Table 1 Laboratory data on admission

TSH (0.5-5.0 μIU/mL)	1.72	FPG (70-109 mg/dL)	50
FT3 (2.3-4.3 pg/mL)	2.58	F-IRI (3.1-16.9 μU/mL)	0.5
FT4 (0.9-1.7 pg/mL)	0.98	Proinsulin (5-10 pmol/mL)	6.7
		Serum-CPR (0.6-2.1 ng/mL)	0.11
ACTH (7.2-63.3 pg/mL)	42.6	Urine-CPR (29.2-167 μg/day)	51.5
Cortisol (4.0-18.3 μg/dL)	19.7	Insulin antibody (<0.4%)	(-)
		Insulin receptor antibody (<24.2%)	39.6%
Glucagon (50-150 pg/mL)	120	PA IgG (9-25 ng/10^7 platelets)	149.6
LH (1.1-14.2 mIU/mL)	0.20	GAD antibody (<1.5 U/mL)	(-)
FSH (1.5-8.5 mIU/mL)	<0.05	ICA antibody (<1.25 JDF U)	(-)
PRL (4.9-29.3 ng/mL)	157.2	Pituitary antibody (-)	(-)
ADH (0.3-3.5 pg/mL)	0.7	Anti-thyroglobulin antibody (-)	(+)
GH (0.66-3.68 ng/mL)	0.46	Ant-microsome antibody (-)	(+)
IGF-1 (73-311 ng/mL)	180	Anti-DNA antibody (-)	(-)
IGF-2 (-)	(-)	Anti-nuclear antibody (-)	(-)
		CEA (<5.0 ng/mL)	0.6
Urine-cortisol (11.2-80.3 μg/day)	117	CA19-9 (<37.0 U/mL)	6.5
Urine-17-OHCS (2.6-7.8 mg/day)	3.1	CA125 (<35.0 U/mL)	25.6
Urine-17-KS (1.0-10.9 mg/day)	7.7		

(Normal range)

Abbreviations: S, serum; U, urine; TSH, thyroid stimulating hormone; FT3, free triiodothyronine; FT4, free thyroxine; ACTH, adrenocorticotropic hormone; LH, luteinizing hormone; FSH, follicle-stimulating hormone; PRL, prolactin; ADH, antidiuretic hormone; GH, growth hormeone; IGF, insulin-like growth factor; OHCS, hydroxycorticosteroid; KS, ketosteroid; FPG, fasting plasma glucose; IRI, immunoreactive insulin; CPR, connecting peptide immunoreactivity; PA IgG, platelet-associated IgG; GAD, glutamic acid decarboxylase; ICA, islet cell antibody; CEA, carcinoma embryonic antigen

at 15 years of age. She was brought into the advanced emergency center of Iwate Medical University Hospital due to a sudden loss of consciousness at 9 weeks of the first pregnancy in December 2001. Her blood glucose level was as low as 26 mg/dl and she was immediately treated for hypoglycemia, and then hospitalized in Morioka City Hospital on the same day for close examination. An intrauterine fetal death was found during the examination. The hypoglycemia did not occur thereafter. The glucose tolerance test and various hormone measurements conducted thereafter showed that all results were within the normal ranges. Also, no abnormal findings suggesting insulinoma were observed in the imaging tests, including abdominal ultrasonography, computed tomography (CT) and angiography.

Two years later, her hypoglycemia had recurred at 8 weeks into the second pregnancy in September 2003, and she was re-hospitalized in the Morioka City Hospital for treatment of hypoglycemia. In order to prevent night-time hypoglycemia, glucose solution was intravenously infused every day. However, due to the persistence of the low blood glucose level, at 15 weeks of pregnancy (October 2003) the patient was transferred to the Department of Obstetrics and Gynecology of the Iwate Medical University Hospital for whole-body care and close examination, and was referred to the Department of Diabetes and Metabolism on the same day.

On admission, physical examination revealed that she had a low grade fever (37.2°C), but no particular abnormalities relating to acanthosis nigricans, hirsutism, and Sjogren syndrome. Laboratory data showed that there were no abnormalities in peripheral blood and biochemical examinations except a mild anemia (RBC: 367 x 10^4/μl; Hb: 11.3 g/dl), thrombocytopenia (11.3 × 10^4/μl) and an increase in serum γ-globulin fraction (27%). Fasting plasma glucose (FPG) level was 50 mg/dl, and fasting serum insulin (IRI) and serum C-peptide levels were lower than normal, whereas the pro-insulin level was within the normal range (Table 1). The growth hormone (GH) level was lower than normal, whereas the insulin-like growth factor-1 (IGF-1)

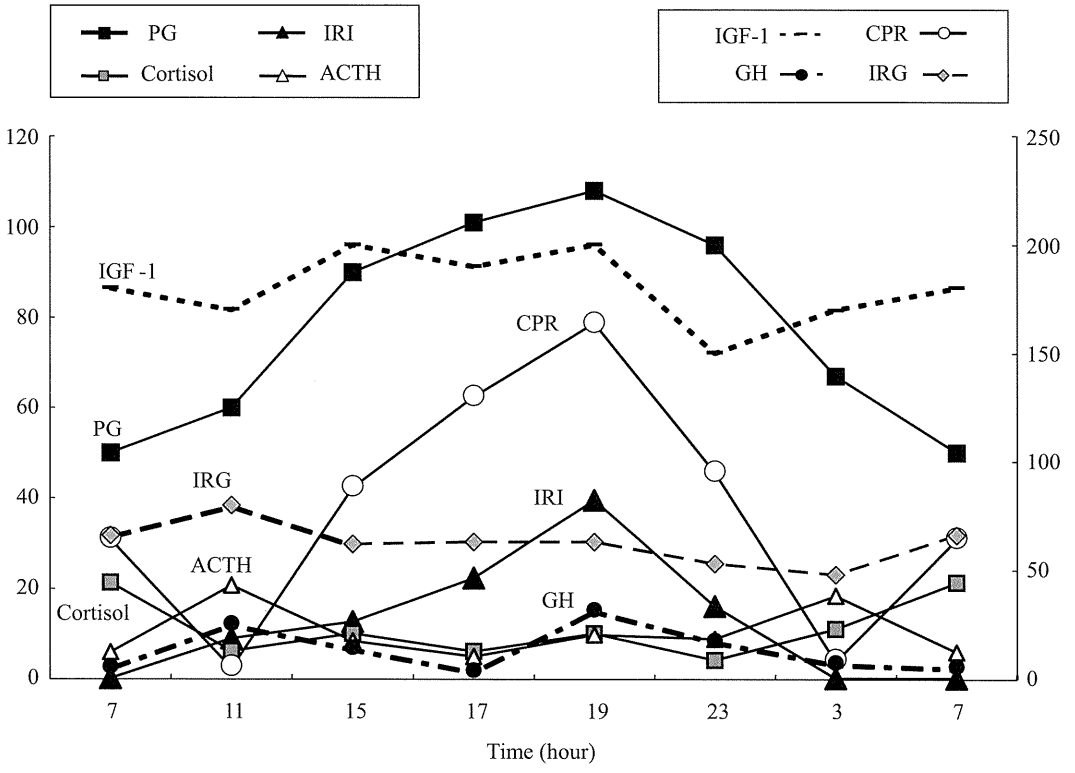

Fig. 1 Circadian variations of plasma hormone levels in the patient.
Abbreviations: PG, plasma glucose (mg/dL); IRI, immunoreactive insulin (μU/mL); GH, growth hormone ($\times 10^{-1}$ ng/mL); IRG, immunoreactive glucagon (pg/mL); ACTH, adrenocorticotropic hormone (pg/mL); CPR, connecting peptide immunoreactivity ($\times 10^{-2}$ ng/mL); IGF, insulin-like growth factor (ng/mL).

level was within the normal range and IGF-2 was not detected (measured by Prof. Naomi Hizuka, Tokyo Women's Hospital). The thyroid hormone levels (free T_3 [FT_3] and free T_4 [FT_4]) were normal. The luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were lower and the prolactin level was higher than normal. As for autoantibodies, insulin antibody was negative, whereas anti-insulin receptor antibody was positive; the inhibition rate of insulin binding was 39.6% (measured by BML, Inc., Tokyo, Japan). Anti-thyroglobulin antibody and anti-microsome antibody were positive, whereas anti-DNA and anti-nuclear antibodies were negative. No abnormality was observed in the abdominal ultrasonography and magnetic resonance imaging of the brain. CT was not performed because of the pregnancy.

After admission, since hypoglycemia (50-60 mg/dl at 4:00 a.m.) persisted even with supplementary meals at 3 p.m. and 9 p.m. and a 5% glucose drip-infusion from midnight to 6 a.m., 5% glucose was changed to 10% glucose. But the mean glucose level at 4 a.m. remained as low as 50-60 mg/dl. At one time, she lost consciousness because of hypoglycemia when she was out of the hospital in the daytime without the glucose infusion, and was brought back to the hospital by an ambulance.

In January 2004, since the platelet count fell to as low as 7.0×10^4/μl and anti-platelet antibody (PA IgG; 149.6 fg/platelet) was positive, a bone marrow puncture was performed at the Hematology Department of our hospital and she was diagnosed as having idiopathic thrombocytopenic purpura (ITP).

Since the blood and urinary cortisol levels were lower than those during usual pregnancy, although the serum aldosterone level was as high as that during usual pregnancy, secondary adrenocortical insufficiency was suspected. Although the baseline levels of serum ACTH and cortisol were within the normal range, the circadian rhythm of these hormones showed low levels as a whole (Fig. 1). Therefore, we considered hypocortisolemia as a part of the possible causes of her hypoglycemia, and administered orally a small

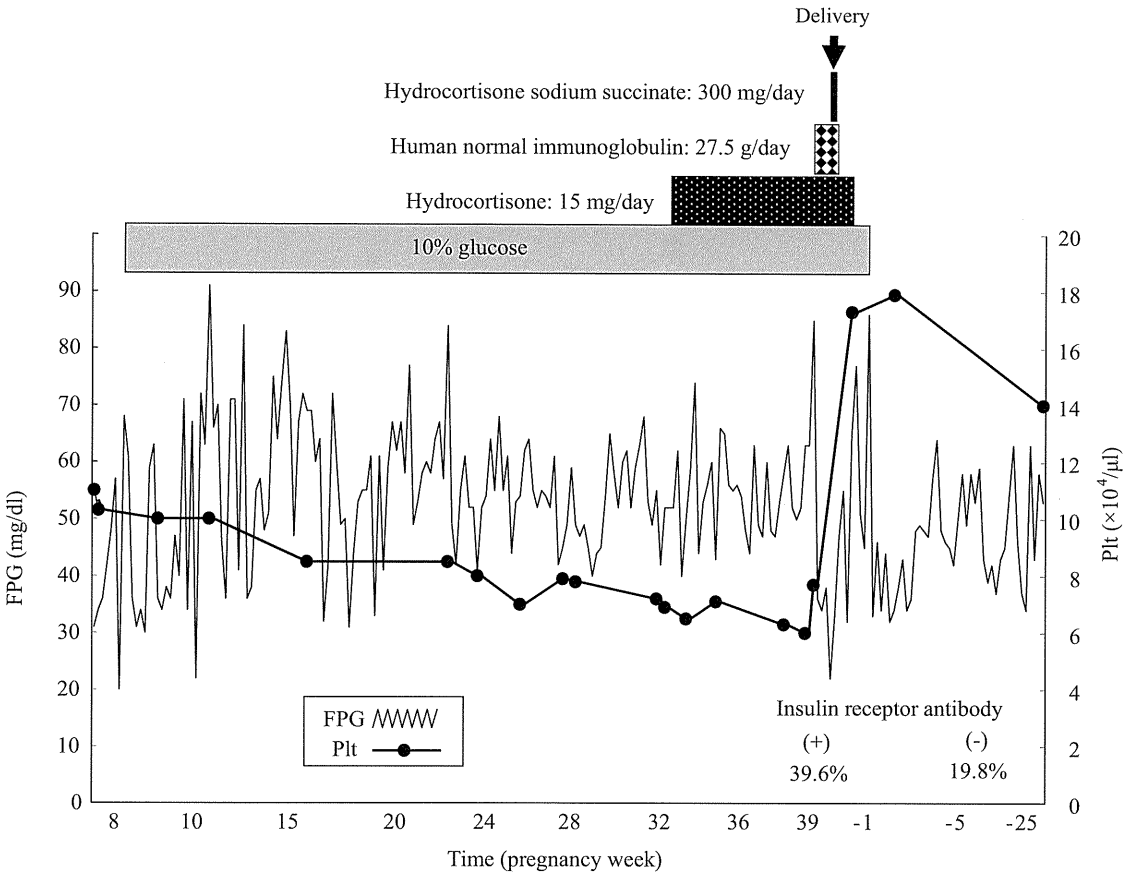

Fig. 2 Time course of FPG and platelet counts according to pregnancy week of the patient.
Abbreviations: FPG, fasting plasma glucose; Plt, platelet

dose of hydrocortisone (15 mg/day) to the patient from March 2004 (at 33 weeks of pregnancy) (Fig. 2).

Because the platelet count further decreased to as low as $6.0 \times 10^4/\mu l$, we decided to carry out a planned delivery by Caesarian section. To increase the platelet count, human immunoglobulin was administered to the patient at a dose of 27.5 g/day for three days from April 3, 2004 (39 weeks of pregnancy), and the platelet count increased to $15.2 \times 10^4/\mu l$. Also, on April 6, 2004, to avoid any adrenal crisis which could be induced by stress at delivery, hydrocortisone sodium succinate was administered to the patient at a dose of 300 mg/day, and on the same day a healthy baby was delivered by Caesarian section (Fig. 2).

