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

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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

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

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

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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.

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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.

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REGULAR ARTICLE

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

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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

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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

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(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

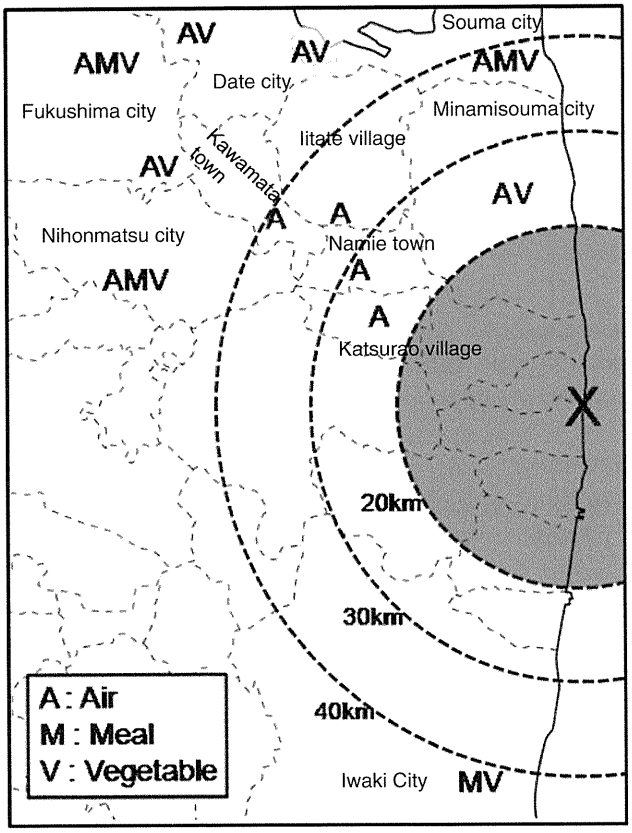

Fig. 1 Geographical locations of the field study areas. "A" represents sites where air sampling was conducted. "M" represents grocery stores where meals were purchased. Tap water (12 L) was collected in the same towns where meals were purchased. "V" represents commercial vender where vegetables were 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

inspired at all sampling sites at a height of 1.5 m above ground. An Andersen low-volume sampler was used to collect dust of various aerodynamic diameters to estimate the respirable portion of dust in Fukushima Prefecture. This sampler was fixed at a sampling site in Fukushima City. Dust samples were weighed, and their radioactivities were measured.

Determination of ^{137}Cs and ^{134}Cs

Aliquots of 100–200 g from each sample of food and cow's milk (dry weight), and soil (fresh weight) were weighed and sealed in cylindrical plastic containers. Filters from aerosol sampling were pressed into small cylindrical plastic containers. Radiometric determinations were performed using a high-purity, low-background, high-resolution germanium detector (0.7 keV). The detector was protected by a lead shield, 10 cm thick internally, covered with 0.5 mm electrolytic copper. A multichannel analyzer (4,096 channels, range 0–3,000 keV, MCA8000; Princeton Gamma Technologies, NJ, USA) was used for gamma-spectrum acquisition and processing. Characteristic gamma-ray energies were monitored to identify and quantify the radionuclides (^{134}Cs 604.7 and 795.9 keV, ^{137}Cs 661.7 keV). The detector was calibrated using a gamma-ray reference source from the Japan Radioisotope Association (Tokyo, Japan). The gamma spectrum of each sample was measured for >20,000 s for food and dust samples and for >2,000 s for soil samples. The lower limits of detection were 0.05 Bq/kg, 0.2 Bq/kg, 0.2 Bq/kg, 0.2 mBq/m^3, and 1 Bq/kg for food, vegetable, milk, dust, and soil samples, respectively. All samples were assumed to be in radioactive equilibrium. All activities were corrected to March 15, 2011 using physical half-lives (^{134}Cs 2.06 years, ^{137}Cs 30.1 years).

Effective dose coefficients for exposures by ingestion and inhalation

Radioactivities were converted into effective doses using effective dose coefficients of 0.019 μSv/Bq for ^{134}Cs and 0.013 μSv/Bq for ^{137}Cs by ingestion, respectively [7]. For inhalation, we assumed that a standard adult resident inhaled 20 m^3 air per day and used the effective dose coefficients of 0.02 μSv/Bq for ^{134}Cs and 0.039 μSv/Bq for ^{137}Cs for inhalation [7]. For the two routes of exposure, we postulated conservatively that all the radionuclides were retained in the body or in the lung, with no elimination.

Results and discussion

A total of 74 sets of whole-day meals were collected and analyzed. Their menus and components are presented in

