Table 1 Socio-demographic characteristics

Variable	All (n=188)
Gender	
Boys	111 (53.7%)[1]
Girls	87 (46.3%)
Age	
6-8.9 mo	64 (34.0%)
9-11.9 mo	34 (18.1%)
12-14.9 mo	30 (16.0%)
15-18.9 mo	60 (31.9%)
Birth weight, g	3000 (2700, 3375)[2]
Low Birth Weight	20 (10.6%)
Number of children aged under 5 y	
1 child	149 (79.3%)
≧2 children	39 (20.7%)
Family income, 1000VND/capita/d[3]	11.1 (6, 22.2)
Mother's age, y	25.0 (22, 28)
Mother's education level	
Primary school (1-5 y)	26 (13.8%)
Secondary school (6-9 y)	130 (69.1%)
More (> 10 y)	32 (17.0%)
Mother's BMI[4]	
6-11.9 mo postpartum, kg/m^2	19.7 (18.4, 20.8)
12-18.9 mo postpartum, kg/m^2	19.0 (17.8, 20.1)

Abbreviation : BMI, Body Mass Index
[1] Number, % in parentheses (all such values).
[2] Median, 25th and 75th percentile in parentheses (all such values).
[3] Exchange rate as of May-June 2007 : US1$=VND17,803.
[4] n=98 for 6-11.9 mo postpartum and n=90 for 12-18.9 mo postpartum

of children belonged to the "Kinh" ethnic group. As to the main family occupation, 55.3% were farmers or agricultural workers. Water from wells was used by 98.4% of the participant households and 99% of mothers answered that they boiled the water for drinking. The latrine types were mainly traditional pit latrine (76.1%) and ventilated improved pit latrine (13.3%). The family income per capita was < 17,800 VND, 17,800-35,600 VND and > 35,600 VND for 67.0%, 20.7% and 12.2% of the study participants, respectively. Coverage of immunization in the study site was 99.5% for BCG, 97.4% for DPT 3rd, 97.9% for OPV 3rd, 97.3% for Measles and 63.7% for Hepatitis B 3rd. The coverage of Vitamin A distribution among the children aged 6 to 36 mo was 96.7%.

Z-scores and the prevalence of undernutrition calculated by WHO/CDC/NCHS reference (13) among the children are shown in Table 2. The prevalence of underweight, stunting and wasting

Table 2 Z-scores and prevalence of undernutrition

	All	6-11.9 mo	12-18.9 mo
n	188	98	90
WAZ	-1.21± 1.01[1]	-0.89± 1.01	-1.57± 0.90 **
HAZ	-1.20± 1.06	-0.94± 1.04	-1.50± 1.01 **
WHZ	-0.52± 0.88	-0.21± 0.86	-0.99± 0.77 **
Underweight	37 (19.7%)[2]	14 (14.3%)	23 (25.6%) *
Stunting	44 (23.4%)	18 (18.4%)	26 (28.9%)
Wasting	10 (5.3%)	3 (3.1%)	7 (7.8%)

Abbreviation : WAZ, Weight-for-Age ; HAZ, Height-for-Age ; WHZ, Weight-for-Height
[1] Mean± SD (all such values).
[2] Number, % in parentheses (all such values).
*P< 0.05 ; ** P< 0.01. vs. children aged 6-11.9 month (unpaired t-test)

was 19.7%, 23.4% and 5.3%, respectively. The WAZ, HAZ and WHZ among the children aged 12 to 18.9 mo were significantly lower than those among the children aged 6 to 11.9 mo (P<0.05). When the Z-score was calculated by the recent WHO growth reference (14) for all the children (n=188), the mean WAZ, HAZ and WHZ were -0.91± 1.08, -1.21± 1.19 and -0.37± 1.09, respectively ; and the prevalence of underweight, stunting and wasting was 25.5% (n=48), 14.9% (n=28), 6.9% (n=13), respectively. Comparing these scores with the NCHS growth reference, the mean WAZ and WAZ were significantly lower (P<0.001), the mean HAZ was similar (P=0.772), and a higher proportion of children was classified as stunted (P<0.001) and fewer children were classified as underweight (P<0.001).