After the delivery, her blood glucose level rose gradually to the point at which the patient did not develop hypoglycemia even with regular meals and without glucose infusion. Since a starvation test conducted one month after the delivery showed that the patient did not develop hypoglycemia during 7 hours of fasting, she was discharged from the hospital.

An examination conducted in July 2004 (3 months after the delivery) showed that anti-platelet antibody was negative (<25 ng/10^7 platelets) and the platelet count had returned to normal. Furthermore, an examination in October 2004 (6 months after the delivery) showed that anti-insulin receptor antibody was also negative (19.8%). Thereafter, we did not follow her laboratory data because of her moving to a city far from our hospital. However, according to the recent telephone information from her on June, 2011, the patient had not become pregnant and not developed hypoglycemia and her boy grew satisfactorily since the delivery on 2004.

Materials and Methods

Measurement of anti-insulin receptor antibody

Anti-insulin receptor antibody in serum was mea-

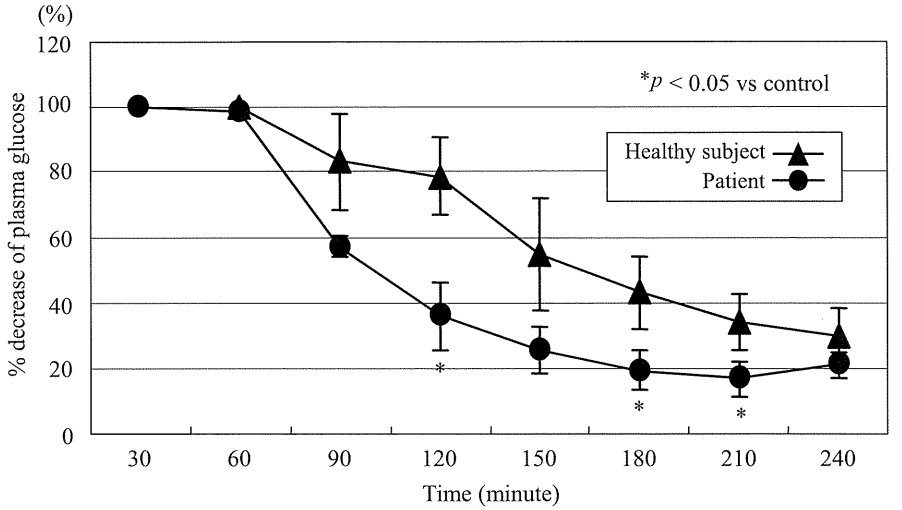

Fig. 3 Percent decrease of plasma glucose levels after injection with serum from the patient or a healthy subject.

sured in the BML, Inc. (Tokyo, Japan) by a radioreceptor assay as previously reported [2]. Binding activity of insulin receptor antibody with insulin receptor from human B lymphoblastic IM-9 cells [3] was expressed as % inhibition by insulin receptor antibody of ^{125}I-insulin binding with human insulin receptor. Cutoff value of this assay was 24.2% (mean + 3SD).

Treatment of mice with human serum

Sera were taken from the patient during the pregnancy and a person with normal glucose tolerance (control), and one ml of each serum was intraperitoneally administered to 5 mice in each group, and the blood glucose level of the mice was measured by a glucometer. The mice were fasted after the administration of the serum (water was fed freely), and the changes in their blood glucose level were shown as % decrease in comparison with the level before the serum administration. The protocol of the animal experiment was approved by the Animal Care and Use Committee of the Iwate Medical University.

Phosphorylation of IR of CHO-IR cells

The patient's serum and the healthy subject's (control) serum were dialyzed in with Ham' sF12 buffer. After incubation of CHO-IR cells for 5 hours in a serum-free culture medium (serum starvation), the medium was replaced with the sera from the patient or control subjects after dialysis and incubated for 5-30 minutes. After incubation, the cells were solubilized, subjected to immunoprecipitation using anti-IR ß chain antibody, subjected to electrophoresis in 7.5% acrylamide gel and blotted using an anti-phosphotyrosine antibody (4G10) as reported previously [4].

Results

The patient's serum lowered mouse blood glucose levels

To determine whether the serum of the patient had any hypoglycemic effect. One ml of each serum was intraperitoneally administered to 5 mice in each group. As shown in Fig. 3, the % decrease in the blood glucose level of the mice administered with the patient's serum was significantly greater than that of the control. This result suggested the presence of a factor lowering the blood glucose level in the serum of the patient.

The effect of the patient's serum on phosphorylation of insulin receptors of CHO-IR cells.

To determine whether the patient's serum, which was positive for the IR antibody and had the hypoglycemic effect in mice (Fig. 3), phosphorylates IR, CHO-IR cells were incubated with the serum. As shown in Fig. 4, the patient's serum (lane 3 and 5) showed a positive blot band in the 90-kDa region just like insulin as the positive control (lane 1), and the serum with longer incubation (30 min. with lane 5 vs. 5 min. with lane 3) showed a more distinct positive band. On the other hand, for negative control (lane 4 for 5 min. and lane 6 for 30 min.), the blot band was negative. This suggested that the patient's serum stimulated the IR and induced the tyrosine phosphorylation of IR ß chain.

Discussion

Hypoglycemia is induced by a variety of endogenous and exogenous causes [5]. Endogenous causes are classified as insulin-mediated (insulinoma, nesidoblastosis, non-insulinoma pancreatogenous hypoglycemia syndrome [NIPHS], insulin antibody and reactive hypoglycemia) and insulin-independent causes (critical organ failure, sepsis, hormone deficiency such as cortisol, growth hormone and hypopituitarism, insulin receptor antibodies, and non-islet cell tumor). Exogenous causes include therapeutic drugs such as oral hypoglycemic agents and others, factitious cause, and alcohol or toxins.

In this case, exogenous causes could be neglected, because she was not administered any medicines. Among endogenous causes, insulin-mediated causes such as insulinoma, nesidoblastosis and NIPHS are unlikely, because serum insulin and c-peptide levels were not high at the time of hypoglycemia and anti-insulin antibody was negative. As insulin-independent causes, she had no critical organ failure, sepsis and hormone deficiencies. Although serum cortisol levels were relatively low as a pregnant woman, serum ACTH levels were within normal limits and she had no signs and symptoms of Addison's disease, nor autoimmune polyglandular syndrome type 1 and type 2. A relationship between hypocortisolemia and autoantibodies such as anti-insulin receptor antibody and anti-platelet antibody is unknown. The relative hypocorticosolemia may not be a major cause of hypoglycemia, rather it might be a factor exacerbating hypoglycemia.

It is most likely in this case that anti-IR antibody may have induced hypoglycemia, because of several lines of evidence. First, the patient's serum positive for anti-IR antibody lowered plasma glucose levels of mice as compared with control serum from a healthy subject (Fig. 3). Second, the patient's serum phosphorylated tyrosineof IR of CHO-IR cells as did insulin (Fig. 4). Finally, the improvement of hypoglycemia was associated with decreased titer of anti-IR antibody. Eventually she can be diagnosed as having type B insulin resistance syndrome associated with hypoglycemia [6].

There remain some questions, e.g., why hypoglycemia was induced and why multiple autoantibodies such as anti-IR antibody and PA IgG were produced only during the period of pregnancy, although anti-IR antibody and PA-IgG was not measured at time of the first pregnancy, when she had no thrombocytopenia. There

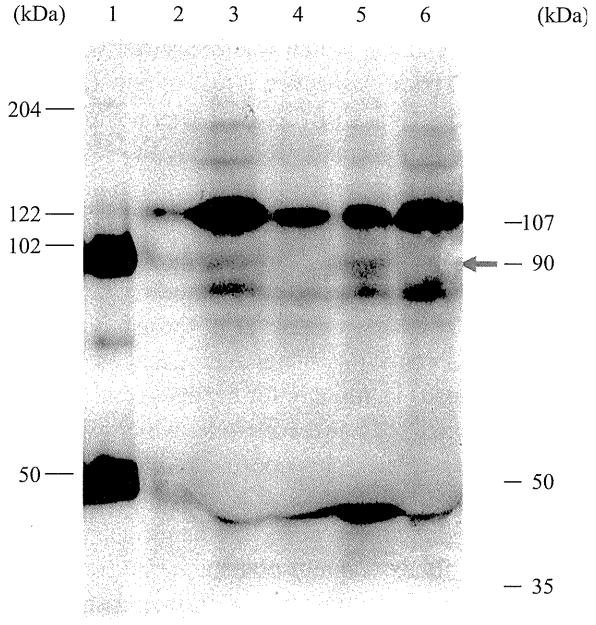

Fig. 4 Tyrosine phosphorylation of the insulin receptor of CHO cells by the treatment with the patient's serum.
M: Markers Lane 1: Insulin (positive control) Lane 2: Blank Lane 3 and 5: Patients serum incubated 5 min. (lane 3) and 30 min. (lane 5). Lane 4 and 6: Control serum incubated 5 min. (lane 4) and 30 min. (lane 6).

has been implications that the immune system becomes aberrant and some autoimmune diseases such as systemic lupus erythematosus (SLE) are exacerbated during pregnancy [7]. SLE is associated with various multiple autoantibodies and there are some reports on cases with SLE associated anti-insulin receptor antibody [8-10]. Our case did not show symptoms of SLE and did not fulfill the criteria of SLE by the American Rheumatoid Association [9].

It has been reported that approximately 7-8% of pregnant woman have thrombocytopenia, the cause of which includes gestational thrombocytopenia and ITP [12]. Pregnancy does not increase the risk of ITP, but it exacerbates preexisting ITP [12]. Therefore, this case is not rare in terms of pregnancy associated with ITP. However, there has not been reported a pregnant woman associated with both anti-IR antibody and anti-platelet antibody. Recently, a very rare case of type B insulin resistance syndrome and ITP has been reported [13]. In this case, helicobacter pylori (HP) infection was indicated as a cause of ITP, and eradication therapy of HP resulted in an increase of platelet number and decrease of anti-IR antibody. In our case, low titer of anti-HP antibody (14 U/mL) (normal < 10 U/ml, SRL

Co. Ltd, Tokyo, Japan) was detected in the serum during the pregnancy, which was measured years later in the stored serum. However, a role of anti-HP antibody in the pathogenesis of this case was unclear, because platelet number and anti-IR antibody improved without eradication therapy of HP.

After the successful delivery, hypoglycemia was improved and the anti-IR antibody anti-platelet antibody became negative. The completion of the pregnancy may have resulted in decrease of these autoantibodies. However, a possibility is not denied that administration of a low dose (15 mg/day for 6 weeks) and a high dose (300 mg once) of hydrocortisone, which was given to compensate relative hypocortisolemia and to prevent adrenal crisis during the Caesarian section, respectively, might have reduced autoanitbodies including anti-IR antibody and anti-platelet antibody.

In summary, we report here an interesting rare case of a pregnant woman who suffered from severe hypoglycemia only during two occasions of her pregnancies. She had anti-IR antibody and anti-platelet antibody. Administration of the serum lowered blood glucose levels in mice, and the serum phosphorylated tyrosine of insulin receptor of CHO-IR cells. These autoantibodies and both the hypoglycemia and thrombocytopenia disappeared after the delivery. From these findings, we concluded that anti-insulin receptor antibody and anti-platelet antibody during pregnancies might have lead to hypoglycemia and thrombocytopenia, respectively.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Prof. N. Hizuka, Tokyo Women's Hospital, for the measurement of IGF-II, Prof. T. Suda, Department of Endocrinology, Hirosaki University, for the helpful discussion and Dr. Paul Langman, Ph.D. for his kind assistance with English usage.

References

1. Service FJ (1995) Hypoglycemia disorders. Hypoglycemia disorders. *N Engl J Med* 332: 1144-1152.
2. Satoh K, Kawaguchi R, Yonezawa M, Kubono K, Hikiji K, Ishigami T, Tsukada Y, Takahashi M (1990) A determination of anti-insulin receptor antibody in serum--a radioreceptor assay excluded the influence of insulin and anti-insulin antibody. *Rinsho Byori* 38: 311-316.
3. Van Obberghen E, De Meyts P, Roth J (1976) Cell surface receptors for insulin and human growth hormone. Effect of microtubule and microfilament modifiers. *J Biol Chem* 251: 6844-6851.
4. Yamada T, Katagiri H, Asano T, Inukai K, Tsuru M, Kodama T, Kikuchi M , Oka Y (2001) 3-phosphoinositide-dependent protein kinase 1, an Akt1 kinase, is involved in dephosphorylation of Thr-308 of Akt1 in Chinese hamster ovary cells. *J Biol Chem* 276: 5339-5345.
5. Glaser B, Leibowitz G (2005) Hypoglycemia. In: Kahn CR, Weir GC, King GL, Jacobson AM, Moses AC, Smith RJ (eds) Joslin's Diabetes Mellitus 14th edn, Lippincott Williams & Wilkins, New York: 1147-1175.
6. Lupsa BC, Chong AY, Cochran EK, Soos MA, Semple RK, Gorden P (2009) Autoimmune forms of hypoglycemia. *Medicine* 88: 141-153.
7. Carvalheiras G, Vita P, Marta S, Trovão R, Farinha F, Braga J, Rocha G, Almeida I, Marinho A, Mendonça T, Barbosa P, Correia J, Vasconcelos C (2010) Pregnancy and systemic lupus erythematosus: Review of clinical features and outcome of 51 pregnancies at a single institution. *Clin Rev Allergy Immunol* 38: 302-306.
8. Moller DE, Ratner RE, Borenstein DG, Taylor, SI (1988) Autoantibodies to the insulin receptor as a cause of autoimmune hypoglycemia in systemic lupus erythematosus. *Am J Med* 84: 334-338.
9. Varga J, Lopatin M, Boden G (1990) Hypoglycemia due to antiinsulin receptor antibodies in systemic lupus erythematosus. *J Rheumatol* 17: 1226-1229.
10. Kato Y, Ichiki, Kitajima Y (2008) A case of systemic lupus erythematosus presenting as hypoglycemia due to anti-insulin receptor antibodies. *Rheumatol Int* 29: 103-105.
11. Parodi A, Rebora A (1997) ARA and EADV criteria for classification of systemic lupus erythematosus in patients with cutaneous lupus erythematosus. *Dermatology* 194: 217-220.
12. Sukenik-Halevy R, Ellis MH, Fejgin MD (2008) Management of immune thrombocytopenic purpura in pregnancy. *Obstet Gynecol Surv* 63: 182-188.
13. Imai J, Yamada T, Saito T, Ishigaki Y, Hinokio Y, Kotake H, Oka Y, Katagiri H (2009) Eradication of insulin resistance. *Lancet* 374: 264.