Table 1 Dietary intake of radioactive cesium in Fukushima Prefecture

Sampling site	*n*		Food volume (g/day)	Water content (%)	Daily intake (Bq/day)		Estimated dose (μSv/year)
					^{134}Cs	^{137}Cs	
Fukushima total	55	*n* > MDL (%)	–	–	36 (65.5)	35 (63.6)	
		Median (range)	2,053 (1,100–3,145)	80.8 (73.3–97.6)	0.2 (ND–7.2)	0.3 (ND–7.0)	3.0 (ND–83.1)
		Mean ± SD	2,178 ± 400	81.9 ± 4.5	0.5 ± 1.1	0.6 ± 1.0	6.4 ± 12.5
Iwaki	10	*n* > MDL (%)	–	–	9 (90.0)	9 (90.0)	
		Median (range)	2,241 (1,879–2,690)	82.1 (76.8–86.1)	0.4 (ND–2.5)	0.7 (ND–1.6)	6.5 (ND–24.7)
		Mean ± SD	2,238 ± 272	81.5 ± 3.3	0.7 ± 0.8	0.7 ± 0.5	8.6 ± 7.8
Souma	10	*n* > MDL (%)	–	–	7 (70.0)	8 (80.0)	
		Median (range)	2,451 (2,044–2,795)	80.5 (73.3–87.1)	0.6 (ND–7.2)	0.9 (ND–7.0)	8.2 (ND–83.1)
		Mean ± SD	2,395 ± 293	80.1 ± 4.2	1.4 ± 2.2	1.6 ± 2.2	17.4 ± 25.3
Nihonmatsu	10	*n* > MDL (%)	–	–	5 (50.0)	4 (40.0)	
		Median (range)	2,611 (1,964–3,145)	79.4 (75.1–82.6)	0.1 (ND–0.9)	ND (ND–0.9)	1.7 (ND–10.4)
		Mean ± SD	2,529 ± 423	78.9 ± 2.3	0.3 ± 0.4	0.2 ± 0.3	2.9 ± 3.6
Fukushima	25	*n* > MDL (%)	–	–	15 (60.0)	14 (56.0)	
		Median (range)	1,954 (1,100–3,051)	83.7 (77.9–97.6)	0.1 (ND–0.8)	0.2 (ND–1.3)	1.3 (ND–11.3)
		Mean ± SD	1,927 ± 308	84.1 ± 4.8	0.2 ± 0.2	0.2 ± 0.3	2.6 ± 3.1
Kyoto (Uji)	19	*n* > MDL (%)	–	–	1 (5.3)	1 (5.3)	–
		Maximum	–	–	0.4	0.5	5.3
		Mean ± SD	2,955 ± 652	87.2 ± 2.5	–	–	–

Estimated dose is the total for doses attributable to exposure to ^{134}Cs and ^{137}Cs. The effective dose coefficients for ^{134}Cs and ^{137}Cs by oral route were 0.019 and 0.013 μSv/Bq, respectively

MDL method detection limit, *ND* less than MDL

Table 2 Radioactive cesium in local commercial products purchased in Fukushima Prefecture

Sampling site	n		Weight (g)	Radioactivity (Bq/kg)			Recommended standard[a] (Bq/kg)
				^{134}Cs	^{137}Cs	Total	
Milk							200
Fukushima total	21	n > MDL (%)	–	20 (95.2)	19 (90.5)	–	
		Median (range)	–	1.8 (ND–4.9)	1.9 (ND–5.5)	4.1 (ND–10.1)	
		Mean ± SD	985 ± 119	2.1 ± 1.7	2.4 ± 1.9	4.5 ± 3.6	
Iwaki	3	n > MDL (%)	–	3 (100.0)	3 (100)		
		Median (range)	–	0.9 (0.6–1.2)	1.2 (1.1–1.3)	2.0 (1.9–2.3)	
		Mean ± SD	752 ± 202	0.9 ± 0.3	1.2 ± 1.1	2.1 ± 0.2	
Souma	6	n > MDL (%)	–	6 (100.0)	6 (100.0)	–	
		Median (range)	–	3.1 (1.4–3.8)	3.1 (1.9–4.4)	6.1 (3.3–8.2)	
		Mean ± SD	1,019 ± 29	2.8 ± 1.0	3.1 ± 1.0	5.9 ± 1.9	
Nihonmatsu	3	n > MDL (%)	–	3 (100.0)	1 (33.3)		
		Median (range)	–	0.2 (0.2–1.3)	ND (ND–1.1)	0.2 (0.2–2.4)	
		Mean ± SD	1,047 ± 15	0.5 ± 0.7	0.4 ± 0.6	0.9 ± 1.3	
Fukushima	9	n > MDL (%)	–	8 (88.9)	8 (88.9)	–	
		Median (range)	–	3.4 (ND–4.9)	3.9 (ND–5.5)	7.3 (0.2–10.1)	
		Mean ± SD	1,021 ± 18	2.6 ± 2.0	2.3 ± 4.4	5.6 ± 4.4	
Kyoto (Uji)	3	n > MDL (%)	–	1 (33.3)	1 (33.3)	–	
		Median (range)	–	ND (ND–0.7)	ND (ND–0.7)	ND (ND–1.4)	
		Mean ± SD	1,037 ± 21	0.2 ± 0.4	0.2 ± 0.4	0.5 ± 0.8	

	Weight (g)	Radioactivity (Bq/kg weight)			Recommended standard[a] (Bq/kg)
		^{134}Cs	^{137}Cs	Total	
Vegetable/fruit					500
Kyoto (Uji)					
Spinach	1,249	ND	ND	ND	
Japanese mustard spinach	3,044	ND	ND	ND	
Fukushima (n = 43)					
Date					
Japanese mustard spinach	1,828	2.6	2.2	4.8	
Spinach	1,677	0.2	0.3	0.5	
New Zealand spinach	1,097	29.9	32.7	62.6	
Ceylon spinach	826	2.1	3.1	5.2	
Cucumber	1,643	3.4	4.5	7.9	
Welsh onion	1,770	3.3	2.8	6.1	
Kawamata					
Mizuna	504	5.9	7.7	13.7	
Shiitake	1,012	140.4	164.2	304.6	
Ceylon spinach	503	4.4	3.0	7.4	
Cucumber	1,007	1.3	1.6	2.8	
Broccoli	831	6.4	6.6	12.9	
Chinese chives	704	7.2	4.5	11.7	
Partially dried Japanese persimmon	332	1.8	1.7	3.5	
Welsh onion	1,455	5.7	6.6	12.3	
Fukushima					
Chinese chives	436	1.9	2.0	3.9	
Cucumber	493	2.9	3.9	6.8	