Table 3 shows the incidence of diarrhea and ARIs

Table 3 Incidence of infectious disease during the last 14 days of the survey

	Total	age group	
		6-11.9 mo	12-18.9 mo
n	188	98	90
Diarrhea			
acute diarrhea	23 (12.2%)[1]	10 (10.2%)	13 (14.4%)
dysentery	5 (2.7%)	2 (2.0%)	3 (3.3%)
chronic diarrhea	1 (0.5%)	1 (1.0%)	0 (0.0%)
Acute Respiratory Infectious disease			
cough and difficult breathing	38 (20.2%)	21 (21.4%)	17 (18.9%)
bronchial infection	5 (2.7%)	2 (2.0%)	3 (3.3%)
pneumonia	11 (5.9%)	8 (8.2%)	3 (3.3%)
throat infection	15 (8.0%)	8 (8.2%)	7 (7.8%)
nose infection	3 (1.6%)	4 (2.0%)	2 (1.1%)

[1] Number, % in parentheses (all such values).

during the last 14 days of the interview. The incidence of diarrhea and ARI was 12.2% and 20.2%, respectively. The incidence of diarrhea and ARIs did not differ for older children and younger children. There was no child with measles or pertussis during the time of the survey.

Ninety nine percent of the children had been breastfed or were being breastfed at the time of interview. The percentage of currently breastfed children was 99.0% among those aged 6 to 11.9 mo, while that was 73.3% among those aged 12 to 18.9 mo. Breastfeeding status in the first 4 and 6 mo is shown in Table 4. In the first 4 mo, 21.3% of the children were exclusively breastfed and 18.6% were almost exclusively breastfed. The prevalence of exclusively and almost exclusively breastfed children declined rapidly as at 6 mo, there was no child who had been exclusively breastfed and only 3.2% who were almost exclusively breastfed, while the percentage of partially breastfed children increased to 95.2%.

A logistic regression model was used to identify the risk factors related to a child's underweight (Table 5). As independent variables, incidence of infectious disease, exclusive breastfeeding in the first 4 mo, gender, age of children, low birth weight, number of children age under 5 y, family income, mother's age and mother's education level were applied. For the analysis, incidence of infectious disease was defined if the child had diarrhea or ARIs. Exclusive breastfeeding was defined if the child had exclusive breastfeeding or almost exclusive breastfeeding in the first 4 mo. Non-exclusive breastfeeding in the first 4 mo (OR 3.95, p=0.025) and low birth weight (OR 4.38, p=0.009) were associated with underweight in the children, while incidence of infectious disease was not (OR 1.16, p=0.734).

Table 4 Breastfeeding status (Total n=188)

	Breastfeeding at age 4 mo	Breastfeeding at age 6 mo
Exclusive	40 (21.3%)[1]	0 (0%)
Almost exclusive	35 (18.6%)	6 (3.2%)
Predominant	4 (2.1%)	2 (1.1%)
Partial	108 (57.4%)	179 (95.2%)
Artificial	1 (0.5%)	1 (0.5%)

[1] Number, % in parentheses (all such values).

Table 5 Odds ratio of the risk factors for underweight (Total n=188)

	Underweight		
	Odd Ratio	95% CI	*P*-value
Morbidity[1]			
No	1.00		
Yes	1.16	0.48, 2.81	0.734
Exclusive breast feeding at age 4 mo			
Yes	1.00		
No	3.95	1.19, 13.16	0.025
Gender			
Boys	1.00		
Girls	0.66	0.79, 3.96	0.329
Age			
6 to 11.9 mo	1.00		
12 to 18.9 mo	2.11	0.94, 4.75	0.072
Low Birth Weight			
Normal	1.00		
Birth weight< 2500 g	4.38	1.45, 13.24	0.009
Number of children aged under 5 y			
1 child	1.00		
2 children	2.27	0.82, 6.26	0.113
Family income			
more than 11,100 VND/capita/d	1.00		
under 11,100 VND/capita/d	0.86	0.37, 2.02	0.734
Mother's age at birth			
less than 25 y	1.00		
over 25 y	1.37	0.60, 3.14	0.451
Mother's education level			
Up to primary school	1.00		
More	0.69	0.19, 2.48	0.568

[1] Morbidity was defined as "Yes" if the child had the incidence of diarrhea or ARI during the last 14 days of the survey.