Hypertension Research (2011) 34, 1302–1308

www.nature.com/hr

Open

ORIGINAL ARTICLE

Reduction of circulating superoxide dismutase activity in type 2 diabetic patients with microalbuminuria and its modulation by telmisartan therapy

Hiroki Fujita[1], Takuya Sakamoto[2], Koga Komatsu[2], Hiromi Fujishima[1], Tsukasa Morii[1], Takuma Narita[1], Takamune Takahashi[3] and Yuichiro Yamada[1]

Growing evidence indicates that oxidative stress induced by excessive superoxide has a central role in the pathogenesis of diabetic nephropathy (DN). Telmisartan, one of the currently available angiotensin II type 1 receptor blockers (ARBs), has been shown to exert a more powerful proteinuria (albuminuria) reduction in patients with DN, but whether the prominent renoprotective effect of telmisartan is mediated through enhancing antioxidant defense capacity and reducing oxidative stress has not been fully elucidated. The present study first revealed that the serum activity of superoxide dismutase (SOD) responsible for superoxide removal is reduced in the DN stage of microalbuminuria, but not in normoalbuminuria in type 2 diabetic patients. We next examined the alteration of SOD and oxidative stress following an 8-week treatment with telmisartan (40 mg per day) in 12 type 2 diabetic patients with microalbuminuria. Interestingly, the telmisartan treatment not only reduced the circulating levels of two oxidative stress markers, 8-hydroxy-2′-deoxyguanosine (8-OHdG) and nitrotyrosine (NT), but also enhanced serum SOD activity. Notably, a significant correlation was observed between the increase in serum SOD activity and the reduction in albuminuria. We further compared the anti-oxidative effect of telmisartan with that of losartan, another member of the ARB class, by implementing an 8-week interval crossover treatment with these ARBs in another 12 microalbuminuric type 2 diabetic patients. The patients showed higher serum SOD activity, and lower circulating levels of 8-OHdG and NT, during treatment with telmisartan than with losartan. These results suggest that telmisartan has a more potent antioxidative effect through its ability to enhance SOD activity in type 2 diabetic patients with microalbuminuria.
Hypertension Research (2011) **34**, 1302–1308; doi:10.1038/hr.2011.127; published online 4 August 2011

Keywords: angiotensin II type 1 receptor blocker; diabetic nephropathy; oxidative stress; superoxide dismutase; telmisartan

INTRODUCTION

Oxidative stress induced by superoxide anion ($O_2^{\bullet -}$) overproduction is considered a major cause of diabetic vascular injury, including diabetic nephropathy (DN). An excess of the superoxide anion causes vascular cell injury through the formation of cytotoxic secondary reactive oxygen species, such as peroxynitrite ($ONOO^-$) and hydroxyl radicals ($^{\bullet}OH$).[1] The superoxide is produced by multiple pathogenic pathways, including increased nicotinamide adenine dinucleotide phosphate (NAD(P)H) oxidase activity, uncoupled endothelial nitric oxide (NO) synthase and enhanced angiotensin II signaling.[2] In contrast to the superoxide-producing enzymes such as NAD(P)H oxidase and endothelial NO synthase, superoxide dismutase (SOD) serves as an antioxidant enzyme responsible for superoxide removal. The SOD converts superoxide anion into hydrogen peroxide (H_2O_2) and molecular oxygen.[3,4] The hydrogen peroxide is further detoxified into water (H_2O) by catalase in peroxisomes or glutathione peroxidase in mitochondria.[1,5] Growing evidence indicates that chronic hyperglycemia causes superoxide overproduction by activating NAD(P)H oxidase[6–9] and uncoupling endothelial NO synthase.[9] Therefore, the SOD antioxidant defense system has a key role in protecting vascular cells from increased oxidative stress in the diabetic state.

Telmisartan is a unique angiotensin II type 1 (AT1) receptor blocker (ARB) that functions as a partial agonist of the peroxisome proliferator-activated receptor-γ.[10–12] A recent clinical study showed that telmisartan is superior to another ARB, losartan, in reducing proteinuria effect in patients with DN, despite a comparable blood pressure reduction.[13] Given this compelling evidence, it was expected that telmisartan, among various ARBs, may exert a more powerful protective effect against oxidative stress in the diabetic state. In the present study, we first determined the stage of DN that alters the SOD antioxidant defense capacity and enhances oxidative stress. Our data demonstrate that reduced SOD antioxidant defense capacity and markedly increased oxidative stress are observed in the DN stage of

[1]Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University Graduate School of Medicine, Akita, Japan; [2]Division of Gastroenterology, Honjo Daiichi Hospital, Akita, Japan and [3]Division of Nephrology, Vanderbilt University Medical Center, Nashville, TN, USA
Correspondence: Dr H Fujita, Division of Endocrinology, Metabolism and Geriatric Medicine, Akita University Graduate School of Medicine, 1-1-1 Hondo, Akita 010-8543, Japan.
E-mail: hirofuji@gipc.akita-u.ac.jp
Received 29 January 2011; revised 13 May 2011; accepted 6 June 2011; published online 4 August 2011

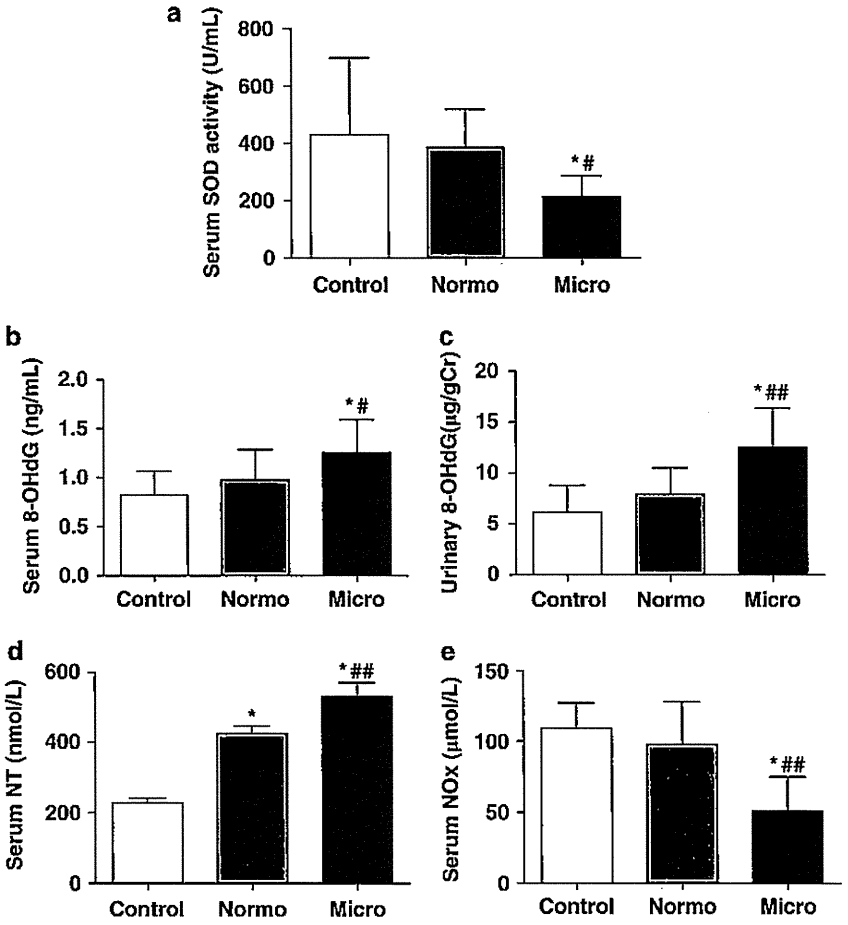

Figure 1 Oxidative stress markers in non-diabetic healthy subjects (control, n=18) and type 2 diabetic patients with normoalbuminuria (normo, n=19) and microalbuminura (micro, n=16). (a) serum SOD activity; (b) serum 8-OHdG; (c) urinary 8-OHdG; (d) serum NT; (e) serum NOx. Data are presented as the means ± s.d. $^{*}P<0.001$ *vs.* control; $^{\#}P<0.05$ *vs.* normo; $^{\#\#}P<0.001$ *vs.* normo. NT, nitrotyrosine.

Not surprisingly, the telmisartan treatment lowered systolic and diastolic blood pressure and reduced albuminuria. Figure 2 shows the changes in the oxidative stress markers after 8 weeks of telmisartan treatment in the diabetic patients with microalbuminuria. Notably, an increase in serum SOD activity was observed after 8 weeks of telmisartan treatment (Figure 2a). Interestingly, there was a significant correlation between the increase in serum SOD activity and the reduction in albuminuria (Figure 2b). In agreement with the improvement in antioxidant defense capacity, serum and urinary 8-OHdG levels and serum NT levels were significantly reduced after 8 weeks of telmisartan treatment (Figures 2c–e). The serum NOx levels were significantly increased after 8 weeks of telmisartan treatment (Figure 2f). Thus, we found that the telmisartan treatment can improve systemically increased oxidative stress and reduce SOD antioxidant defense capacity in microalbuminuric type 2 diabetic patients.

Changes in oxidative stress markers by crossover treatment with telmisartan and losartan in type 2 diabetic patients with microalbuminuria

To compare the antioxidative effects of telmisartan with those of other ARBs, we performed a crossover treatment with telmisartan and losartan in type 2 diabetic patients with microalbuminuria, and investigated changes in oxidative stress markers. Table 3 shows clinical and biochemical parameters at the end of each 8-week treatment period: telmisartan 40 mg per day (first period), losartan 50 mg per day (second period) and telmisartan 40 mg per day (third period). There were no significant differences in body mass index, blood pressure, plasma glucose or serum lipid levels between the three

Table 2 Changes in clinical parameters after 8 weeks of telmisartan treatment in type 2 diabetic patients with microalbuminuria

	Baseline	*Telmisartan 8W*
n	12	
Age (years)	64±7	
Gender (male/female)	6/6	
Body mass index (kg m^{-2})	24.8±2.6	24.8±2.6
Systolic blood pressure (mm Hg)	138±5	127±10*
Diastolic blood pressure (mm Hg)	73±7	69±7†
Fasting plasma glucose (mg dl^{-1})	116±12	115±13
HbA1c (%)	7.2±0.7	7.2±0.7
LDL-cholesterol (mg dl^{-1})	97.1±24.2	92.7±26.3
HDL-cholesterol (mg dl^{-1})	54.7±8.8	56.5±7.9
Triglyceride (mg dl^{-1})	130.8±66.7	121.4±61.5
Serum creatinine (mg dl^{-1})	0.71±0.16	0.70±0.14
Urinary albumin (mg g^{-1} creatinine)	110.5±72.5	66.8±59.7*

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data were presented as means ± s.d. $^{*}P<0.001$, $^{\dagger}P<0.01$ *vs.* baseline.

Table 3 Changes in clinical parameters by crossover treatment with telmisartan and losartan in type 2 diabetic patients with microalbuminuria

	First period telmisartan 8W	*Second period losartan 8W*	*Third period telmisartan 8W*
n	12		
Age (years)	68±6		
Gender (male/female)	5/7		
Body mass index ($kg\,m^{-2}$)	23.9±3.7	23.7±3.8	23.8±3.8
Systolic blood pressure (mm Hg)	126±12	132±13	128±11
Diastolic blood pressure (mm Hg)	69±8	75±8	71±7
Fasting plasma glucose ($mg\,dl^{-1}$)	113±13	119±17	115±17
HbA1c (%)	6.5±0.8	6.6±0.9	6.6±0.9
LDL-cholesterol ($mg\,dl^{-1}$)	107.9±24.7	107.9±24.5	110.0±24.5
HDL-cholesterol ($mg\,dl^{-1}$)	59.8±14.7	58.6±14.6	58.2±13.5
Triglyceride ($mg\,dl^{-1}$)	86.3±38.4	72.4±25.2	79.7±22.7
Serum creatinine ($mg\,dl^{-1}$)	0.65±0.19	0.61±0.15	0.62±0.15
Urinary albumin ($mg\,g^{-1}$ creatinine)	68.4±38.5	86.0±53.1*	73.5±49.3†

Abbreviations: HbA1c, hemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data were presented as means±s.d. *$P<0.05$ *vs.* first period; †$P<0.05$ *vs.* second period.

stood. Recent experimental studies have shown that telmisartan attenuates oxidative stress by downregulating NAD(P)H oxidase, a major superoxide-producing enzyme.[22,23] By contrast, the effect of telmisartan on a superoxide-scavenging enzyme, SOD, has not been clarified. Therefore, we next tested whether telmisartan treatment ameliorates the reduced SOD antioxidant defense capacity in the DN stage of microalbuminuria. Our data clearly demonstrate that telmisartan treatment enhances serum SOD activity and systemically reduces oxidative and nitrosative stress in patients with microalbuminuric DN. Importantly, the increase in serum SOD activity by telmisartan treatment showed a correlation with the reduction of albuminuria in these patients with microalbuminuric DN. This finding indicates that the mechanism by which telmisartan provides renoprotective effects may involve improvement of the SOD antioxidant defense capacity. Considering the present results along with recent compelling evidence, it is likely that telmisartan exerts antioxidative effects by modulating both superoxide-producing and superoxide-scavenging enzymes.