Table 2 continued

	Weight (g)	Radioactivity (Bq/kg weight)			Recommended standard[a] (Bq/kg)
		^{134}Cs	^{137}Cs	Total	
Iwaki					
Spinach	1,903	0.5	0.9	1.4	
Snap bean	860	3.5	3.6	7.1	
Shiitake	89	ND	ND	ND	
Green onion	571	7.3	8.5	15.8	
Chinese chives	615	2.8	3.5	6.3	
Broccoli	1,479	0.9	1.1	2.0	
Ceylon spinach	1,079	1.5	2.6	4.0	
Garlic	691	0.8	0.5	1.3	
Souma					
Welsh onion	1,543	4.1	2.6	6.7	
Peach	794	9.3	7.9	17.2	
Cherry	244	29.3	37.3	66.6	
Broad beans	418	4.9	6.0	10.9	
Onion (large)	835	0.5	0.6	1.1	
Onion (small)	430	9.1	9.2	18.3	
Red onion (large)	589	3.3	5.0	8.3	
Red onion (small)	524	9.6	11.6	21.3	
Garlic	256	9.4	7.2	16.6	
Potato	1,258	1.0	0.8	1.8	
Minamisouma					
Carrot	1,271	1.4	2.1	3.5	
Shiitake	417	127.1	154.7	281.8	
Bell pepper	502	ND	ND	ND	
Nihonmatsu					
Asparagus	637	1.3	1.5	2.8	
Bell pepper	390	12.0	10.7	22.7	
Ceylon spinach	1,533	1.7	3.2	4.9	
Cucumber	2,064	3.6	4.3	7.9	
Welsh onion	1,309	5.4	5.0	10.5	
Cherry	352	24.5	28.5	52.9	

MDL method detection limit, *ND* less than MDL

[a] Recommended by Ministry of Health, Labor, and Welfare of Japan [8]

Table S1. Radioactivity per daily intake (Bq/day) is also summarized in Table 1. ^{134}Cs or ^{137}Cs was detected in 36 of 55 whole-day meal samples from Fukushima Prefecture, compared with only one of 19 from Kyoto. The estimated median dose levels was 3.0 μSv/year, ranging from not detectable (ND) to 83.1 μSv/year in Fukushima, while the maximum dose level in Kyoto was 5.3 μSv/year.

The levels of ^{134}Cs and ^{137}Cs in cow's milk and vegetables were also determined (Table 2). The median total activity in milk from Fukushima Prefecture was 4.1 Bq/kg, ranging from ND to 10.1, which was an order of magnitude lower than the recommended limit set by the Ministry of Health, Labor, and Welfare of Japan [8]. Trace radioactivity was detected in only one sample from Kyoto. No vegetables in Fukushima Prefecture exceeded 100 Bq/kg, except for shiitake mushrooms (*Lentinula edodes*), which contained relatively high levels of radioactivity, up to 60% of the recommended limit (Table 2). Radioactivities in shiitake at Kawamata or Minamisouma were larger than at Iwaki, indicating that a radioactive plume was transferred by northeasterly winds from the nuclear plant. No radioactivity was detected in vegetables from Kyoto. These results indicate that the levels of radioactive Cs ingested were well below the recommended limits [8] in various towns in Fukushima Prefecture, except in the case of shiitake.

Table 3 Particle size distribution and respiratory deposition estimate for radioactive cesium in Fukushima Prefecture

Sampling site		Date (2011)	Andersen low-volume sampler, 224 m^3			
			Fraction (μm)	Dust amount (mg)	Radioactivity (mBq/m^3-air)	
					^{134}Cs	^{137}Cs
Fukushima	37°45′42″N 140°28′18″E	7/2–7/8	100–11.4	0.7	0.4	0.3
			11.4–7.4	1.1	0.3	0.3
			7.4–4.9	1	1.0	0.4
			4.9–3.3	0.9	0.5	0.6
			3.3–2.2	0.6	0.3	0.2
			2.2–1.1	0.8	0.3	0.2
			1.1–0.7	1.3	0.8	0.4
			0.7–0.46	1.3	1.5	1.1
			<0.46	0.9	1.5	1.3
		Total		8.6	6.5	4.7
		Respirable	<4.9	5.8	4.8	3.8

Sampling site		Date (2011) (weather)	High-volume air sampler							Ambient dose rate	Radioactivity in soil (Bq/kg)		
			Air volume sampled (m^3)	Dust amount (mg)	Radioactivity in air (mBq/m^3-air)		Estimated dose[a] (μSv/year)						
					^{134}Cs	^{137}Cs	^{134}Cs	^{137}Cs	Total	(μSv/h)	^{134}Cs	^{137}Cs	*n*
Fukushima	37°45′42″N 140°28′18″E	2011/7/2 (F)	473	6.8	1.9	3.0	0.3	0.8	1.1	1.2	NA	NA	
Date	37°47′10″N 140°33′26″E	2011/7/3 (CL)	94	3.5	7.9	6.4	1.1	1.8	3.0	0.9	3,232 ± 2,666	3,855 ± 3,047	5
Fukushima	37°39′26″N 140°32′11″E	2011/7/3 (CL)	83	1.9	4.7	1.5	0.7	0.4	1.1	1.0	2,515 ± 859	3,059 ± 1,077	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/4 (R)	450	8	1.6	1.5	0.2	0.4	0.6	1.2	NA	NA	
Souma	37°46′1″N 140°57′2″E	2011/7/5 (F)	88	0.7	0.6	0.2	0.1	0.1	0.1	0.5	1,710 ± 2,365	2,116 ± 2,976	5
Minami-Souma	37°38′29″N 140°55′30″E	2011/7/5 (F)	84	2.4	0.7	1.1	0.1	0.3	0.4	0.9	1,772 ± 411	2,151 ± 546	5
Souma	37°46′8″N 140°43′1″E	2011/7/5 (F)	84	1.3	1.1	2.3	0.2	0.7	0.8	1.6	1,723 ± 1,792	2,047 ± 2,174	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/5 (F)	220	4	2.9	3.4	0.4	1.0	1.4	1.2	NA	NA	
Nihonmatsu	37°33′21″N 140°27′34″E	2011/7/6 (F)	93	0.1	0.6	0.6	0.1	0.2	0.3	1.2	12,184 ± 12,170	14,202 ± 14,025	5
Nihonmatsu	37°33′21″N 140°30′43″E	2011/7/6 (F)	53	0.3	4.2	7.3	0.6	2.1	2.7	1.9	1,895 ± 674	2,244 ± 755	5
Kawamata	37°36′14″N 140°38′49″E	2011/7/6 (CL)	72	0.4	6.3	6.1	0.9	1.7	2.7	2.0	3,931 ± 4,856	4,741 ± 5,929	5
Fukushima	37°45′42″N 140°28′18″E	2011/7/6 (CL)	246	4	5.3	7.6	0.8	2.2	2.9	1.2	NA	NA	
Fukushima	37°45′42″N 140°28′18″E	2011/7/7 (CL)	259	5.3	1.9	2.5	0.3	0.7	1.0	1.2	NA	NA	
Iitate	37°36′44″N 140°44′52″E	2011/7/7 (CL)	84	1.7	24.6	38.9	3.6	11.1	14.7	9.0	18,531 ± 11,235	23,185 ± 15,664	5
Namie	37°33′38″N 140°45′39″E	2011/7/7 (CL)	84	1.7	148.2	194.2	21.6	55.3	76.9	13.0	13,548 ± 10,469	16,216 ± 12,653	5
Katsurao	37°31′33″N 140°48′21″E	2011/7/7 (CL)	84	1.5	65.0	64.0	9.5	18.2	27.7	10.0	16,332 ± 11,170	16,799 ± 10,058	5