DISCUSSION

To our knowledge, this is the first study to assess the nutritional status, feeding practice and incidence of infectious diseases simultaneously in order to determine the risk of undernutrition among children aged 6 to 18 mo in northern mountainous Vietnam. In mountainous areas, the prevalence of undernutrition among children is remarkably higher compared to other regions (19). Growth faltering in Vietnam occurs early in their life and the prevalence of undernutrition accumulates with age; it starts from 3 to 4 mo of age and increases quickly from 6 to 12 mo of age, then obtains the highest level at age 24 mo (20); therefore, early prevention and control of undernutrition are expected. The present study indicated that high prevalence of undernutrition in terms of underweight, stunting and wasting among children aged 6 to 18 mo in northern mountainous Vietnam. Non-exclusive breastfeeding status in the first 4 mo and low birth weight were predictors for the child undernutrition, while incidence of infectious disease was not.

The prevalence of underweight and stunting in the study site were relatively lower compared to those nationwide as well as a previous report in Bac Giang province on children under 5 y (4). This was due to the fact that the prevalence of malnutrition was relatively higher among children older than 12 mo compared to younger children, which is consistent with a previous report of Vietnam (21), and that our study participants were relatively younger than those in the previous reports. Another report on Vietnamese children of the same age in a mountainous region demonstrated a similar prevalence of undernutrition (22). Additionally, using the recent WHO growth reference resulted both in a difference in mean Z scores for WAZ and WHZ and in changes in the prevalence of stunting and underweight. The differences in results between the WHO/CDC/NCHS growth reference and the recent WHO growth reference were similar to those in a previous report in Vietnam (23).

Since suitable and low-cost alternatives to breastfeeding are not available and non-breast milk food such as unhygienic water and low nutrient-density food has several problems in most of developing countries, WHO (24) has recommended giving only breast milk during the first 4 to 6 mo. The benefits of exclusive breastfeeding in the first 6 mo for the child's nutritional status were also reported both in industrialized countries and developing countries (25). In this study, non-exclusive breastfed children in the first 4 mo had 3.95 times higher incidence of underweight (P=0.025). The result supports the previous findings that Vietnamese children who were not exclusively breastfed or predominantly breastfed in the first 4 mo showed significantly lower anthropometric measurements at the age of 6 to 12 mo than the children who were exclusively or predominantly breastfed (5). It has been reported that an ideal method of identifying exclusive breastfeeding is a descriptive longitudinal and prospective study design with an indicator of "exclusively breastfeeding since birth" (26). In this study, we developed a standardized definition of exclusive breast feeding status following the definition that WHO has proposed (17), while we determined the status by retrospectively asking the time of introduction of non-breast milk food. Since this study has a cross-sectional design, the assessments might have retrospective bias. Although we could not draw a direct epidemiological inference for causality between exclusive breastfeeding and underweight, our findings indicate that advantages of the practice of exclusively breastfeeding would include amelioration of undernutrition among the children. Exclusive breastfeeding has been recommended through a childhood undrernutrition control program provided by the government of Vietnam since 2000 to the present and covering all communes in the country (27); however, the percentage of mothers giving exclusive breastfeeding in the first 4 mo is still low in Vietnam and even lower than the global rate : 51% (28). In the present study, chronic energy malnutrition among mothers was also highly prevalent and mothers complained that "the child appeared hungry just after breastfeeding" and gave this as the reason for non-exclusive breastfeeding (data not shown). Further efforts to scale-up exclusive breastfeeding, such as monitoring and evaluation with a feedback system that allow for periodic program corrections and continued innovation (29), are expected.

As to the other risk factors of underweight in this study, low birth weight was the strong predictor of underweight. This observation is consistent with the findings of other studies in Vietnam (21) and other Asian countries (30, 31). In Vietnam, thanks to the economic improvement during the last two decades, the prevalence of low birth weight has been decreasing, especially in urban areas ; however, the prevalence of low birth weight remains high in rural areas (32). It has been demonstrated that the identification

in an early stage and immediate direct intervention such as extra macro- and micro-nutrition can help infants of low birth weight catch up with their heavier contemporaries (33). It has been also shown that appropriate breastfeeding and the quality of breast milk are also important to catch up to the normal nutritional status (34).

Although a marked negative relationship between diarrhea and the physical growth of children has been demonstrated in clinical and epidemiological studies (35-38) and the relative risk of diarrhea mortality is significantly increased for malnourished children especially among children aged 6 to 11 mo (39), this relationship was not observed in the present study. Since the incidence of diarrhea has seasonal variation and the survey was conducted in May, colder and drier season with a lower incidence of diarrhea, a further prospective longitudinal survey is needed.

