 54 (1982) 909–913.
[13] B. Gebhard, J.J. Holst, C. Biegelmayer, J. Miholic, Postprandial GLP-1, norepinephrine, and reactive hypoglycemia in dumping syndrome, Dig. Dis. Sci. 46 (2001) 1915–1923.

BASIC RESEARCH www.jasn.org

Reduction of Renal Superoxide Dismutase in Progressive Diabetic Nephropathy

Hiroki Fujita,*† Hiromi Fujishima,* Shinsuke Chida,‡ Keiko Takahashi,† Zhonghua Qi,† Yukiko Kanetsuna,§ Matthew D. Breyer,† Raymond C. Harris,† Yuichiro Yamada,* and Takamune Takahashi†

*Division of Endocrinology, Metabolism and Geriatric Medicine, and ‡Bioscience Education Research Center, Akita University School of Medicine, Akita, Japan; †Division of Nephrology and Hypertension, Vanderbilt University Medical Center, Nashville, Tennessee; and §Division of Pathology, Jikei University School of Medicine, Tokyo, Japan

ABSTRACT

Superoxide excess plays a central role in tissue damage that results from diabetes, but the mechanisms of superoxide overproduction in diabetic nephropathy (DN) are incompletely understood. In the present study, we investigated the enzyme superoxide dismutase (SOD), a major defender against superoxide, in the kidneys during the development of murine DN. We assessed SOD activity and the expression of SOD isoforms in the kidneys of two diabetic mouse models (C57BL/6-Akita and KK/Ta-Akita) that exhibit comparable levels of hyperglycemia but different susceptibility to DN. We observed down-regulation of cytosolic CuZn-SOD (SOD1) and extracellular CuZn-SOD (SOD3), but not mitochondrial Mn-SOD (SOD2), in the kidney of KK/Ta-Akita mice which exhibit progressive DN. In contrast, we did not detect a change in renal SOD expression in DN-resistant C57BL/6-Akita mice. Consistent with these findings, there was a significant reduction in total SOD activity in the kidney of KK/Ta-Akita mice compared with C57BL/6-Akita mice. Finally, treatment of KK/Ta-Akita mice with a SOD mimetic, tempol, ameliorated the nephropathic changes in KK/Ta-Akita mice without altering the level of hyperglycemia. Collectively, these results indicate that down-regulation of renal SOD1 and SOD3 may play a key role in the pathogenesis of DN.

J Am Soc Nephrol 20: 1303–1313, 2009. doi: 10.1681/ASN.2008080844

Diabetic nephropathy (DN) is the leading cause of end-stage renal disease. Although hyperglycemia is clearly a prerequisite for the development of DN, alone it is insufficient for its development. Epidemiologic studies demonstrate only 10% to 40% of all diabetic patients get DN, despite comparable levels of glucose control in those subjects developing DN versus spared. In addition, sibling studies show a strong familial component for the risk of developing persistent proteinuria, suggesting a genetic basis for DN risk.[1,2] However, the molecular or cellular mechanisms coupled with the genetic susceptibility to DN are incompletely understood.

There is compelling evidence that superoxide excess induced by diabetic hyperglycemia plays a central role in diabetic vascular cell damage.[3] High glucose flux increases the production of superoxide anion ($O_2^{\bullet -}$) by mitochondrial electron-transport chain, and the overproduced superoxide enhances the major pathways of hyperglycemic vascular cell

Received August 12, 2008. Accepted February 2, 2009.

Published online ahead of print. Publication date available at www.jasn.org.

Correspondence: Dr. Hiroki Fujita, Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan. Phone: +81-18-884-6040; Fax: +81-18-884-6449; E-mail: hirofuji@gipc.akita-u.ac.jp; or Dr. Takamune Takahashi, Division of Nephrology and Hypertension, Vanderbilt University Medical Center, S-3223, MCN, Nashville, Tennessee 37232. Phone: 615-343-4312; Fax: 615-343-7156; E-mail: takamune.takahashi@vanderbilt.edu

damage, including protein kinase C, advanced glycation end (AGE) products, and hexosamine pathways.[4] In addition, superoxide is produced by multiple pathogenic pathways of diabetes. These include increased nicotinamide adenine dinucleotide phosphate [NAD(P)H] oxidase activity, uncoupled endothelial nitric oxide synthase (eNOS), and enhanced signaling of AGEs, angiotensin II, and oxidized-LDL receptors.[5] Excessive production of superoxide anion results in the formation of secondary reactive oxygen species (ROS) including peroxynitrite and hydroxyl radicals, leading the damage of DNA, proteins, and lipids, and causes vascular cell injury.[6] Thus, superoxide overproduction is considered as a major pathogenic pathway in diabetic vascular complication.