CL cloudy, *F* fine, *R* rainy, *NA* not available

[a] It was assumed that radioactive cesium was in respirable fraction and that a standard human inhales 20 m^3 air

We collected 16 dust samples using the high-volume sampler (Table 3; Fig. 1). Data obtained with the low-flow-volume sampler suggested that a large proportion of the radionuclides from the crippled Fukushima nuclear power plant was in the respirable fraction: 74% (4.8/6.5) of the total ^{134}Cs and 81% (3.8/4.7) of the total ^{137}Cs (Table 3). To estimate the exposure doses for humans, we therefore selected a conservative scenario whereby all ^{134}Cs and ^{137}Cs activities in the dust samples collected using the high-volume sampler were allocated to the respirable fraction (aerodynamic diameter <4.9 μm). The highest dose level of 76.9 μSv/year was recorded in a sample collected at Namie. However, this value was still less than one-tenth of the permissible dose level of 1 mSv/year [8]. The estimated dose levels for ^{137}Cs were significantly correlated with ambient dose rate (μSv/h) ($n = 10$, $r^2 = 0.79$, $p < 0.05$) but not with mean radioactivity levels in soil (Bq/kg) ($n = 11$, $r^2 = 0.32$, $p > 0.05$).

Given that the samples in this study were obtained in early July, about 4 months after the major release of radioactivity, airborne radioactivity was likely to represent resuspended deposited radioactivity, rather than direct transport from the source. Several studies have investigated resuspension from a flat surface [5], but information on resuspension from ecological systems including forests and paddy fields is scant.

We demonstrated the radioactivity levels due to ^{134}Cs and ^{137}Cs in Fukushima Prefecture in July 2011. The maximum total exposure dose through inhalation and ingestion was estimated to be 160 μSv/year (83.1 by ingestion and 76.9 by inhalation) in zones outside a 20-km radius of the crippled Fukushima nuclear power plant.

The amounts of radioactivity in the daily meals consumed by residents of the study regions were well below the regulation limit. However, many food items are now imported globally, such that a high portion of foodstuffs comes from uncontaminated areas. It is possible that the radioactivity in some highly contaminated foodstuffs may be diluted by other "clean" foods. However, the ingested doses estimated in the present study would underestimate the exposure of residents whose daily foods are mostly supplied locally from within the contaminated areas. The conclusions of this study may therefore not be applicable to people in such a situation. Furthermore, the current study only utilized air monitoring in a few, geographically limited areas. All meal samples were obtained from outside a 30-km radius of the nuclear power plant, because no commercial venders were present between 20 and 30 km from the power plant, which had been defined as the planned emergency evacuation zone. In addition to the small number of air samples collected, the survey was conducted in the rainy season when "resuspension" is relatively low. The current study is thus subject to the above limitations and biases. However, the conservative approach adopted in this study maximized the estimated dose levels and would thus partially mitigate the effects of any biases and limitations. In conclusion, the estimated dose levels in residents of Fukushima Prefecture as a result of ingestion and inhalation were much lower than the 1 mSv/year, recognized as a publicly permissible dose [8]. Further studies are needed to perform qualitative risk assessments based on more accurate exposure estimates.

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Conflicts of interest The authors declare that there are no conflicts of interest.

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Glucose and Fatty Acids Synergize to Promote B-Cell Apoptosis through Activation of Glycogen Synthase Kinase 3β Independent of JNK Activation

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Abstract

Background: The combination of elevated glucose and free-fatty acids (FFA), prevalent in diabetes, has been suggested to be a major contributor to pancreatic β-cell death. This study examines the synergistic effects of glucose and FFA on β-cell apoptosis and the molecular mechanisms involved. Mouse insulinoma cells and primary islets were treated with palmitate at increasing glucose and effects on apoptosis, endoplasmic reticulum (ER) stress and insulin receptor substrate (IRS) signaling were examined.

Principal Findings: Increasing glucose (5–25 mM) with palmitate (400 μM) had synergistic effects on apoptosis. Jun NH2-terminal kinase (JNK) activation peaked at the lowest glucose concentration, in contrast to a progressive reduction in IRS2 protein and impairment of insulin receptor substrate signaling. A synergistic effect was observed on activation of ER stress markers, along with recruitment of SREBP1 to the nucleus. These findings were confirmed in primary islets. The above effects associated with an increase in glycogen synthase kinase 3β (Gsk3β) activity and were reversed along with apoptosis by an adenovirus expressing a kinase dead Gsk3β.

Conclusions/Significance: Glucose in the presence of FFA results in synergistic effects on ER stress, impaired insulin receptor substrate signaling and Gsk3β activation. The data support the importance of controlling both hyperglycemia and hyperlipidemia in the management of Type 2 diabetes, and identify pancreatic islet β-cell Gsk3β as a potential therapeutic target.