In addition, socioeconomic factors such as older age, male gender, higher number of children aged under 5 yrs, lower family income, young mothers' age at giving birth and mothers' poor education those observed association with childhood undernutrition in previous studies in Vietnam (19, 21) and also in other Asian countries (27, 28, 40-42) were assumed to be predictors of childhood underweight in the present study. However, we did not observe any association between these socioeconomic factors and children's underweight in the logistic regression model. For economic status, inequality of income was reported in Vietnam and it was the lowest in the northern mountainous area compared to other regions (43). The mean family income in the present study was similar to the reported income in northern mountainous areas : 10.9000 VND (40). Significant association between economic status and childhood undernutrition has been observed when across different economic areas are plotted (19) ; however, the present study was conducted in a particular rural mountainous area, and the difference of family income might be too small to show the significant association to childhood underweight. Mother's young age at birth was assumed to be a risk factor for underweight because of lack of experience in child care ; however, younger mothers in the study area usually lived with their parents and could have support from them. Therefore the mother's younger age at birth may not be a risk factor for the childhood underweight. Lower education is considered to create difficulties in accessing skills, information and health care services (44), and several previous studies have reported a relationship between the mother's education level and the childhood underweight ; however, the small sample size may limit the opportunity to observe such a statistical association in the present study.

Other factors such as vitamin A deficiency, zinc deficiency and anemia continue to be serious problem in the public health of preschool children, particularly in children aged under 2 y in mountainous areas, and contribute to underweight in this population (22, 45). A further research is needed to assess their micronutrient status and efficacy of nutritive intervention for children aged under 2 y to be considered in the health system in northern mountainous Vietnam.

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Effects of Two-Month Consumption of 30 g a Day of Soy Protein Isolate or Skimmed Curd Protein on Blood Lipid Concentration in Russian Adults with Hyperlipidemia

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Summary Recently the American Heart Association has reported that favorable effects of soy protein on blood lipids were characteristic only for high amounts of soy protein and not observed for an intake less than 30 g/d. However, the period of the studies with the smaller amount was 4–6 wk and we thought a longer study was necessary for the conclusion. The death rate by heart disease is very high in Russia; therefore, we have done this study in Russian subjects with hyperlipidemia. Prior to the study we tried to find a favorable method for subjects to take 30 g protein a day from soybean protein isolate (SPI) or skimmed curd protein (SMP) and decided to use Russian style cookies. Thirty subjects with hyperlipidemia were recruited; however, due to the 5-mo long study 28 of them (19 females and 9 males aged 50±2 y) could complete the trial. They were randomly assigned to two groups and were given either cookie for 2 mo separated by a month-long washout interval in a cross-over design. Fasting blood samples were drawn before and after the dietary treatments. Fasting blood samples at 1 mo were also measured as a health check and to observe the trends of the blood parameters in the middle of the study period. Serum samples were used for the lipid and other biochemical measurements. Every month for 3 non-consecutive days, energy and nutrient intakes were assessed and physical activity was estimated by pedometer. With the consumption of SPI for 2 mo, concentrations of total-cholesterol changed from 280±7 to 263±8 mg/dL (−6.5%, p=0.0099), HDL-cholesterol from 57.4±2.5 to 62.6±2.9 mg/dL (+9%, p=0.0047), non-HDL-cholesterol (total-cholesterol−HDL-cholesterol) from 223±7 to 201±8 mg/dL (−11%, p=0.0023) and triglycerides from 204±23 to 173± 19 mg/dL (−18%, p=0.022). There were no significant changes with SMP (p>0.05). Thus, administration of 30 g SPI a day for 2 mo confirmed its favorable effects on serum lipids in Russians with hyperlipidemia.

Key Words soy protein isolate, skimmed milk curd, hyperlipidemia, Russian

It is well established that soy protein foods can decrease blood cholesterol (*1–3*) and mortality rates from cardiovascular diseases (CVD) (*4, 5*) both in Asians (*6, 7*) and Westerners (*8–10*). The Food and Drug Administration of the USA and the American Heart Association recommend daily consumption of 25 g of soy protein to control blood cholesterol (*11, 12*). However, this conclusion is based mainly on studies with whole soy foods, which contains several components of soy beans that influence blood lipids (*13*). The results of studies with soy protein isolate (SPI) are less consistent and recent meta-analysis shows that favorable results were observed only in studies with quite high amounts of soybean protein (*13*) and that there were no effects when the participants consumed about 25 g of SPI daily for 6 wk (*14, 15*). These findings suggest that at least 30 g of soy protein and more than 6 wk administration are necessary to achieve favorable changes in blood lipid concentrations.