A net accumulation of superoxide anion is determined by a balance between superoxide production and antioxidant capacity. In this context, antioxidant defense system could play a critical role in diabetic vascular damage. Superoxide dismutase (SOD) is the major antioxidant enzyme for superoxide removal, which converts superoxide into hydrogen peroxide (H_2O_2) and molecular oxygen.[7,8] The hydrogen peroxide is further detoxified to water (H_2O) by catalase or glutathione peroxidase.[6,9] In mammals, three SOD isoforms exist: cytoplasmic CuZnSOD (SOD1), mitochondrial MnSOD (SOD2), and extracellular CuZnSOD (SOD3, ecSOD).[8,10] Each SOD isoform is derived from distinct genes but catalyzes the same reaction, producing H_2O_2 from $O_2^{\bullet -}$.[10] There is substantial evidence that SOD activity in peripheral blood cells is reduced in the diabetic patients with DN as compared with those without diabetic complication.[11–14] In addition, recent studies have implicated SOD (SOD1 and SOD2) gene polymorphism in human DN risk.[15–17] Furthermore, it was shown that the transgenic mice with SOD (SOD1 or SOD2) gene are resistant to diabetes-induced vascular injuries, including nephropathy.[4,18,19] In aggregate, these findings suggest a pivotal role of SOD enzyme in the pathogenesis of DN. However, the changes in renal SOD enzymes in DN and their significance are poorly described.

Recent studies of streptozotocin (STZ)-induced diabetic mice have shown that genetic factors significantly affect the development and the severity of DN in mice as well as in human.[20] Among the inbred strains of mice, KK/HlJ and DBA/2 strains have been identified as DN-prone strains, whereas the widely used C57BL/6 strain is relatively resistant to DN.[21] Compared with the STZ model, spontaneously diabetic mice offer a unique opportunity to assess the pathogenic pathways in DN without the potential nonspecific tissue toxicity of STZ. *Ins2*^{Akita} mouse (Akita mouse) is a well-studied nonobese hypoinsulinemic diabetic mouse.[22,23] This diabetic strain has a mutation in cysteine 96 to tyrosin in the insulin 2 gene (Akita mutation) and exhibits marked hyperglycemia as early as 4 wk of age.[23] However, Akita mouse does not develop overt DN due to C57BL/6 background.[24] To investigate renal alteration of SOD enzyme in advanced DN without the nonspecific tissue toxicity of STZ, we here generated a new congenic strain of the Akita mutation that exhibits progressive DN by backcrossing C57BL/6-strain Akita mouse (C57BL/6-Akita) to the nephropathy prone KK/Ta strain mouse.[25] Our data demonstrate that renal expression of SOD1 and SOD3, but not SOD2, is prominently down-regulated in KK/Ta-strain Akita mouse (KK/Ta-Akita), which exhibits progressive DN, whereas renal SOD expression was not altered in the DN-resistant C57BL/6-Akita mouse. Furthermore, the present study demonstrates that treatment with tempol, a SOD mimetic, remarkably ameliorates the nephropathic changes in KK/Ta-Akita mice. Taken together, these results suggest that downregulation of renal SOD1 and SOD3 may play a key role in the pathogenesis of DN.

RESULTS

Development of the KK/Ta-Akita Mouse

To investigate the alterations of renal SOD enzyme in advanced DN, first we backcrossed C57BL/6-Akita mouse to KK/Ta strain,[25] a DN-prone strain mouse, for 10 generations and developed an Akita mouse strain that accompanies progressive DN. The male mice were characterized and used for the study. As shown in Figure 1, A and B, KK/Ta-Akita males developed hyperglycemia (>300 mg/dl) at around 5 wk of age as did C57BL/6-Akita males, and both strains exhibited markedly elevated blood glucose and HbA_{1c} levels after 10 wk of age. KK/Ta-Akita and C57BL/6-Akita mice showed comparable blood glucose levels, and there was no difference in the severity of hyperglycemia between these two groups of mice. KK/Ta and C57BL/6 wildtype (WT) mice exhibited normal blood glucose levels during the study period, although KK/Ta-WT mice showed higher blood glucose levels than C57BL/6-WT mice at 20 wk of age. As shown in Figure 1C, both KK/Ta-Akita and C57BL/6-Akita mice exhibited significantly lower body weight as compared with nondiabetic WT mice, and an increase in body weight was not observed in these mice after 10 wk of age. No difference was observed in body weight between C57BL/6-Akita and KK/Ta-Akita males. Table 1 shows systolic BP and blood parameters for each group of mice. KK/Ta-Akita mice showed increases in systolic BP, total cholesterol, triglyceride, blood urea nitrogen (BUN), and creatinine as compared with KK/Ta-WT mice. Significant differences were not observed in these parameters between C57BL/6-WT and C57BL/6-Akita mice, although C57BL/6-Akita mice showed higher level of systolic BP. KK/Ta-WT mice showed higher BP and plasma triglyceride levels than C57BL/6-WT mice.

Renal Phenotype in KK/Ta-Akita and C57BL/6-Akita Mice

Renal changes in KK/Ta-Akita and C57BL/6-Akita males were assessed by the measurements of urine albumin excretion, FITC-inulin clearance to estimate GFR, kidney-to-body weight ratio, and renal histopathology. Compared with KK/Ta-WT males, KK/Ta-Akita males showed significantly increased urine albumin excretion as early as 5 wk of age, and