Angiotensin II has been shown to promote superoxide generation through NAD(P)H oxidase activation, independently of its systemic vasoconstriction ability.[24,25] Therefore, blockade of the angiotensin II signaling pathway via AT1 receptors is expected to reduce the superoxide-induced oxidative stress. Although all ARBs may share the antioxidative effects to some extent, there seems to be a difference

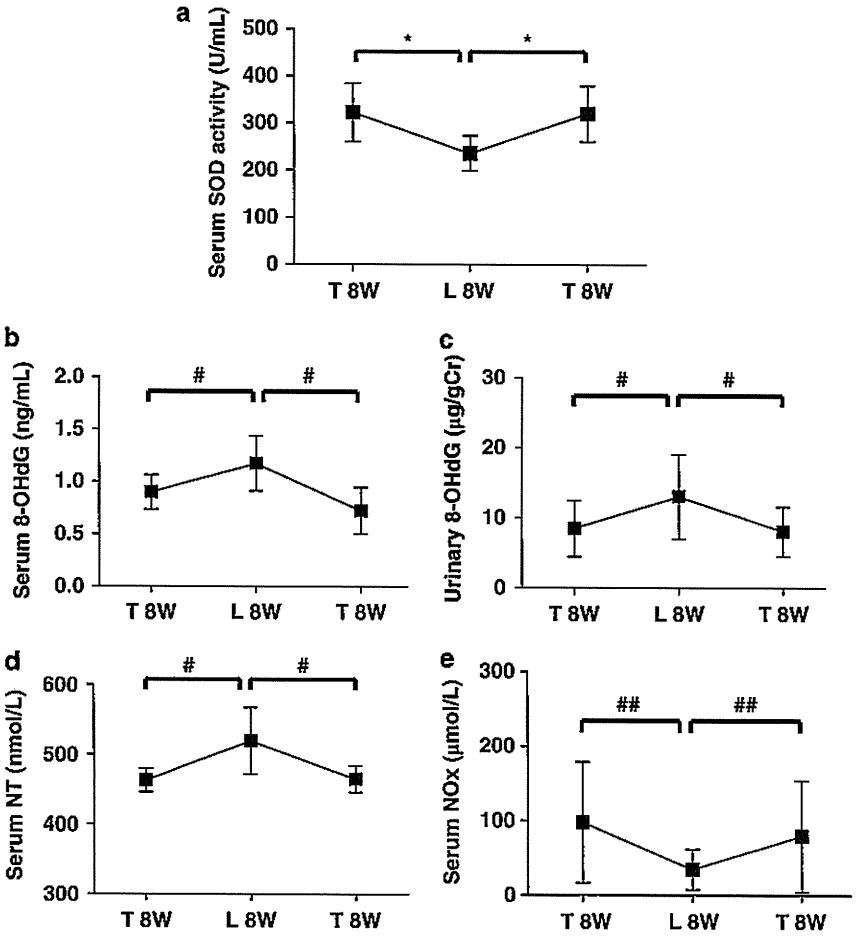

Figure 3 Changes in oxidative stress markers by crossover treatment with telmisartan and losartan in type 2 diabetic patients with microalbuminuria. (a) serum SOD activity; (b) serum 8-OHdG; (c) urinary 8-OHdG; (d) serum NT; (e) serum NOx. Data are presented as the means±s.d. ($n=12$). *$P<0.01$, #$P<0.001$, ##$P<0.05$. T 8W, telmisartan treatment for 8 weeks; L 8W, losartan treatment for 8 weeks; NT, nitrotyrosine.

levels with cardiovascular disease and modulation by statin therapy. *JAMA* 2003; **289**: 1675–1680.
18 Cosentino F, Hishikawa K, Katusic ZS, Luscher TF. High glucose increases nitric oxide synthase expression and superoxide anion generation in human aortic endothelial cells. *Circulation* 1997; **96**: 25–28.
19 Fujita H, Fujishima H, Chida S, Takahashi K, Qi Z, Kanetsuna Y, Breyer MD, Harris RC, Yamada Y, Takahashi T. Reduction of renal superoxide dismutase in progressive diabetic nephropathy. *J Am Soc Nephrol* 2009; **20**: 1303–1313.
20 Galle J, Schwedhelm E, Pinnetti S, Boger RH, Wanner C. Antiproteinuric effects of angiotensin receptor blockers: telmisartan versus valsartan in hypertensive patients with type 2 diabetes mellitus and overt nephropathy. *Nephrol Dial Transplant* 2008; **23**: 3174–3183.
21 Makino H, Haneda M, Babazono T, Moriya T, Ito S, Iwamoto Y, Kawamori R, Takeuchi M, Katayama S. Prevention of transition from incipient to overt nephropathy with telmisartan in patients with type 2 diabetes. *Diabetes Care* 2007; **30**: 1577–1578.
22 Sugiyama H, Kobayashi M, Wang DH, Sunami R, Maeshima Y, Yamasaki Y, Masuoka N, Kira S, Makino H. Telmisartan inhibits both oxidative stress and renal fibrosis after unilateral ureteral obstruction in acatalasemic mice. *Nephrol Dial Transplant* 2005; **20**: 2670–2680.
23 Takaya T, Kawashima S, Shinohara M, Yamashita T, Toh R, Sasaki N, Inoue N, Hirata K, Yokoyama M. Angiotensin II type 1 receptor blocker telmisartan suppresses superoxide production and reduces atherosclerotic lesion formation in apolipoprotein E-deficient mice. *Atherosclerosis* 2006; **186**: 402–410.
24 Griendling KK, Minieri CA, Ollerenshaw JD, Alexander RW. Angiotensin II stimulates NADH and NADPH oxidase activity in cultured vascular smooth muscle cells. *Circ Res* 1994; **74**: 1141–1148.
25 Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest* 1996; **97**: 1916–1923.
26 Kakuta H, Sudoh K, Sasamata M, Yamagishi S. Telmisartan has the strongest binding affinity to angiotensin II type 1 receptor: comparison with other angiotensin II type 1 receptor blockers. *Int J Clin Pharmacol Res* 2005; **25**: 41–46.
27 Burnier M. Telmisartan: a different angiotensin II receptor blocker protecting a different population? *J Int Med Res* 2009; **37**: 1662–1679.
28 Yano Y, Hoshide S, Ishikawa J, Noguchi C, Tukui D, Takanori H, Tada M, Kanemaru Y, Yano A, Ishikawa S, Shimada K, Kario K. The differential effects of angiotensin II type 1 receptor blockers on microalbuminuria in relation to low-grade inflammation in metabolic hypertensive patients. *Am J Hypertens* 2007; **20**: 565–572.
29 Dobrian AD, Schriver SD, Khraibi AA, Prewitt RL. Pioglitazone prevents hypertension and reduces oxidative stress in diet-induced obesity. *Hypertension* 2004; **43**: 48–56.
30 Tessari P, Cecchet D, Cosma A, Vettore M, Coracina A, Millioni R, Iori E, Puricelli L, Avogaro A, Vedovato M. Nitric oxide synthesis is reduced in subjects with type 2 diabetes and nephropathy. *Diabetes* 2010; **59**: 2152–2159.
31 Prabhakar S, Starnes J, Shi S, Lonis B, Tran R. Diabetic nephropathy is associated with oxidative stress and decreased renal nitric oxide production. *J Am Soc Nephrol* 2007; **18**: 2945–2952.
32 Kanetsuna Y, Takahashi K, Nagata M, Gannon MA, Breyer MD, Harris RC, Takahashi T. Deficiency of endothelial nitric-oxide synthase confers susceptibility to diabetic nephropathy in nephropathy-resistant inbred mice. *Am J Pathol* 2007; **170**: 1473–1484.
33 Wago T, Yoshimoto T, Akaza I, Tsuchiya K, Izumiyama H, Doi M, Hirata Y. Improvement of endothelial function in patients with hypertension and type 2 diabetes after treatment with telmisartan. *Hypertens Res* 2010; **33**: 796–801.

The Journal of Nutrition
Nutrition and Disease

Rice Intake Is Associated with Reduced Risk of Mortality from Cardiovascular Disease in Japanese Men but Not Women[1–3]

Ehab S. Eshak,[4] Hiroyasu Iso,[4]* Chigusa Date,[5] Kazumasa Yamagishi,[6] Shogo Kikuchi,[7] Yoshiyuki Watanabe,[8] Yasuhiko Wada,[9] Akiko Tamakoshi,[7] and JACC Study Group[10]

[4]Public Health, Department of Social and Environmental Medicine, Osaka University Graduate School of Medicine, Yamadoka, 2-2 Suita-shi, Osaka, Japan 565-0871; [5]Department of Food Science and Nutrition, Faculty of Human Life and Environment, Nara Women's University, Kitauoya Nishimachi, Nara, Japan 630-8506; [6]Department of Public Health Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Ibaraki,Tsukuba City, Japan 305-8577; [7]Department of Public Health, Aichi Medical University School of Medicine. Nagakute, Aichi, Japan 480-1195; [8]Department of Epidemiology for Community Health and Medicine, Kyoto Prefectural University of Medicine Graduate School of Medical Sciences, Kajii-cho, Kawaramachi-Hirokoji, Kamigyo-Ku, Kyoto, Japan 602-8566; and [9]Department of Health Science, Faculty of Human Life and Environmental Science, Kochi Women's University, Eikokuji-cho, 5-15 Kochi-shi, Japan 780-8515

Abstract

Rice is a staple food in Japan and provides 43% of carbohydrate and 29% of energy intake in the Japanese population. In a prospective study encompassing 83,752 Japanese men and women aged 40–79 y, rice intake was determined by self-administered FFQ. Median follow-up time was 14.1 y from 1988–1990 to the end of 2003, and HR and 95% CI of mortality were calculated according to quintiles of energy-adjusted rice intake. A total of 3514 cardiovascular deaths [1640 strokes, 707 coronary heart disease (CHD), and 560 heart failure] were documented. There was a gender difference on the effect of rice intake on the risk of cardiovascular disease (CVD). Overall, rice intake was inversely associated with CHD, heart failure, and total CVD in men but not in women. Rice intake was not associated with risk of stroke in either gender. The multivariable HR (95% CI) for the extreme quintiles of rice intake in men were 0.70 [(0.49–0.99); *P*-trend = 0.02] for CHD, 0.70 [(0.46–1.05); *P*-trend = 0.05] for heart failure, and 0.82 [(0.70–0.97); *P*-trend = 0.006] for total CVD. For women, rice was not associated with reduced risk of mortality from CVD after adjusting for lifestyle and dietary variables. In conclusion, the consumption of steamed rice was associated with reduced risk of mortality from CVD in Japanese men but not women. This finding necessitates further investigations on the mechanisms leading to this gender difference. J. Nutr. 141: 595–602, 2011.

Introduction

Carbohydrate consumption is high in Japan and rice is its major source (1). Rice provides fully 60% of energy of the food intake in Southeastern Asia and 35% in Eastern Asia and Southern Asia (2). In Japan, rice provides 43 and 29% of carbohydrate and energy intake, respectively (3). Japanese people call their 3 daily meals morning rice (Asa Gohan), lunch rice (Hiru Gohan), and evening rice (Ban Gohan). The Japanese word 'Gohan' means cooked rice. Cooked rice is equal to meal, implying how important rice is for the Japanese people (3).

A high intake of total carbohydrates was positively associated with risk of cardiovascular disease (CVD)[11] (4–6), but the association between carbohydrate intake from refined grain, as white rice, with risk of CVD showed mixed results among women: a nonsignificant positive association with risk of coronary heart disease (CHD) (7), a nonsignificant inverse association with risk of CHD (8), and no associations with ischemic stroke (9) and total CVD (10). On the other hand, among men, carbohydrate intake was not associated with either incidence (6,11,12) or mortality (13) from CHD. Rice intake

[1] 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) to The Japan Collaborative Cohort (JACC) Study.

[2] Author disclosures: E. S. Eshak, H. Iso, C. Date, K. Yamagishi, S. Kikuchi, Y. Watanabe, Y. Wada, A. Tamakoshi, and JACC Study Group, no conflicts of interest.

[3] Supplemental Tables 1 and 2 and Supplemental Figure 1 are available with the online posting of this paper at jn.nutrition.org.

[10] Members of the JACC Study Group are as follows: A. Tamakoshi, M. Mori, F. Sakauchi, Y. Motohashi, I. Tsuji, Y. Nakamura, H. Iso, H. Mikami, M. Kurosawa, Y. Hoshiyama, N. Tanabe, K. Tamakoshi, K. Wakai, S. Tokudome, K. Suzuki, S. Hashimoto, S. Kikuchi, Y. Wada, T. Kawamura, Y. Watanabe, K. Ozasa, T. Miki, C. Date, K. Sakata, Y. Kurozawa, T. Yoshimura, Y. Fujino, A. Shibata, N. Okamoto, and H. Shio.

* To whom correspondence should be addressed. E-mail: fvgh5640@mb.infoweb.ne.jp.

[11] Abbreviations used: CHD, coronary heart disease; CVD, cardiovascular disease; DR, dietary record; JACC, Japan Collaborative Cohort Study.