From the practical point of view the amount of soy protein that should be consumed daily is very important. If the amount is too high, few people can follow a program in daily life and the results will remain only as research findings without practical substance. This is especially important for people who are not familiar with soy foods and in particular for Russians. In international journals there are no publications on the effect of soy protein foods on blood lipids in Russians, whose dietary habits and life-style, i.e. high consumption of saturated fats (*16*) and abuse of alcohol (*17–19*), associated with excessive body mass (*20*), predispose to short longevity, as a result of a high mortality rate especially from CVDs (*21*), which is characteristic of present-day Russians. According to the WHO Statistical

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Information System Mortality Database, the mortality rate per 100,000 from CVDs in Russian adults under 65 in 1986–1991 was 146–161 and subsequently gradually increased, reaching 232–251 in 2001–2005 (22). The mortality rates from CVDs for males and females in other countries were 136.3 and 138.0 respectively in Japan, 241.8 and 244.4 in the USA, 262.7 and 235.2 in England and 172.4 and 176.0 in France (22). In the late 1990 s the average life-spans of Russians were only 58 y for males and 72 y for females (21), while they were 79.0 and 85.8 y for Japanese, 75.2 and 80.4 y for Americans, and 76.6 and 81.0 y for Frenchmen (23). This study was aimed at investigating whether SPI (30 g/d) is effective in controlling serum lipids and preventing heart disease in Russians. Russia is able to produce abundant soybeans but the consumption of soy foods by Russians is very limited partly because of the low quality of the soy foods produced. To promote the use of soybean and soy products, production of good quality products is necessary as well as scientific evidence that SPI is good for the health of Russians.

MATERIALS AND METHODS

Subjects. Fifty-three male and female subjects aged 32–67 were recruited by a cardiologist on a voluntary basis from people visiting a private diagnostic center. These subjects were instructed to follow their usual life and nutrition styles, to minimize differences in energy intake from day to day, to maintain their body weight, and to avoid the use of lipid-lowering drugs throughout the study. Their physical characteristics, nutrient intake, and physical activities were documented. After a lead-in period of 2–3 wk blood samples were taken. Serum samples were frozen until analyses of lipids, total protein and glucose concentrations, and GPT and GOT activities were made. Of these 53 subjects, 30 subjects (9 males and 21 females) aged between 32 and 64 were selected on the basis of their ability to follow the protocol. The inclusion criteria were overweight (BMI 25–34 kg/m^2), fasting serum total cholesterol 240–330 mg/dL, non-HDL-cholesterol 150–280 mg/dL, HDL-cholesterol 40–70 mg/dL, and triglycerides 100–280 mg/dL. The exclusion criteria were the presence of endocrine, liver, renal and gastrointestinal diseases.

Ethics. In accordance with the Helsinki Declaration on human studies, the protocol was approved by the Amur State Medical Academy Ethics Committee. The participants were informed in detail about the purpose, the advantages and disadvantages of the study, and their rights and duties concerning their lifestyle. Informed consent from all participants was obtained in writing.

Study design. This study was done by a crossover design. Subjects were given either SPI- or skimmed curd protein (SMP)-enriched cookies (protein content 30%) for the first 2 mo. After a month-long washout period, the subjects received the opposite test food for another 2 mo.