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Introduction

The natural history of Type 2 diabetes mellitus (T2D) includes a progressive decline in β-cell function associated with peripheral insulin resistance. The β-cell dysfunction has been attributed in part to loss of β-cell mass via apoptosis [1] with inadequate insulin secretion leading to hyperglycemia and other diabetes symptoms [2]. Insulin resistance is at the core of obesity associated diabetes and is thought to reflect impaired insulin signaling due to chronically increased levels of free fatty acids (FFA). High FFA are also implicated in the reduction in β-cell mass that has been referred to as lipotoxicity. The combination of elevated glucose and FFA, or "glucolipotoxicity" that is prevalent in T2D has been suggested to be a major contributor to β-cell death [3,4,5,6].

The search for molecular mechanisms for glucose potentiation of FFA- induced β-cell dysfunction has been the subject of several recent studies (see [7] for review). One area of investigation has focused on the insulin receptor substrate-PI3K-Akt signaling pathway. The first study showing that the FFA oleate impaired insulin signaling in insulinoma cells demonstrated that the cells were protected from FFA-induced apoptosis by expressing a constitutively active Akt [8]. Several biochemical and genetic studies subsequently showed that saturated FFA could promote ER stress in insulinoma cells and in primary rodent and human islets [9,10,11,12]. More recently, it was shown that high glucose

potentiated FFA induced apoptosis by enhancing ER stress [13]. ER stress in insulinoma cells was shown to impair insulin signaling through activation of ATF3, an ER stress response protein that was implicated in suppression of IRS2 expression [14]. ATF3 is another stress inducible gene that is activated in different tissues by a variety of stresses [15].

How glucose potentiates FFA induced ER stress, reduced insulin receptor substrate signaling, and apoptosis is incompletely understood. Our recent study showed that there was a dose-dependent effect of FFA in the presence of high glucose on apoptosis in insulinoma cells and primary islets [16] that was associated with JNK activation, ER stress, and reduced insulin signaling. In the current study, we found a dose-dependent effect of glucose in the presence of palmitate on cell death that appeared to be over and above JNK activation. We observed glucose dose-dependent synergistic effects on palmitate inhibition of receptor substrate signaling and activation of Gsk3β. Co-treatment with an adenovirus expressing a kinase dead Gsk3β significantly protected β-cells from cell death. Our data support importance of Gsk3β in the synergistic effects of glucose and FFA.

Materials and Methods

Cell Culture

Mouse insulinoma cell line MIN6 (passage 24–32) were grown in monolayer cultures as described previously [17] in Dulbecco's modified Eagle's medium (Sigma Aldrich) supplemented with 15% fetal bovine serum, 50 mmol/L β-mercaptoethanol at 37°C in a humidified (5% CO_2, 95% air) atmosphere. Rat insulinoma INS-r3 cells were grown as previously described [18]. The palmitic acid (palmitate), formalin, propidium iodide, IL-1b, tunicamycin and TNFα were purchased from Sigma (Saint Louis, MO). Tauroursodeoxycholic Acid Sodium Salt (TUDCA) was purchased from CALBIOCEM (Darmstadt, Germany).

Fatty acids (FFA) Treatment of MIN6 Cells and Islets

The complete protocol was previously described [16]. Briefly a 20 mM solution of the FFA in 0.01 M NaOH was incubated at 70°C for 30 minutes. Then, 330 µL of 30% BSA and 400 µL of the free FFA/NaOH mixture was mixed together and filter sterilized with 20 mL of either the DMEM or RPMI media. The approximate molar ratio of FFA:BSA is 6:1 with 400 µM palmitate. The addition of BSA or a FFA:BSA mixture has not been shown to affect the pH of the media.

Propidium iodide/Cell Death Assay

MIN6 cells were grown on glass cover slips within the wells of a 6-well plate and incubated with either BSA alone or 400 µM both FFAs complexed with BSA for 24 hours as previously described [16]. After treating cells for 24 hours with various treatments, the cells were incubated with 10 µg/ml (1 to 1000 dilution) Propidium Iodide (PI) and 20 ug DAPI added directly to the media at 37°C, 5% CO2 for 1 hour. The medium was then removed by aspiration, and the cells were washed once with PBS and then fixed by incubation with 3.7% formaldehyde for 15 min at room temperature. After fixation, the MIN6 cells were mounted with anti-fading gel solution including DAPI (Biomeda Corporation, Foster City, CA) on to glass slides. Each condition reported represents over 3000 cells counted by randomized field selection. The percentage of cell-death is reported as the number of PI stained nuclei over the total number of nuclei stained by DAPI as quantitated by Image J software 1.37 [19].

Western blot analysis

MIN6 were washed twice in ice-cold phosphate-buffered saline and were lysed in ice-cold cell lysis buffer consisting of 50 mM HEPES (pH 7.5), 1% (v/v) Nonidet P-40, 2 mM activated sodium orthovanadate, 100 mM sodium fluoride, 10 mM sodium pyrophosphate, 4 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 µg/mL leupeptin, and 1 µg/mL aprotinin, then passed through syringe with a 21 gauge needle 10 times while INS-r3 cells were sonicated (Misonix, Farmingdale, NY) and particulate material from both cell lines were removed by centrifugation (10,000× g; 10 min; 4°C). The supernatants were collected. Protein concentrations were determined using the Bio-Rad protein assay (Bio-Rad, Hercules, CA).