Manuscript received September 2, 2010. Initial review completed October 5, 2010. Revision accepted January 13, 2011.
First published online February 23, 2011; doi:10.3945/jn.110.132167.

was not associated, in either gender, with mortality from stroke among Japanese (14) or acute myocardial infarction among Italians (12).

Among women, high-carbohydrate intake may have adverse effects on lipid and glucose metabolism (15,16), which is expected to increase CVD risk. On the other hand, carbohydrate intake was not associated with blood concentrations of fasting TG, HDL-cholesterol, insulin, or glucose levels among men (11). In an Indian study, rice had the least potential of increasing postprandial hyperglycemia and TG levels compared with other carbohydrate sources such as white bread (17). These findings taken together warrant gender-specific analyses to examine the rice-disease associations.

Furthermore, it was reported that the adverse effects of a high-carbohydrate diet on CVD risk are more evident for obese than for lean participants (4,5). Because Japanese populations have a lower mean BMI than Western populations do, the examination of the rice-disease associations by BMI stratification may be useful to determine whether rice intake would be protective among a high-CVD risk group with higher BMI.

In our population, rice intake was associated with lower age-adjusted mortality from stroke and CHD in men but not women, but other confounding variables were not taken into account (18). We benefited from the large sample size and long follow-up of participants in the Japan Collaborative Cohort (JACC) Study to examine the relationship between rice intake, as a major source of carbohydrate, and subsequent risk of mortality from CVD in this cohort.

Participants and Methods

Study population. The JACC Study, a large prospective study sponsored by the Ministry of Education, Sports and Science, was conducted from 1988 to 1990. A total of 110,792 participants (n = 46,465 men and 64,327 women) aged 40–79 y from 45 communities across Japan completed self-administered questionnaires about their lifestyles and medical histories. The sampling methods and protocols of the JACC Study were previously described in detail (19). Informed consent was obtained from participants or community leaders. The ethics committees of the Nagoya University School of Medicine and Osaka University approved the protocol of this investigation.

We excluded from analysis 16,109 participants (4683 men and 11,426 women) who reported a medical history of cancer, stroke, or CHD. We also excluded those whose responses to the FFQ were insufficient, which means 1 or more of the following: failure to give an answer to 5 or more items of the 40 food items of the FFQ, and/or no answer for current rice intake, and/or no answer for current miso soup intake, and/or no answer for current alcohol consumption. Participants with implausible energy intake [<500 kcal/d (<2096 kJ/d) or >3500 kcal/d (>14,645 kJ/d)] were excluded (189 participants). A total of 83,752 individuals (35,064 men and 48,688 women) were eligible for the study (**Supplemental Fig. 1**).

Mortality surveillance. Investigators reviewed death certificates, which were 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 according to the International Statistical Classification of Diseases and Related Health Problems, 10th revised edition (ICD-10). Participants who died after removal from their original communities were treated as censored cases; of the total 83,752 participants, 3436 (4.1%) moved out. Cause-specific mortality was categorized as stroke (I60 to I69), CHD (I20 to I25), heart failure (I50 to I59), and total CVD (I01 to I99).

Diet and baseline survey. Each participant was asked to fill in a self-administered questionnaire, including a FFQ, to collect the baseline data for demographic characteristics, past and familial medical histories, and other data. Participants were asked to record the frequency of the intake of 40 foods without specifying portion size. The response was based on the usual food intake for the past year. Five responses were possible for each food item: rarely, 1–2 times/mo, 1–2 times/wk, 3–4 times/wk, and almost every day (19). The consumption of each food item was calculated by multiplying the frequency score of consumption of each food by 0, 0.38, 1.5, 3.5, and 7.0/wk, respectively. Each portion size was estimated from a validation study (19) conducted in 85 participants of the baseline participants. As for the consumed amount of rice, it was calculated according to the number of medium-sized bowls of rice consumed in the replies in the FFQ. Each bowl was estimated to contain 140 g of steamed rice (19). The energy-adjusted intakes of rice and other foods were calculated by the residual method (20). Key's dietary score, a method of expressing the fat quality of the diet, was calculated using this formula: Key's dietary score = $1.35\times$ [2 × SFA (% energy) − PUFA (% energy)] + 1.52 × [cholesterol intake (mg/1000 kcal)]^2. A high score indicates that the diet caused increasing total blood cholesterol levels (21).

The FFQ was validated by using four 3-d weighed dietary records (DR) over a 1-y period as a reference standard. The Spearman rank correlation coefficients for rice intake between the FFQ and four 3-d DR were 0.63 ($P < 0.001$) for 85 individuals in the validation study and 0.62 ($P < 0.001$) between 2 FFQ conducted 1 y apart (19). The rice intake from the second FFQ (mean ± SD) (336 ± 99 g/d) did not differ from that for the DR (317 ± 87 g/d) ($P = 0.20$).

Statistical analysis. The analyses were carried out separately for men and women, and the amount of gender-specific, energy-adjusted rice intake was modeled as categorical (5 quintile groups) variables in primary analysis. For each participant, the person-years of follow-up were calculated from the date that the baseline questionnaire was completed until the time of death, the participant moved out of the community, or the end of 2003 or 1999 (in 4 study areas), whichever occurred first.

Because most distributions for dietary variables are skewed, gender-specific medians with IQR or proportions of cardiovascular risk factors were calculated. The HR and 95% CI for mortality by disease outcome were calculated by using the Cox proportional hazard model with reference to the risk according to quintiles of rice intake. Estimates were presented as 3 models; the first model was adjusted for age only. The second model was adjusted for age, cardiovascular risk factors, and selected lifestyle and dietary variables, including history of hypertension, history of diabetes, quintiles of BMI, smoking status (never, ex-smoker, current smoker of 1–19 and ≥20 cigarettes/d), alcohol consumption (never, ex-drinker, current drinker of 0.1–22.9, 23.0–45.9, 46.0–68.9, and ≥69.0 g ethanol/d), hours of exercise (almost never, 1–2, 3–4 and ≥5 h/wk), hours of walking (almost never, 0.5, 0.6–0.9, and ≥1 h/d), perceived mental stress (low, moderate, and high), education level (primary school, junior high school, high school, and college or higher), sleep duration (≤6, 6 to <7, 7 to <8, 8 to >9, and ≥9 h/d), and energy-adjusted quintiles of selected food intakes including fish, fruit, vegetable, meat, milk and dairy products, soy, and total energy intake (quintiles); this was to exclude potential confounding that may arise from differences in dietary patterns and food choices among participants with high rice consumption and those without it. The last model was further adjusted for sodium intake and Key's dietary score to examine whether the rice and CVD association was independent of dietary lipid factors. We conducted tests for trend in means or proportions for each confounding variable considered across quintiles of rice intake after assigning median values for each quintile. To assess potential effect modification by BMI, we further conducted a stratified analysis by BMI tertiles for both genders. Tests for effect modification by gender or BMI were conducted with an interaction term generated by multiplying the median of each quintile of rice intake by gender or BMI. Sensitivity analyses were performed by excluding persons with a history of diabetes (n = 3659); the rationale for their exclusion was potential effect modification and potential changes in dietary habits as a result of diagnosis and treatment. Further, we tested the association between rice intake (g/d, continuous) with CVD by a 1-SD increment of energy-adjusted rice intake. We also calculated the multivariable HR of

TABLE 1 Baseline characteristics of Japanese men and women according to quintiles of energy-adjusted rice intake[1]

	Men					Women				
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	Q1 (low)	Q2	Q3	Q4	Q5 (high)
Rice intake, *g/d*	280 (0–396)	420 (397–446)	449 (447–551)	583 (552–655)	711 (656–1680)	279 (0–309)	259 (310–397)	420 (398–420)	453 (421–493)	560 (494–1680)
Participants at risk, *n*	7012	7013	7013	7013	7013	9737	9738	11016	8460	9737
Age, *y*	59 (49–66)	60 (51–67)	56 (47–63)	56 (48–63)	57 (49–63)	59 (50–66)	56 (48–63)	60 (52–67)	56 (48–64)	57 (50–63)
BMI, kg/m^2	22.6 (20.7–24.4)	22.3 (20.6–24.2)	22.5 (20.8–24.4)	22.5 (20.7–24.3)	22.5 (20.8–24.3)	22.6 (20.8–24.6)	22.7 (20.8–24.7)	22.8 (20.8–24.8)	22.8 (20.9–24.9)	22.8 (20.8–24.9)
History of hypertension, %	22	24	21	19	17	23	20	26	23	22
Hypertension medication, %	14	14	13	11	9	19	21	21	18	20
History of diabetes mellitus, %	7	8	7	6	4	5	3	4	4	3
Ethanol intake, *g/d*	46 (23–69)	34 (23–46)	29 (23–46)	23 (11–46)	23 (10–46)	7 (3–23)	5 (2–11)	5 (2–11)	5 (2–11)	5 (2–11)
Current smoker, %	55	53	54	54	55	6	4	5	5	5
College or higher education, %	21	19	20	16	13	14	12	8	9	8
Exercise ≥5 h/wk, %	35	35	33	30	25	28	25	25	21	19
Walking ≥1 h/d, %	69	67	68	69	72	71	72	72	72	73
Food group intakes[2], *g/d*										
Fish	63 (39–85)	48 (30–75)	44 (27–69)	36 (22–53)	33 (21–53)	69 (43–88)	48 (34–75)	44 (28–70)	39 (25–53)	33 (21–49)
Vegetables	117 (66–773)	98 (58–733)	85 (51–146)	70 (74–117)	64 (39–105)	214 (93–808)	123 (80–772)	105 (68–744)	87 (57–149)	69 (46–116)
Fruit	80 (34–127)	80 (34–127)	61 (34–114)	54 (23–88)	50 (23–88)	127 (88–161)	114 (80–161)	107 (61–127)	80 (46–127)	61 (34–107)
Meat	32 (20–49)	27 (16–40)	25 (16–36)	22 (13–32)	20 (11–32)	36 (23–54)	29 (19–42)	26 (16–38)	23 (13–33)	20 (10–31)
Milk and dairy products	146 (32–152)	106 (31–150)	78 (12–147)	37 (7–146)	31 (1–130)	147 (78–171)	146 (73–154)	98 (31–151)	73 (11–146)	31 (1–95)
Soy	63 (38–85)	48 (30–75)	43 (27–69)	36 (22–53)	33 (21–53)	69 (43–88)	48 (34–75)	44 (28–70)	39 (25–53)	33 (21–49)
Egg	37 (18–37)	18 (8–37)	18 (8–37)	18 (8–37)	18 (8–37)	37 (18–37)	37 (18–37)	18 (8–37)	18 (8–37)	18 (8–37)
Sodium intake,[2] *mg/d*	2743 (2115–3320)	2368 (1768–2954)	2183 (1576–2769)	1935 (1330–2553)	1589 (1094–2229)	2658 (2080–3240)	2197 (1652–2741)	2021 (1530–2541)	1823 (1357–2354)	1517 (1100–2101)
Energy intake, *kJ/d*	7293 (7093–7817)	7293 (7093–7393)	6619 (5853–8269)	7293 (5686–8340)	7293 (7293–7545)	6029 (6029–6397)	6376 (5011–6820)	6029 (6029–6029)	5623 (5263–5949)	6029 (5078–7021)
Keys dietary score,[3] *mmol/L*	0.80 (0.71–0.91)	0.77 (0.72–0.88)	0.72 (0.61–0.83)	0.63 (0.54–0.73)	0.53 (0.44–0.61)	0.97 (0.88–1.1)	0.88 (0.79–0.97)	0.81 (0.72–0.90)	0.74 (0.65–0.83)	0.59 (0.50–0.69)

[1] Values are median (IQR) or percentage.
[2] Values are adjusted for energy intake.
[3] Key's dietary score in mg/dL was calculated by this formula: Key's dietary score = 1.35 x [2 x SFA (% energy) − PUFA (% energy)] + 1.52 x [cholesterol intake (mg/1000kcal)] [2] and transformed into mmol/L by multiplying by 0.0259.

mortality after the exclusion of deaths that occurred within 5 y from baseline to check potential reverse causation for rice intake and mortality risk. All *P*-values were 2-sided and $P < 0.05$ was the significance level.

Results

At baseline, compared with men with the lowest rice intake, men with higher rice intakes were younger, less educated, and less likely to smoke, to practice sports, and to have a history of diabetes or hypertension. Women in the highest quintile of rice intake were younger, had a higher BMI, were less educated, and were more likely to smoke and to have a history of diabetes. Furthermore, higher rice intake in both men and women was associated lower intakes of alcohol, fish, meat, vegetables, fruit, and soy and a lower Key's dietary score (**Table 1**).

Among the 83,752 adults aged 40–79 y at the baseline examination, there were 3514 (1927 men and 1587 women) total CVD deaths during the 14.1-y follow-up, comprising 1640 (874 men and 766 women) deaths from stroke, 707 (429 men and 278 women) from CHD, and 560 (295 men and 265 women) from heart failure.

Among men, age-adjusted HR for mortality from CHD, heart failure, and total CVD were lower in the highest compared with the lowest quintiles of rice intake, and after adjustment for cardiovascular risk factors and selected lifestyle and dietary variables, these inverse associations were slightly strengthened (**Table 2**). After further adjustment for sodium intake and Key's dietary score, the associations did not materially change. There was no association between rice intake and mortality from ischemic or hemorrhagic stroke (data not shown). Among women, in the age-adjusted model, an excess risk of mortality from total CVD was observed at the highest quintile of rice intake. However, after adjustment for lifestyle and dietary variables, the excess risk disappeared. No associations were found for rice intake with stroke, CHD, or heart failure in either age- or multivariable-adjusted models. The results did not change for either men or women after the exclusion of participants with a history of diabetes mellitus from the total participants (**Supplemental Table 1**).