Intervention. We tried to make various kinds of foods with 30% SPI or SMP in cooperation with local food specialists and assessed their acceptability with our associates. We finally decided on cookies with a low energy content (340 kcal/100 g) due to the use of sorbitol instead of sucrose. Special SPI-enriched cookies (protein content 30%) were supplied by the local authorized confectioner and used as the test food for the experimental group (composition per 1 kg of cookies: wheat flour 333 g, SPI 333 g, margarine 183 g, sorbitol 233 g, egg 33 g, salt 6.7 g, baking soda 3.3 g, ammonia carbonate 1.7 g, energy value 340 kal in 100 g). The daily total amount of cookies made up 30 g in terms of protein content and was divided into 2 or 3 servings throughout the day. SPI (FUJIPRO) was obtained from Jilin Fuji Protein Co., Ltd. China. Preparation of cookies with casein was difficult because of its low solubility and its tendency to form glue-like structures. Therefore we used SMP (protein 18%, fat 0.6% and carbohydrate 1.5%). Originally the producer suggested the following formula for skimmed curd-enriched cookies: skimmed curd 500 g, wheat flour 150 g, sorbitol 100 g, egg 33 g, salt 3 g, baking soda 2 g, ammonia carbonate 1 g. However, the composition of these cookies (ratio protein : fat : carbohydrates) was not identical to the composition of SPI-enriched cookies, SMP content in these cookies was insufficient, and storage time was limited. For this reason, we allowed the subjects to prepare SMP cookies by themselves by mixing the daily amount of skimmed curd (170 g) with a 33 g of wheat flour, 1 g of egg solids, 23 g of sorbitol, salt 0.7 g, baking soda 0.3 g, ammonia carbonate 0.2 g, and frying the obtained dough. The composition of these SMP-enriched cookies was identical to the composition of SPI-enriched cookie with the exception of test protein. For the convenience of participants the mixtures of minor ingredients for daily portions of skimmed curd cookies were prepared by the confectionary producer, packed in small packets and delivered to the participants. Skimmed curd was purchased from an authorized local provider.

Nutrition survey. A nutrition survey was done every month, including the wash-out period, for 3 non-consecutive days (two weekdays and one weekend day) by the 24-h recall method. A specialist instructed the subjects on how to record their food on the day prior to the interview.

Physical activity monitoring. To monitor physical activity of the subjects, we measured the number of steps per day using a pedometer (Omron HJ-005-E, China).

Chemical analysis. Blood samples were drawn in the morning after overnight fasting (10 h) before the beginning of the intervention, as well as after the first and second month. Serum samples were frozen until analyses. TC was measured by the cholesterol-oxidase method, HDL-C by the same method after precipitating low density and very low density lipoproteins, TG by the lipoprotein lipase and glycerophospate-oxidase method, glucose by the glucose-oxidase method, TP by the biuret colorimetric method, GOT and GPT by the enzymatic kinetic methods.

Table 1. Characteristics of the participants at baseline.[1]

Index	Males	Females
Number of participants	9	19
Physiological indices		
Age (y)	51±2	50±2
Weight (kg)	87.2±3.8	77.6±2.4
Height (cm)	173±2	164±1
BMI	29.6±1.2	28.7±0.9
Energy consumption (kcal per day)	2,022±218	1,409±93
Nutrient intake (g per day)		
Proteins	107.0±6.7	78.9±5.9
Fats	80.0±7.4	52.5±2.9
Carbohydrates	272.0±20.0	152.7±8.9
Steps per day	9,397±1,373	8,569±642
Biochemical indices		
Total cholesterol (mg/dL)	301±26	247±7
HDL-cholesterol (mg/dL)	54±3	63±4
Non-HDL-cholesterol (mg/dL)	247±25	214±9
Triglycerides (mg/dL)	255±33	183±30
Glucose (mg/dL)	80±9	94±8
Total protein (g/L)	78±2	77±1
GOT (U/L)	20±1	20±2
GPT (U/L)	16±2	18±3

[1] Values are means±SE. Total number of participants in each pooled group was 28.

Table 2. BMI, energy and nutrient intakes and physical activity of the participants at baseline and after 1 and 2 mo of the dietary treatments.[1]

Indices	Groups	Initial values	After 1 mo	After 2 mo
BMI (kg/m^2)	SPI	28.7±0.7	28.5±0.7	28.8±0.7
	SMP	29.2±0.7	29.0±0.7	28.9±0.7
Energy intake (kcal/d)	SPI	1,713±110	1,644±115	1,670±121
	SMP	1,574±92	1,612±97	1,555±138
Protein intake (g/d)	SPI	86.9±4.8	89.9±4.8	88.1±4.6
	SMP	82.4±3.0	88.4±3.0	81.7±3.5
Fat intake (g/d)	SPI	62.1±3.3	63.1±3.3	67.4±3.8
	SMP	56.6±2.1	58.6±2.1	57.0±3.0
Carbohydrate intake (g/d)	SPI	194.8±7.3	189.9±10.5	182.4±11.8
	SMP	185.0±8.9	182.0±8.9	172.4±9.7
Activity (steps/d)	SPI	8,757±955	8,629±845	8,710±640
	SMP	8,910±805	8,727±910	9,157±702

[1] Values are means±SE. Total number of participants in each pooled group was 28.