The extracts (20 µg of total protein) were resolved on 7.5% or 4–15% gradient polyacrylamide gels and were blotted onto a nitrocellulose membrane (Bio-Rad, CA), and incubated overnight at 4°C in Tris-buffered saline containing a 1:1000–5000 dilution of antibody as listed below. The membrane was then incubated at 4°C for 60 min in Tris-buffered saline with a 1:2000 dilution of anti-rabbit IgG or anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (Cell Signaling Technology). Antibodies used were anti-total Akt, anti-phospho-Akt (S473), anti-cleaved Caspase3, anti-phospho-PERK (980Thr), anti-phospho-eIF2α(51Ser), anti-total JNK1/2, anti-phospho-JNK, anti-phospho-AMPK, total AMPK, anti-phospho-ACC, anti-total ACC from Cell Signaling Technology (Beverley, MA), anti-SREBP1 from Neo Markers (Fremont, CA), anti-IRS1, anti-IRS2 from Upstate (Billerica, MA), anti-ATF3, anti-Insig1, anti-Lamin from Santa Cruz (Santa Cruz, CA) and anti-α-Tubulin and from Sigma (Saint Louis, MO).

Immune complexes were revealed using ECL Advance Western Blot Detection kit (Amersham Plc, Buckinghamshire UK) and the images were acquired using a FluoroChem 8800 digital camera acquisition system (Alpha Innotech, San Leandro, CA, USA). Band intensities in the blots were later quantified using ImageJ 1.38× [19] and α-Tubulin or β-Actin bands were used to adjust for loading differences.

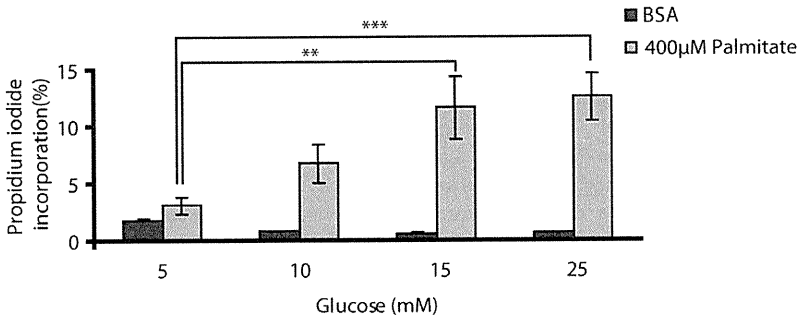
Nuclear and cytoplasmic fractions from MIN6

MIN6 cells were cultured in 60-mm diameter culture dishes until 80% confluency. For isolation of nuclear extracts, the cells were then collected into microtubes, centrifuged for 20 s in a microcentrifuge, and resuspended in 200 µl of 10.0 mM Hepes, pH 7.9, containing 10.0 mM KCl, 1.5 mM $MgCl_2$, and 0.5 mM dithiothreitol. After incubation at 4°C for 15 min, the cells were lysed by passing 10 times through a 21-gauge needle. Next, the cells were centrifuged for 20s in a microcentrifuge, and the supernatant (cytoplasmic fraction) was removed and frozen in small aliquots. The pellet, which contained the nuclei, was resuspended in 150 µl of 20 mM Hepes, pH 7.9, containing 20% v/v glycerol, 0.1 M KCl, 0.2 mM EDTA, 0.5 mM dithiothreitol, and 0.5 mM phenylmethanesulfonyl fluoride and then stirred at 4°C for 30 min. The nuclear extracts were then centrifuged for 20 min at 4°C in a microcentrifuge. The supernatant was collected, aliquoted into small volumes, and stored at −80°C.

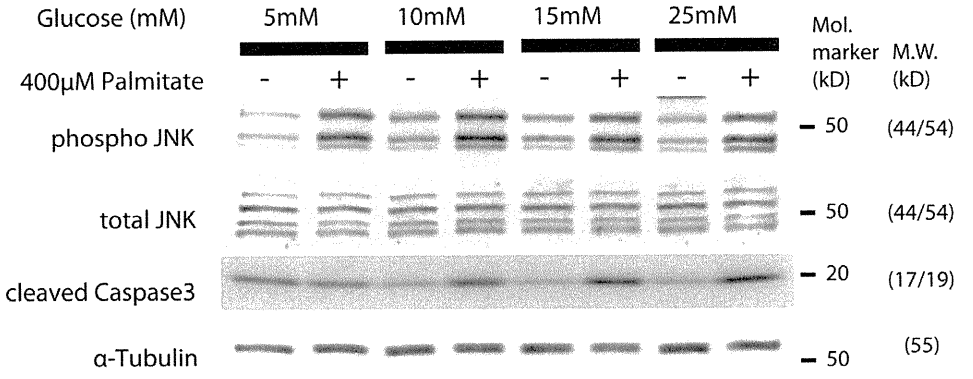
Islet isolation and culture

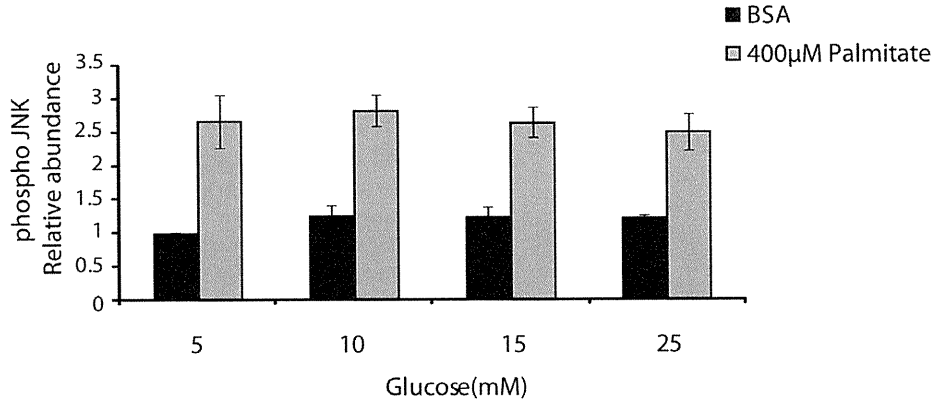
Islets from 12 weeks of age C57BL/6 male mice were isolated by ductal collagenase distension/digestion of the pancreas [20] followed by filtering and washing through a 70 mm Nylon cell strainer (BD Biosciences, San Jose, CA). Isolated islets were then maintained in RPMI medium containing 11 mM glucose, 10% FBS, 200 units/ml penicillin, and 200/ml streptomycin in humidified 5% CO2, 95% air at 37C. The palmitate treatments were carried out 15 hours after isolation. Adenovirus infections

A.