Furthermore, the multivariable-adjusted HR (95% CI) for mortality of CVD by a 1-SD increment of energy-adjusted rice intake (174.4 g for men and 131.5 g for women) were 0.97 (0.90–1.04) for stroke, 0.90 (0.81–0.99) for CHD, 0.86 (0.76–0.97) for heart failure, and 0.92 (0.88–0.97) for total CVD in men and were 0.97 (0.89–1.06) for stroke, 0.99 (0.88–1.17) for CHD, 1.08 (0.94–1.24) for heart failure, and 1.02 (0.96–1.09) for total CVD in women (data not shown).

TABLE 2 Gender-specific HR (95% CI) for mortality from CVD among Japanese men and women according to quintiles of energy-adjusted rice intake

	Men						Women					
	Q1	Q2	Q3	Q4	Q5	*P*-trend	Q1	Q2	Q3	Q4	Q5	*P*-trend
Person-years	85,336	85,777	87,065	88,077	90,017		123,200	124,881	142,025	107,131	124,314	
Stroke												
Cases, *n*	198	223	160	128	165		173	114	208	116	155	
Age-adjusted HR (95% CI)	1.00	0.96 (0.79–1.16)	0.98 (0.80–1.21)	0.81 (0.64–1.01)	0.98 (0.80–1.21)	0.48	1.00	0.86 (0.77–1.09)	0.94 (0.77–1.15)	0.96 (0.76–1.21)	1.15 (0.92–1.42)	0.16
Multivariable-adjusted HR (95%CI)[1]	1.00	0.95 (0.78–1.15)	0.95 (0.75–1.20)	0.75 (0.58–0.95)	0.97 (0.78–1.22)	0.43	1.00	0.84 (0.64–1.10)	0.86 (0.70–1.07)	0.84 (0.61–1.15)	0.91 (0.70–1.19)	0.49
Multivariable-adjusted HR (95%CI)[2]	1.00	0.96 (0.79–1.17)	0.96 (0.76–1.22)	0.78 (0.61–1.00)	1.02 (0.82–1.31)	0.88	1.00	0.85 (0.64–1.12)	0.89 (0.72–1.11)	0.89 (0.64–1.26)	0.99 (0.75–1.31)	0.93
CHD												
Cases, *n*	97	123	63	78	68		62	42	64	52	58	
Age-adjusted HR (95% CI)	1.00	1.10 (0.85–1.44)	0.76 (0.55–1.04)	0.95 (0.71–1.29)	0.79 (0.58–1.07)	0.07	1.00	0.93 (0.63–1.37)	0.80 (0.57–1.14)	1.24 (0.86–1.80)	1.27 (0.89–1.82)	0.13
Multivariable-adjusted HR (95%CI)[1]	1.00	1.03 (0.79–1.36)	0.71 (0.50–1.02)	0.82 (0.59–1.14)	0.70 (0.50–0.98)	0.02	1.00	0.95 (0.60–1.49)	0.79 (0.55–1.14)	1.05 (0.64–1.75)	0.97 (0.62–1.52)	0.71
Multivariable-adjusted HR (95%CI)[2]	1.00	1.04 (0.79–1.37)	0.73 (0.51–1.05)	0.85 (0.60–1.19)	0.70 (0.49–0.99)	0.02	1.00	1.01 (0.64–1.59)	0.83 (0.57–1.21)	1.25 (0.74–2.10)	1.08 (0.66–1.77)	0.98
Heart failure												
Cases, *n*	76	77	52	42	48		57	36	76	45	51	
Age-adjusted HR (95% CI)	1.00	0.86 (0.63–1.18)	0.85 (0.60–1.22)	0.71 (0.49–1.04)	0.78 (0.54–1.13)	0.09	1.00	0.85 (0.56–1.30)	1.04 (0.74–1.47)	1.15 (0.78–1.70)	1.19 (0.81–1.73)	0.22
Multivariable-adjusted HR (95%CI)[1]	1.00	0.82 (0.59–1.13)	0.85 (0.56–1.28)	0.62 (0.41–0.94)	0.68 (0.46–1.00)	0.02	1.00	0.88 (0.54–1.43)	0.92 (0.65–1.32)	1.40 (0.81–2.43)	0.99 (0.62–1.58)	0.95
Multivariable-adjusted HR (95%CI)[2]	1.00	0.83 (0.60–1.15)	0.89 (0.58–1.35)	0.65 (0.42–1.00)	0.70 (0.46–1.05)	0.05	1.00	0.89 (0.54–1.46)	0.96 (0.66–1.38)	1.61 (0.90–2.82)	1.15 (0.70–1.90)	0.61
Total CVD												
Cases, *n*	464	494	331	307	331		348	233	418	274	314	
Age-adjusted HR (95% CI)	1.00	0.91 (0.80–1.03)	0.86 (0.75–0.99)	0.81 (0.70–0.94)	0.83 (0.72–0.96)	0.002	1.00	0.88 (0.75–1.05)	0.94 (0.81–1.08)	1.14 (0.97–1.33)	1.18 (1.01–1.37)	0.007
Multivariable-adjusted HR (95%CI)[1]	1.00	0.89 (0.78–1.02)	0.85 (0.72–1.00)	0.76 (0.65–0.89)	0.80 (0.69–0.93)	0.001	1.00	0.91 (0.75–1.11)	0.87 (0.75–1.01)	1.09 (0.87–1.35)	0.99 (0.83–1.20)	0.85
Multivariable-adjusted HR (95%CI)[2]	1.00	0.90 (0.79–1.03)	0.87 (0.74–1.02)	0.79 (0.67–0.93)	0.82 (0.70–0.97)	0.006	1.00	0.94 (0.78–1.14)	0.90 (0.77–1.05)	1.20 (0.94–1.51)	1.07 d(0.88–1.34)	0.66

[1] Adjusted for history of hypertension, history of diabetes, BMI, alcohol consumption, smoking status, hours of exercise, hours of walking, education level, perceived mental stress, sleep duration, selected food intakes (fish, meat, vegetable, fruit, dairy products, and soy), and total energy intake.
[2] Adjusted further for sodium intake and Key's dietary score.

TABLE 3 Gender-specific multivariable-adjusted HR (95% CI) for mortality from CVD among Japanese men and women according to quintiles of energy-adjusted rice intake stratified by tertiles of BMI

	Men						Women					
	Q1 (low)	Q2	Q3	Q4	Q5 (high)	*P*-trend	Q1 (low)	Q2	Q3	Q4	Q5 (high)	*P*-trend
Stroke												
BMI T1[1] Person-years	27,025	28,859	28,252	29,081	28,106		41,252	41,535	45,759	35,161	40,554	
Cases, *n*	80	101	72	45	73		63	44	86	49	52	
HR (95% CI)[2]	1.00 (referent)	1.09 (0.80–1.48)	1.06 (0.74–1.53)	0.70 (0.47–1.06)	1.25 (0.86–1.80)	0.59	1.00 (referent)	0.98 (0.63–1.54)	1.00 (0.70–1.42)	1.07 (0.62–1.87)	0.98 (0.60–1.62)	0.96
BMI T2[1] Person-years	28,544	28,372	27,697	29,118	32,174		43,709	41,758	48,075	34,294	40,404	
Cases, *n*	65	76	44	45	50		61	39	70	31	57	
HR (95% CI)[2]	1.00 (referent)	1.05 (0.74–1.48)	0.80 (0.51–1.24)	0.82 (0.52–1.28)	0.96 (0.63–1.47)	0.60	1.00 (referent)	1.08 (0.66–1.76)	0.85 (0.59–1.22)	0.93 (0.50–1.72)	1.15 (0.71–1.87)	0.87
BMI T3[1] Person-years	29,767	28,545	31,116	29,877	29,737		38,238	41,587	48,190	37,675	43,355	
Cases, *n*	53	46	44	38	42		49	31	52	36	46	
HR (95% CI)[2]	1.00 (referent)	0.71 (0.47–1.08)	0.98 (0.61–1.58)	0.86 (0.53–1.41)	0.90 (0.57–1.44)	0.88	1.00 (referent)	0.59 (0.35–0.98)	0.81 (0.53–1.26)	0.64 (0.33–1.21)	0.92 (0.51–1.68)	0.81
CHD												
BMI T1[1] Person-years	27,025	28,859	28,252	29,081	28,106		41,252	41,535	45,759	35,161	40,554	
Cases, *n*	32	42	28	34	25		16	17	19	21	17	
HR (95% CI)[2]	1.00 (referent)	0.97 (0.60–1.56)	0.93 (0.51–1.68)	1.06 (0.61–1.84)	0.74 (0.41–1.34)	0.41	1.00 (referent)	1.71 (0.76–3.86)	1.05 (0.51–2.17)	2.01 (0.78–5.23)	1.12 (0.42–2.95)	0.98
BMI T2[1] Person-years	28,544	28,372	27,697	29,118	32,174		43,709	41,758	48,075	34,294	40,404	
Cases, *n*	30	39	11	22	23		21	9	29	15	24	
HR (95% CI)[2]	1.00 (referent)	1.25 (0.76–2.05)	0.48 (0.22–1.02)	0.79 (0.41–1.51)	0.80 (0.43–1.49)	0.27	1.00 (referent)	1.02 (0.40–2.58)	1.08 (0.59–1.96)	1.33 (0.49–3.60)	1.25 (0.56–2.80)	0.58
BMI T3[1] Person-years	29,767	28,545	31,116	29,877	29,737		38,238	41,587	48,190	37,675	43,355	
Cases, *n*	35	42	24	22	20		25	16	16	16	17	
HR (95% CI)[2]	1.00 (referent)	0.95 (0.59–1.52)	0.74 (0.40–1.36)	0.64 (0.34–1.21)	0.59 (0.32–1.11)	0.05	1.00 (referent)	0.59 (0.28–1.25)	0.50 (0.25–1.01)	0.82 (0.32–2.11)	0.96 (0.38–2.40)	0.59
Heart failure												
BMI T1[1] Person-years	27,025	28,859	28,252	29,081	28,106		41,252	41,535	45,759	35,161	40,554	
Cases, *n*	33	37	24	22	22		17	17	27	16	18	
HR (95% CI)[2]	1.00 (referent)	0.91 (0.56–1.49)	0.76 (0.41–1.41)	0.66 (0.35–1.24)	0.81 (0.44–1.51)	0.34	1.00 (referent)	1.40 (0.60–3.26)	1.09 (0.56–2.09)	2.12 (0.73–6.12)	1.48 (0.61–3.59)	0.49
BMI T2[1] Person-years	28,544	28,372	27,697	29,118	32,174		43,709	41,758	48,075	34,294	40,404	
Cases, *n*	21	28	21	13	19		25	10	24	14	16	
HR (95% CI)[2]	1.00 (referent)	1.17 (0.65–2.09)	1.85 (0.87–3.93)	0.91 (0.42–2.00)	0.92 (0.46–1.87)	0.74	1.00 (referent)	0.70 (0.30–1.68)	0.74 (0.40–1.36)	1.81 (0.67–4.92)	1.09 (0.48–2.45)	0.99
BMI T3[1] Person-years	29,767	28,545	31,116	29,877	29,737		38,238	41,587	48,190	37,675	43,355	
Cases, *n*	22	12	7	7	7		15	9	25	15	17	
HR (95% CI)[2]	1.00 (referent)	0.42 (0.19–0.91)	0.48 (0.17–1.34)	0.38 (0.14–1.06)	0.35 (0.13–0.96)	0.03	1.00 (referent)	0.69 (0.27–1.79)	1.22 (0.61–2.46)	1.23 (0.44–3.45)	0.94 (0.35–2.54)	0.87
Total CVD												
BMI T1[1] Person-years	27,025	28,859	28,252	29,081	28,106		41,252	41,535	45,759	35,161	40,554	
Cases, *n*	172	204	134	126	132		118	91	163	107	103	
HR (95% CI)[2]	1.00 (referent)	0.98 (0.79–1.21)	0.94 (0.73–1.21)	0.86 (0.66–1.11)	0.94 (0.73–1.22)	0.47	1.00 (referent)	1.14 (0.83–1.58)	1.02 (0.79–1.31)	1.48 (1.00–2.19)	1.07 (0.75–1.53)	0.80
BMI T2[1] Person-years	28,544	28,372	27,697	29,118	32,174		43,709	41,758	48,075	34,294	40,404	
Cases, *n*	146	165	94	95	115		127	75	144	78	118	
HR (95% CI)[2]	1.00 (referent)	1.04 (0.82–1.30)	0.90 (0.67–1.21)	0.83 (0.62–1.12)	0.93 (0.71–1.23)	0.40	1.00 (referent)	1.09 (0.77–1.54)	0.84 (0.65–1.08)	1.13 (0.75–1.70)	1.19 (0.85–1.66)	0.67
BMI T3[1] Person-years	29,767	28,545	31,116	29,877	29,737		38,238	41,587	48,190	37,675	43,355	
Cases, *n*	146	125	94	86	84		103	67	111	89	93	
HR (95% CI)[2]	1.00 (referent)	0.71 (0.55–0.91)	0.73 (0.54–0.99)	0.67 (0.49–0.92)	0.64 (0.47–0.88)	0.005	1.00 (referent)	0.68 (0.48–0.96)	0.83 (0.62–1.12)	0.96 (0.63–1.47)	1.00 (0.67–1.49)	0.95

[1] BMI tertiles: T1 ≤ 21.4 kg/m^2 for men and ≤ 21.6 kg/m^2 for women; T2 = 21.5–23.5 kg/m^2 for men and 21.7–23.8 kg/m^2 for women; T3 > 23.5 kg/m^2 for men and > 23.8 kg/m^2 for women.