Among the 21 women, 5 were pre-menopausal. They had regular menstrual cycles, and blood was withdrawn monthly on the same day after menses.

Statistical analysis. The statistical analysis was performed by the StatSoft, Inc. (2001) STATISTICA (data analysis software system), version 6. To evaluate the statistical significance of the observed changes in the studied indices in the same group of participants between the initial and final values (after 2 mo of the dietary intervention) and between the SPI and SMP groups of participants at the end of the trial, we used parametric criteria: paired *t*-test for dependent samples and unpaired *t*-test for independent samples, respectively. The statistically significant level was set at p less than 0.05.

RESULTS

Table 1 shows the baseline characteristics of the subjects. All subjects were overweight (average BMI 29.0±3.87). The average values of serum total cholesterol, non-HDL-cholesterol, HDL-cholesterol, and triglycerides were typical for moderate hyperlipidemia. Six subjects had serum cholesterol higher than 300 mg/dL. Glucose and total protein contents, GPT and GOT activities were normal.

The 30 subjects were assigned to two groups, consisting of 15 persons each. These groups received SPI cookies and skimmed curd cookies for 2 mo. After a month's washout period, the subjects received the opposite test food for another 2 mo.

Table 3. Serum lipid and glucose concentrations and GOT and GPT activities at the beginning and after 1 and 2 mo of the dietary treatments.[1]

Indices	Groups	Initial values	After 1 mo	After 2 mo
Total cholesterol (mg/dL)	SPI	280±7	281±10	263±9**
	SMP	277±9	282±10	272±9
HDL-cholesterol (mg/dL)	SPI	57.4±2.5	60.2±2.6	62.5±2.9***
	SMP	59.3±3.3	60.1±2.4	56.6±2.6
Non-HDL-cholesterol (mg/dL)	SPI	223±7	221±10	201±8.8***
	SMP	219±10	222±10	215±9
Triglycerides (mg/dL)	SPI	204±23	183.3±20*	173±16*
	SMP	192±17	193±20	201±17
Glucose (mg/dL)	SPI	85.8±4.7	83.4±3.0	79.0±3*
	SMP	87.8±5.5	86.1±4.8	88.6±3.6
Total protein (g/L)	SPI	78.0±1.0	78.3±0.8	78.9±0.8
	SMP	76.2±0.8	78.0±0.7	77.6±1.1
GOT (U/L)	SPI	22.1±2.4	20.8±1.7	22.2±2.1
	SMP	17.2±1.4	20.7±1.4	18.9±1.7
GPT (U/L)	SPI	22.6±3.1	18.3±2.6	21.1±1.9
	SMP	16.5±1.7	16.4±1.7	18.9±1.9

[1] Values are means±SE. Total number of participants in each pooled group was 28.
*$p<0.05$, **$p<0.01$, ***$p<0.005$. p values correspond to the difference between the initial values of the index and values after 1 or 2 mo (t-test for dependent samples).

Both the SPI and SMP cookies were well accepted by the majority of the subjects. Among the 30 subjects enrolled in the study, only 2 did not finish. One woman failed to consume the SPI enriched cookies because she developed an intestinal upset, and the other finished only the first half of the trial and was lost to follow-up for personal reasons. Twenty-eight subjects successfully completed the trials. Energy and nutrient intakes, physical activity, and physical characteristics are shown in Table 2. The participants consumed similar amounts of food from day to day within the study period. There were no significant differences in BMI, energy, protein, fat and carbohydrate intakes, or physical activity throughout the trial.

Table 3 shows the changes in serum lipid, glucose and total protein concentrations and GPT and GOT activities before and after the consumption of the test foods. Two-month-long consumption of SPI was followed by decreases in total cholesterol (−17 mg/dL, −6.5%, $p=0.0099$), non-HDL-cholesterol (−22 mg/dL, −11%, $p=0.0023$), and triglycerides (−31 mg/dL, −18%, $p=0.022$), and by an increase in HDL-cholesterol (+5.1 mg/dL, +9%, $p=0.0047$). There were no statistically significant changes in the skimmed curd treatment throughout the course of the study. The initial values for the glucose, total protein contents, GPT and GOT activities in the experimental and control groups were characteristic for healthy persons. Introduction of SPI or skimmed curd did not produce any changes in serum total protein content or in GPT and GOT activities, thereby indicating that consumption of the test foods with a daily SPI amount of 30 g is safe for the liver. The serum glucose contents in the experimental group decreased slightly by 6.8 mg/dL (−8.6%, $p=0.048$), significantly lower (−9.6 mg/dL, −10.8%, $p=0.046$) compared with the milk curd group. We also measured the various blood parameters after 1 mo on dietary intervention as part of a health check and to observe the trends of the blood parameters at mid study period.