BSA
400μM Palmitate
***
**
Propidium iodide incorporation(%)
15
10
5
0
5
10
15
25
Glucose (mM)

B.


Glucose (mM)
5mM
10mM
15mM
25mM
400μM Palmitate
- + - + - + - +
Mol. marker (kD)
M.W. (kD)
phospho JNK
50
(44/54)
total JNK
50
(44/54)
cleaved Caspase3
20
(17/19)
α-Tubulin
50
(55)


BSA
400μM Palmitate
phospho JNK Relative abundance
3.5
3
2.5
2
1.5
1
0.5
0
5
10
15
25
Glucose(mM)

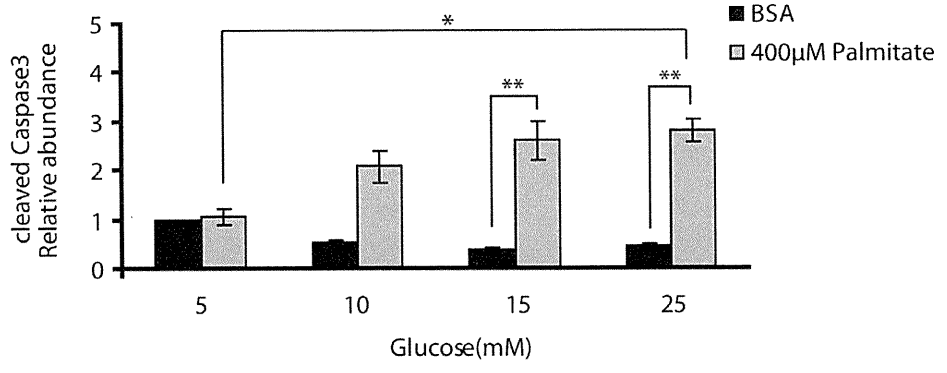
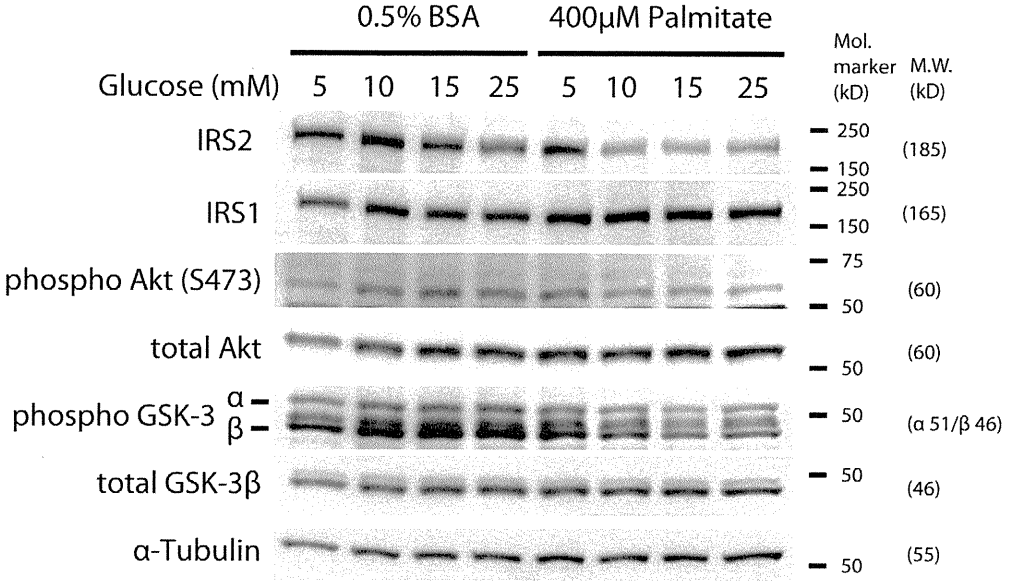

BSA
400μM Palmitate
*
**
**
cleaved Caspase3 Relative abundance
5
4
3
2
1
0
5
10
15
25
Glucose(mM)

Figure 1. Synergistic effects of glucose and palmitate on cell death but not JNK activation in MIN6 cells. MIN6 cells were treated with either control 0.5% BSA or 400 μM palmitate+0.5% BSA at a concentration of 5, 10, 15, 25 mM glucose for 24-h. (**A**) The percentage of cell death was then assessed by adding propidium iodide for the last hour of incubation as described under Methods. The bar graph depicts the averages of the data obtained from five individual experiments, and data are expressed as means ±S.E.M. ** $p<0.01$, *** $p<0.001$; (**B**) The cell lysates were subjected to Western blot analysis using anti-cleaved Caspase3, anti-phospho-JNK, anti-total JNK and anti-α-Tubulin antibodies. Protein level of phospho-JNK was normalized over total JNK. Cleaved Caspase3 levels were normalized over α-Tubulin. The representative result of three individual experiments is shown. The data obtained from three individual experiments are expressed as means ± S.E.M. * $p<0.05$, ** $p<0.01$.
doi:10.1371/journal.pone.0018146.g001

were initiated immediately following isolation at 500 multiplicity of infection (MOI). Infections were incubated for 15 hours and residual virus was removed prior to palmitate treatment. All procedures were performed in accordance with Washington University's Animal Studies Committee. The Principles of laboratory animal care (NIH publication no. 85–23, revised 1985; http://grants1.nih.gov/grants/olaw/references/phspol.htm) were followed.