[2] Adjusted for age, history of hypertension, history of diabetes, BMI, alcohol consumption, smoking status, hours of exercise, hours of walking, education level, perceived mental stress, sleep duration, energy-adjusted quintiles of selected food intakes (fish, meat, vegetable, fruit, dairy products, and soy), total energy intake, sodium intake, and Key's dietary score.

We investigated the associations between rice intake and CVD risk after stratifying by BMI tertiles (**Table 3**). Inverse associations between rice intake with CHD, heart failure, and CVD were more evident for men in the highest BMI tertile than for those in the lowest or modest BMI tertiles. The multivariable-adjusted HR (95% CI) for the highest compared with the lowest quintiles of rice intake among men with the highest BMI tertile were 0.90 (0.57–1.44; *P*-trend = 0.88) for stroke, 0.59 (0.32–1.11; *P*-trend = 0.05) for CHD, 0.35 (0.13–0.96; *P*-trend = 0.03) for heart failure, and 0.64 (0.47–0.88; *P*-trend = 0.005) for CVD. There was a weaker and nonsignificant inverse association between rice intake and CHD risk for women in the highest BMI tertile (*P*-trend = 0.59). There were no interactions with gender or BMI for all endpoints (*P*-interaction > 0.05).

To examine potential reverse causation for rice intake and mortality risk, we calculated the multivariable HR of mortality after the exclusion of deaths that occurred within 5 y from baseline. For men, the inverse associations of rice intake with mortality from CHD and total CVD were slightly attenuated; the HR (95% CI) for the highest compared with the lowest quintiles of rice intake were 0.77 (0.54–1.15; *P*-trend = 0.08) for CHD and 0.84 (0.69–1.00; *P*-trend = 0.03) for total CVD, whereas that from heart failure was slightly strengthened [0.51 (0.28–0.91; *P*-trend = 0.01)]. For women, there were no material changes in risks of mortality (**Supplemental Table 2**).

Discussion

This 14-y prospective cohort study of Japanese men and women aged 40–79 y showed that rice intake was associated with reduced risks of mortality from CHD, heart failure, and total CVD in men but not women. These inverse associations did no change or became slightly stronger after adjustment for cardiovascular risk factors and lifestyle and dietary variables. When stratified by BMI, the strong inverse associations appeared to be evident among men with a BMI in the top tertile (≥23.5 kg/m^2).

To our knowledge, our research should be considered original in investigating the association between rice intake as a specific cereal and CVD. A recent Japanese study found no significant association between rice intake and stroke mortality in either gender after adjustment for potential confounding variables, although a positive association was observed among women in the age-adjusted model (14).

The finding that high-saturated fat diets increase risk of CHD led to recommendations to replace total and saturated fat intake with carbohydrate intake (15,22). However, in postmenopausal women, such low-fat, high-carbohydrate diets affect lipid and glucose metabolism adversely, leading to increased TG, decreased HDL-cholesterol, and enhanced insulin resistance (16). Women in our population were (mean ± SD) 57.5 ± 10 y old and 64% of them were postmenopausal at the baseline examination, which might explain why increased rice intake was not associated with CVD risk. The quality of carbohydrate is critical in that issue. Dietary guidelines advise the substitution of simple sugars and fat with complex carbohydrates (22) and white rice is a starchy food that is considered a desirable complex carbohydrate. Meanwhile, white rice is digested and absorbed quickly and has a relatively high glycemic index (4). Previous studies of women reported protective effects of whole grain consumption on risks of CHD (7,8,10), ischemic stroke (9), heart failure (23), and total CVD (10), whereas total refined grain consumption was not associated with risk of CHD (8,10) or total CVD (10). Interestingly, intake of refined grains, including white rice, was inversely associated with mortality from CHD; however, the association was not significant in the fully adjusted model [HR (95% CI) = 0.69 (0.52–1.21); *P*-trend = 0.29] (8).

The effect of high-carbohydrate intake on risk of CVD differs between genders; it may increase the risk of CHD among women (4,5,11) but not men (6,11). Two previous cohort studies of men suggested a weak inverse association between carbohydrate intake, mainly rice, and risk of CHD (24,25). In the Honolulu Heart Program, 8000 men of Japanese ancestry were followed-up for 10 y; those who later experienced coronary events had lower intakes of carbohydrates (249.8 g/d) than did those who did not develop CHD (265.4 g/d) ($P < 0.001$). In the Puerto Rico Heart Health Program, a significant inverse association between carbohydrate intake, chiefly that derived from rice, and CHD was found; carbohydrate intakes were 253 g/d for men who developed CHD compared with 273 g/d for those who did not ($P < 0.01$). However, neither study adjusted for total energy intake, so those associations may be confounded by physical activity.

A possible reason for this gender difference regarding the effect of carbohydrate intake on risk of CVD could be that lipoprotein changes in response to low-fat and high-carbohydrate diets differ according to gender, with greater increases in TG and VLDL-cholesterol levels and decreases in HDL-cholesterol levels in women than in men (26). Such changes in the blood lipids, i.e. increased TG (27,28) and decreased HDL-cholesterol levels (29), are stronger risk factors for CHD in women than in men.

Rice intake was reported to be positively associated with risk of type 2 diabetes in the Shanghai cohort women (30) and recently among Japanese women but not men (31). Furthermore, diabetic women had a greater risk of developing CVD than diabetic men (32). However, in an Indian study, rice had the least potential of increasing postprandial hyperglycemia and TG levels compared with other carbohydrate sources such as white bread in both diabetic and nondiabetic participants; however, the author did not present gender-specific data (17). Also, a higher carbohydrate intake and dietary glycemic index were not associated with fasting or postload blood concentrations of insulin or glucose in men (11). When we tested the association between rice intake and CVD without adjusting for a history of diabetes, the results did not change materially. Moreover, we found that rice intake was inversely associated with total CVD in both diabetic [HR = 0.54 (95% CI = 0.29–1.01; *P*-trend = 0.02)] and non-diabetic men [HR = 0.86 (95% CI = 0.73–1.04; *P*-trend = 0.04)] but not diabetic [HR = 0.48 (95% CI = 0.16–1.42; *P*-trend = 0.23)] or nondiabetic [HR = 1.13 (95% CI = 0.92–1.40; *P*-trend = 0.42)] women. We could not separately analyze such comparisons regarding risks of stroke, CHD, or heart failure due to the small number of participants in the diabetic category.

In some Western studies, high-carbohydrate diets had more adverse effects on risk of CVD among obese participants than among lean individuals (4,5). However, Japanese populations have a lower mean BMI than Western populations do. In our analysis stratified by BMI, high rice intake was inversely associated with risk of mortality from CHD, heart failure, and total CVD among men in the top tertile of BMI. We also found a weaker and nonsignificant association with risk of CHD among women in the top tertile of BMI. Because male gender along with higher BMI are effects in the high risk group for developing CHD in Japan (33), it is plausible that a protective effect of rice was more evident among men with higher BMI.

Japanese rice is responsible for the provision of many important nutrients, including magnesium, zinc, copper (34),

vitamin B-6 (35), and dietary fiber (36). Dietary vitamin B-6 intake was associated with reduced mortality from CHD in the JACC Study (37) and in another large Japanese cohort (35). Rice was the major source of vitamin B-6 and the greatest contributor to variability (11.5% of total variability) (35). Also, dietary fiber was inversely associated with mortality from CHD and total CVD in the JACC Study, and rice was a major source of dietary fiber and was the second greatest contributor to variability (14.0% of total variability) (36). Rice, which is low in sodium (38), serves as an aid in treating hypertension (39). Moreover, rice is low in fat and is free of cholesterol (40), a dietary risk factor of CHD (41). One of the characteristics of Japanese steamed rice is cooking only with water without addition of butter, margarine, soup, or animal fat.

Limitations of this study include the lack of multiple measurements of dietary variables to reduce measurement errors and to better assess temporal relationships between rice intake and mortality from CVD. Second, no data on glycemic load or index were available because of limited food items in the FFQ for the calculation of these indices. Lastly, rice consumption and food choices associated with rice consumption may substitute food choices that potentially affect risk of CVD. We could not run a complete substitution model due to limited food items in the FFQ. However, our adjustment for selected food groups (fish, fruit, meat, etc.) may partly exclude potential confounding from differences in the underlying dietary patterns.

The present study has several methodological strengths. The exclusion of persons with known CVD or cancer at baseline reduced bias arising from dietary changes due to known diseases. The results from a cohort of community residents are more relevant to generalizability. Furthermore, when we excluded participants who died of CVD within 5 y from the baseline survey to reduce a potential effect of preexisting unknown illness and disease, the associations did not substantially change. This supported that the inverse causality was unlikely.

In summary, we found that the intake of steamed rice was inversely associated with mortality from CHD, heart failure, and total CVD in Japanese men but not women. This gender difference could be explained by the stronger adverse effects of high-carbohydrate intake on lipid metabolism in women but needs further investigation. However, dietary habits are becoming more Westernized with a decrease in rice consumption and an increase in fat consumption in Japan. It might be an important issue in nutritional education to draw attention to the benefits of rice consumption.

Acknowledgments

H.I., C.D., K.Y., S.K., Y.W., Y.W., and A.T. designed the study and conducted the research; E.S.E. analyzed the data and wrote the manuscript; and E.S.E. and H.I. had primary responsibility for the final content. All authors read and approved the final manuscript.

Literature Cited

1. Ministry of Health, Labor and Welfare. Nutrition status based on the national nutrition survey in Japan. Tokyo: Daiichi Shuppan; 2007.
2. Kenneth F, Kriemhild CO. The Cambridge world history of food. 2nd ed. Cambridge: The Cambridge University Press; 2000.
3. Murakami K, Sasaki S, Takahashi Y, Okubo H, Hosoi Y, Horiguchi H, Oguma E, Kayama F. Dietary glycemic index and load in relation to metabolic risk factors in Japanese female farmers with traditional dietary habits. Am J Clin Nutr. 2006;83:1161–9.
4. Liu S, Willett WC, Stampfer MJ, Hu FB, Franz M, Sampson L, Henkens CH, Manson JE. A prospective study of dietary glycemic load, carbohydrate intake, and risk of coronary heart disease in US women. Am J Clin Nutr. 2000;71:1455–61.
5. Oh K, Hu FB, Cho E, Rexrode KM, Stampfer MJ, Manson JE, Liu S, Willett WC. Carbohydrate intake, glycemic index, glycemic load, and dietary fiber in relation to risk of stroke in women. Am J Epidemiol. 2005;161:161–9.
6. Sieri S, Korgh V, Berrino F, Evangelista A, Angoli C, Brighenti F, Pellegrini N, Palli D, Masala G, et al. Dietary glycemic load and index and risk of coronary heart disease in a large Italian cohort: The EPICOR Study. Arch Intern Med. 2010;170:640–7.
7. Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, Hennekens CH, Willett WC. Whole-grain consumption and risk of coronary heart disease: results from the Nurses' Health Study. Am J Clin Nutr. 1999;70:412–9.
8. Jacobs DR, Meyer KA, Kushi LH, Folsom AR. Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Women's Health Study. Am J Clin Nutr. 1998;68:248–57.
9. Liu S, Manson JE, Stampfer MJ, Rexrode KM, Hu FB, Rimm E, Willett WC. Whole grain consumption and risk of ischemic stroke in women: a prospective study. JAMA. 2000;284:1534–40.
10. Jacobs DR, Meyer KA, Kushi LH, Folsom AR. Is whole grain intake associated with reduced total and cause-specific death rates in older women? The Iowa Women's Health Study. Am J Public Health. 1999;89:322–9.
11. van Dam RM, Visscher AW, Feskens EJ, Verhoef P, Kromhout D. Dietary glycemic index in relation to metabolic risk factors and incidence of coronary heart disease: the Zutphen Elderly Study. Eur J Clin Nutr. 2000;54:726–31.
12. Tavani A, Bosetti C, Negri E, Augustin LS, Jenkins DJA, La Veccia C. Carbohydrates, dietary glycemic load and glycemic index, and risk of acute myocardial infarction. Heart. 2003;89:722–6.
13. Levitan EB, Mittleman MA, Hakansson N, Wolk A. Dietary glycemic index, dietary glycemic load, and cardiovascular disease in middle-aged and older Swedish men. Am J Clin Nutr. 2007;85:1521–6.
14. Oba S, Nagata C, Nakamura K, Fuji K, Kawachi T, Takatsuka N, Shimizu H. Dietary glycemic index, glycemic load, and intake of carbohydrate and rice in relation to risk of mortality from stroke and its subtypes in Japanese men and women. Metabolism. 2010;59:1574–82.
15. Liu S, Manson JE, Stampfer MJ, Holmes MD, Hu FB, Hankinson SE, Willett WC. Dietary glycemic load assessed by food frequency questionnaire in relation to plasma high-density-lipoprotein cholesterol and fasting plasma triacylglycerols in postmenopausal women. Am J Clin Nutr. 2001;73:560–6.
16. Jeppesen J, Schaaf P, Jones C, Zhou MY, Reaven GM. Effects of low-fat, high-carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am J Clin Nutr. 1997;65:1027–33.
17. Ezenwaka CE, Kallo R. Carbohydrate-induced hypertriglycridemia among west Indian diabetic and non diabetic after ingestion of three local carbohydrate foods. Indian J Med Res. 2005;121:23–31.
18. Iso H, Kubota Y, Japan Collaborative Cohort Study for Evaluation of Cancer. Nutrition and disease in Japan Collaborative Cohort Study for Evaluation of Cancer (JAAC). Asian Pac J Cancer Prev. 2007;Suppl 8:35–80.
19. Date C, Fukui M, Yamamoto A, Wakai K, Ozeki K, Motohashi Y, Adachi C, Okamoto N, Kurosawa M, et al. Reproducibility and validity of a self-administered food frequency questionnaire used in the JACC Study. J Epidemiol. 2005;15 Suppl 1:9–23.
20. Willett W, Stampfer MJ. Total energy intake: implication for epidemiologic analysis. Am J Epidemiol. 1986;124:17–27.
21. Keys A, Parlin RW. Serum cholesterol response to changes in dietary lipids. Am J Clin Nutr. 1966;19:175–180.
22. AHA. Dietary guidelines for healthy American adults. Circulation. 1996;94:1795–800.
23. Djousse L, Gaziano JM. Breakfast cereals and risk of heart failure in the physicians' health study I. Arch Intern Med. 2007;167:2080–5.
24. McGee DL, Reed DM, Yano K, Kagan A. Ten-year incidence of coronary heart disease in the Honolulu Heart Program: relationship to nutrient intake. Am J Epidemiol. 1984;119:667–76.
25. Garcia-Palmieri MR, Sorlie P, Tillotson J, Costas R Jr, Cordero E, Rodriguez M. Relationship of dietary intake to subsequent coronary

heart disease incidence: The Puerto Rico Heart Health Program. Am J Clin Nutr. 1980;33:1818–27.
26. Knopp RH, Paramsothy P, Retzlaff BM, Fish B, Walden C, Dowdy A, Tsunehara C, Aikawa K, Cheung MC. Gender differences in lipoprotein metabolism and dietary response: basis in hormonal differences and implications for cardiovascular disease. Curr Atheroscler Rep. 2005;7:472–9.
27. Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk. 1996;3:213–9.
28. Iso H, Naito Y, Sato S, Kitamura A, Okamura T, Sankai T, Shimamoto T, Iida M, Komachi Y. Serum triglycerides and risk of coronary heart disease among Japanese men and women. Am J Epidemiol. 2001;153:490–9.
29. Gordon DJ, Probstfield JL, Garrison RJ, Neaton JD, Castelli WP, Knoke JD, Jacobs DR Jr, Bangdiwala S, Tyroler HA. High-density lipoprotein cholesterol and cardiovascular disease. Four prospective American studies. Circulation. 1989;79:8–15.
30. Villegas R, Liu S, Gao YT, Yang G, Li H, Zheng W, Shu XO. Prospective study of dietary carbohydrates, glycemic index, glycemic load, and incidence of type 2 diabetes mellitus in middle-aged Chinese women. Arch Intern Med. 2007;167:2310–6.
31. Nanri A, Mizoue T, Noda M, Takahashi Y, Kato M, Inoue M, Tsugane S. Rice intake and type 2 diabetes in Japanese men and women: the Japan Public Health Center-based Prospective Study. Am J Clin Nutr. 2010;92:1468–77.
32. Wannamethee SG, Perry IJ, Shaper AG. Nonfasting serum glucose and insulin concentrations and the risk of stroke. Stroke. 1999;30:1780–6.
33. Cui R, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S, Kondo T, Watanabe Y, Koizumi A, et al. Body mass index and mortality from cardiovascular disease among Japanese men and women: the JACC study. Stroke. 2005;36:1377–82.
34. Imaeda N, Tokudome Y, Ikeda M, Kitagawa I, Fujiwara N, Tokudome S. Foods contributing to absolute intake and variance in intake of selected vitamins, minerals and dietary fiber in middle-aged Japanese. J Nutr Sci Vitaminol (Tokyo). 1999;45:519–32.
35. Ishihara J, Iso H, Inoue M, Iwasaki M, Okada K, Kita Y, Kokubo Y, Okayama A, Tsugane S. Intake of folate, vitamin B6 and vitamin B12 and the risk of CHD: The Japan Public Health Center-Based Prospective Study Cohort I. J Am Coll Nutr. 2008;27:127–36.
36. Eshak SE, Iso H, Date C, Kikuchi S, Watanabe Y, Wada Y, Wakai K, Tamakoshi A. Dietary fiber Intake is associated with reduced risk of mortality from cardiovascular disease among Japanese men and women. J Nutr. 2010;140:1445–53.
37. Cui R, Iso H, Date C, Kikuchi S, Tamakoshi A, Japan Collaborative Cohort Study Group. Dietary folate and vitamin B6 and B12 intake in relation to mortality from cardiovascular diseases in Japan: Japan Collaborative Cohort Study. Stroke. 2010;41:1285–9.
38. Kempner W. Some effects of the rice diet treatment of kidney disease and hypertension. Bull N Y Acad Med. 1946;22358–70.
39. Cirillo M, Del Guidice L, Bilancio G, Franzese MD, De Santo NG. Low salt diet and treatment of hypertension: an old story. J Nephrol. 2009;22 Suppl 14:136–8.
40. Kik MC. The nutritive value of rice and its by-products. Arkansas Agricultural Experiment Station Bulletin. 589, 1957.
41. Shekelle RB, Stamber J. Dietary cholesterol and ischemic heart disease. Lancet. 1989;1:1177–9.

Environ Health Prev Med
DOI 10.1007/s12199-011-0251-9

REGULAR ARTICLE

Preliminary assessment of ecological exposure of adult residents in Fukushima Prefecture to radioactive cesium through ingestion and inhalation

Akio Koizumi · Kouji H. Harada · Tamon Niisoe · Ayumu Adachi · Yukiko Fujii · Toshiaki Hitomi · Hatasu Kobayashi · Yasuhiko Wada · Takao Watanabe · Hirohiko Ishikawa

Received: 21 October 2011 / Accepted: 23 October 2011

Abstract
Objective This study aims to estimate the ecological exposure of adult residents of Fukushima Prefecture to ^{134}cesium (Cs) and ^{137}Cs through ingestion and inhalation between July 2 and July 8, 2011.
Methods Fifty-five sets of meals with tap water, each representing one person's daily intake, were purchased in local towns in Fukushima Prefecture. Locally produced cow's milk (21 samples) and vegetables (43 samples) were also purchased. In parallel, air sampling was conducted at 12 different sites using a high-volume sampler. Nineteen sets of control meals were collected in Kyoto in July 2011. ^{134}Cs and ^{137}Cs levels in the samples were measured using a germanium detector.
Results Radioactivity was detected in 36 of the 55 sample meals from Fukushima, compared with one of 19 controls from Kyoto. The median estimated dose level (μSv/year) was 3.0, ranging from not detectable to 83.1. None of the cow's milk (21) or vegetable (49) samples showed levels of contamination above the current recommended limits (Bq/kg) of 200 for milk and 500 for vegetables. The total effective dose levels by inhalation were estimated to be <3 μSv/year at nine locations, but samples at three other locations close to the edge of the 20-km radius from the crippled nuclear power plant showed higher levels of contamination (μSv/year): 14.7 at Iitate, 76.9 at Namie, and 27.7 at Katsurao.
Conclusions Levels of exposure to ^{134}Cs and ^{137}Cs in Fukushima by ingestion and inhalation are discernible, but generally within recommended limits.

Keywords ^{134}Cs · ^{137}Cs · Exposure assessment · Fukushima Daiichi nuclear power plant accident · Ingestion · Inhalation

Electronic supplementary material The online version of this article (doi:10.1007/s12199-011-0251-9) contains supplementary material, which is available to authorized users.

A. Koizumi (✉) · K. H. Harada · T. Niisoe · A. Adachi · Y. Fujii · T. Hitomi · H. Kobayashi
Department of Health and Environmental Sciences, Kyoto University Graduate School of Medicine, Kyoto 606-8501, Japan
e-mail: koizumi.akio.5v@kyoto-u.ac.jp

Y. Wada
Department of Lifestyle Design, Faculty of Human Life and Environmental Science, University of Kochi, Kochi 780-8515, Japan

T. Watanabe
Tohoku Bunkyo College, Yamagata 990-2361, Japan

H. Ishikawa (✉)
Severe Storm and Atmospheric Environment Section, Research Division of Atmospheric and Hydrospheric Disaster Division, Disaster Prevention Research Institute, Kyoto University, Uji 611-0011, Japan
e-mail: ishikawa@storm.dpri.kyoto-u.ac.jp

Introduction

Following the Tohoku earthquake and tsunami on March 11, 2011, the Fukushima Daiichi nuclear power plant exploded on March 15, 2011, releasing massive amounts of radionuclides, including iodine, cesium (Cs), strontium, and plutonium into the northern part of Japan and the Pacific Ocean, being the second largest nuclear accident, after the Chernobyl disaster [1, 2]. The total amount of ^{137}Cs released into the environment by the Fukushima Daiichi nuclear plant from March 11 to April 15

Published online: 10 November 2011

(1.3×10^{16} Bq) [3] has been estimated to be 10% of that emitted by the Chernobyl disaster in 1986 [1, 2].

Residents living within a 20-km radius of the nuclear power plant were evacuated soon after the disaster, but people in Fukushima Prefecture have continued to live outside this evacuation zone. Although the direct threat from the radioactive plume is over, it is important to continuously assess the exposure doses due to deposited radioactivity. Contamination with ^{137}Cs has been reported in residential areas in Fukushima Prefecture [4], and the internal doses resulting from inhalation of resuspended deposits [5] and ingestion of contaminated foods need to be monitored.

Residents in particular, but also people in remote areas, are seriously concerned about their levels of internal exposure to radionuclides through ingestion of contaminated food and drink. The ingested dose should be evaluated on the basis of the level of radioactivity contained in complete meals consumed (Bq/day/person), rather than on the radioactive content of an individual item (Bq/kg).

To evaluate potential post-accident internal doses, we conducted a field survey in July 2011, focusing on estimated exposures of adult residents of Fukushima Prefecture to ^{134}Cs and ^{137}Cs through ingestion and inhalation.

Materials and methods

Field survey

We tested whole-day meals, vegetables from local food venders, tap water, and air samples from cities at various distances from the nuclear power plant between July 2 and July 8, 2011 (Fig. 1). In the cities denoted as "M" and "V" in Fig. 1, we purchased whole-day meals and vegetables from local food venders, respectively. Tap water was also collected in the same towns or cities. In the cities denoted by "A," we conducted air sampling using a high-volume sampler (HV-1000F; Sibata, Saitama, Japan) and soil sampling (mixed soil samples from depth of 0–5 cm). We also collected continuous air samples at a fixed point in Fukushima City using a low-volume sampler (SL-30; Sibata, Saitama, Japan) with an eight-stage Andersen cascade impactor sampler (AN-200; Tokyo Dylec Co., Tokyo, Japan).

Food collection and processing for radioactivity determinations

Five male researchers (aged 32–68 years) visited one of the most popular local grocery stores in each city or town and purchased several sets of whole-day meals, according to their personal preferences, as reported previously [6]. A set of whole-day meals comprised prepackaged breakfast, lunch, and dinner, as well as desserts, snacks, and

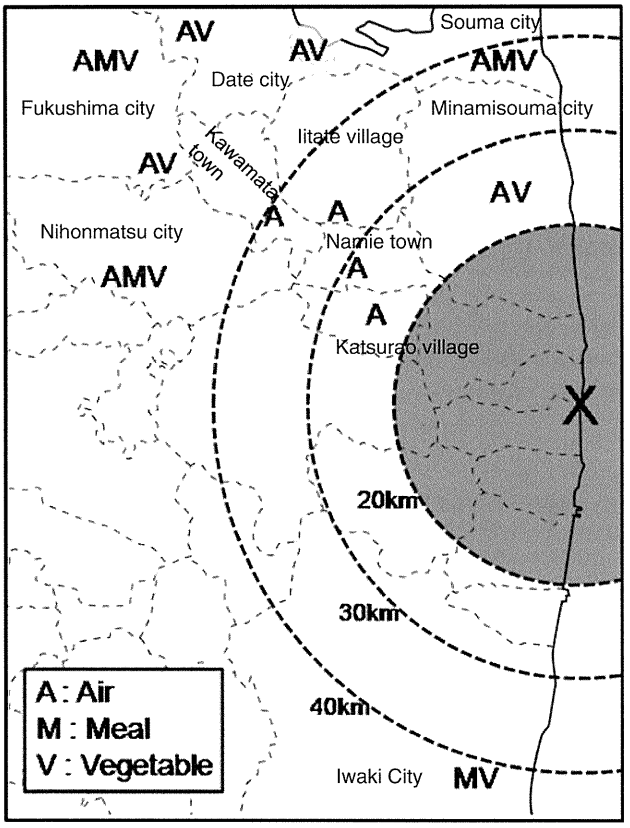

Fig. 1 Geographical locations of the field study areas. "A" represents sites where air sampling was conducted. "M" represents grocery stores where meals were purchased. Tap water (12 L) was collected in the same towns where meals were purchased. "V" represents commercial vender where vegetables were purchased. "X" represents the Fukushima Daiichi nuclear power plant. The *symbols* approximately represent actual geographical positions

beverages. A total of 12 L geographically matched tap water per town was donated by residents of the towns where the grocery stores were located. Locally produced vegetables and cow's milk were also purchased in the same towns. All items were transported daily to Kyoto University at 4°C for processing and analysis.

Daily whole-day meal sets were homogenized with locally collected tap water (approximately 1 L), together with desserts and snacks. The final volumes were recorded, and approximately 1 L of each homogenate was processed for freeze-drying. Vegetables and cow's milk were also freeze-dried. Control meals consisted of whole-day meals collected by 19 females using the food duplicate method, as previously reported [6]. Control meals were collected in July 2011 in Uji, Kyoto, which is located from 540 km to the southwest of the Fukushima nuclear power plant.

Air sampling and determination of radioactivities

A high-volume air sampler was used to collect dust in the air on a quartz membrane filter. A minimum of 50 m^3 was