Loss-of-function of ATF3 with shATF3

INS-r3 cells were seeded 24 hours prior to infection to achieve 70 percent confluence at time of infection. Control and ATF3



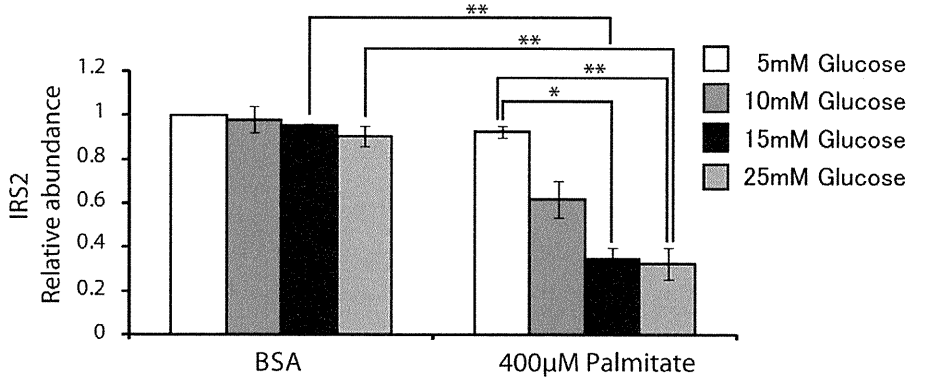


Figure 2. Glucose and palmitate potentiate to reduce insulin signaling. MIN6 cells were treated with either control 0.5% BSA (four lanes on left) or 400 μM palmitate+0.5% BSA (four lanes on right) at a concentration of 5, 10, 15, 25 mM glucose for 24-h. Total cell lysates were obtained and were subjected to Western blot analysis with antibodies to the indicated proteins. Protein level of IRS2 was normalized over α-Tubulin. The representative results of three individual experiments are shown. The results for IRS2 are graphically illustrated, data are expressed as means ±S.E.M. *$p<0.05$, **$p<0.01$.
doi:10.1371/journal.pone.0018146.g002

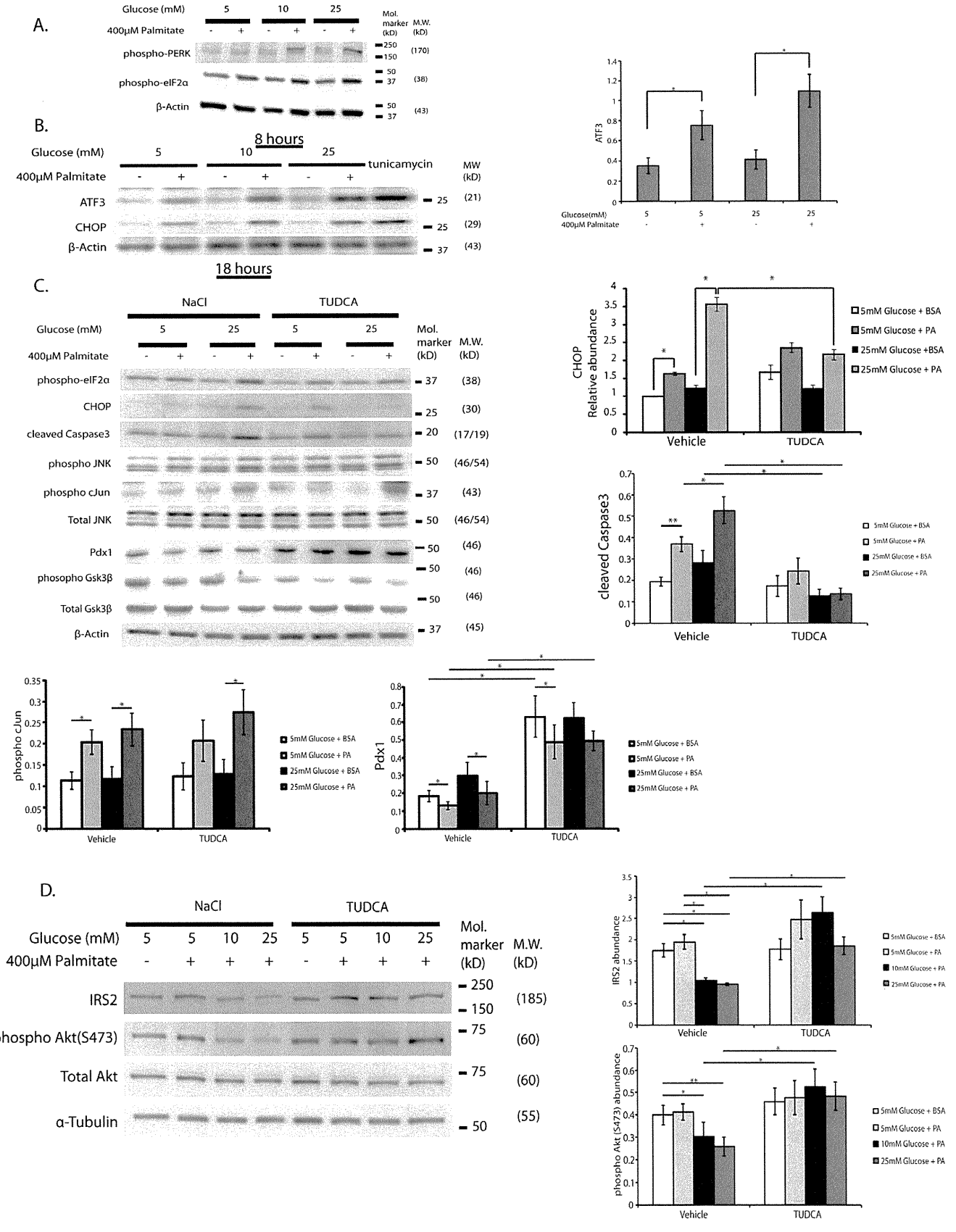


Figure 3. The synergistic effects of glucose and palmitate on ER stress and reduction of insulin signaling is attenuated by addition of a chemical chaperon. (**A**) MIN6 cells were treated with either control 0.5% BSA or 400 μM palmitate+0.5% BSA at a concentration of 5, 10, 25 mM glucose for 8 hours. Total cell lysates were extracted at indicated time points and were subjected to Western blot analysis using anti-