DISCUSSION

This study was aimed at investigating the ability of SPI to decrease blood lipids in Russians, whose dietary habits and life-styles predispose them to low longevity, as a result of a high mortality rate from CVDs, which is characteristic for today's Russia. Although older studies, in which subjects were given high doses of SPI, showed favorable results and Anderson et al. summarized from their meta-analysis that 31–47 g SPI can significantly decrease serum total- and LDL-cholesterol concentrations (*1*), recent studies with 25 g of the protein for 6 wk did not show such effects (*14*, *15*). This was the reason for the American Heart Association experts to question the effectiveness of SPI in decreasing blood cholesterol (*24*). Thus, the daily amount of SPI and the duration of the study seem to be very important for the effect of SPI on blood lipids. In this study with a crossover design we have shown that by consuming 30 g of SPI daily for 2 mo serum concentrations of total-cholesterol, non-HDL-cholesterol and triglycerides decreased, and HDL-cholesterol concentration increased in Russians with moderate hyperlipidemia (Table 3).

In agreement with the results of the majority of studies (*13*), in our study a 1-mo SPI consumption was not enough to decrease blood lipids and only triglycerides in blood serum decreased (by 10%), while serum cholesterol did not change. In contrast to this, 2-mo SPI consumption was followed by statistically significant

changes in serum lipids, namely, by the reduction of total-cholesterol by 17 mg/dL, of non-HDL-cholesterol by 22 mg/dL, and of triglycerides by 31 mg/dL, and by the increase of HDL-cholesterol by 5 mg/dL (Table 3). The observed effect should be considered as strong, bearing in mind the results of the recent meta-analysis of the effect of soy protein supplementation on serum lipids, which shows that the overall pooled net effect of soy protein supplementation on serum lipids was −5.26 mg/dL for total-cholesterol, −4.25 mg/dL for LDL cholesterol, 0.77 mg/dL for HDL cholesterol and −6.26 mg/dL for triglycerides (*13*). The important results in this study were the clear increase in HDL-cholesterol concentration ($p<0.001$) and clear decrease in non-HDL concentration ($p<0.001$) (Table 3). Among previous reports, even those with a high SPI administration, only a few studies observed significant changes in the HDL-cholesterol concentration (*24*). Incidentally, we discovered that consumption of SPI was followed by a decrease in serum glucose level by 6.8 mg/dL (Table 3). The established ability of SPI to decrease serum triglycerides and glucose indicates that soy foods may be useful in the prophylaxis and treatment of metabolic syndrome. The consumption of the same amount of SMP did not induce any changes in serum lipids or glucose concentrations.

The first reason why we could observe favorable effects in this study with 30 g SPI may be the relatively long administration of the experimental diets with a cross-over design. In this type of study, proper care of the subjects is very important. Because of this it is not easy to increase the number of participants. Inter-individual variances are usually large and therefore a large number of subjects is required to have significant effects in a parallel study, while a relatively small number of participants is sufficient for a cross-sectional study because the inter-individual differences are thereby minimized. A review paper by the American Heart Association shows that most cross-sectional studies lasted less than 6 wk (*24*). In our study, the results at 2 mo were effective, suggesting that a study longer than 2 mo may be able to decrease blood lipids of hyperlipidemic Russians at 30 g SPI/d. The second reason for the favorable results of SPI may be the good acceptability of the test diets by the subjects. Sustainability is always required for these types of studies to show the expected results. The reason for the relatively short periods of the previous studies mentioned above might have been the poor acceptability and poor sustainability of SPI by the subjects. SPI at 30 g a day may be easy for Asians to accept but not for Westerners (*12*). In most of the previous studies, there was not sufficient description of the formulas for the test foods enriched with SPI and casein. The authors simply wrote: "The test proteins were incorporated into a variety of baked products and ready-to-mix beverages" (*8*). Drinks with SPI and casein have rather a bad taste and smell and may not be consumed by the participants over a long period. For this reason, we tried to develop some well-accepted foods with a high SPI content (30%). Both SPI- and SMP-enriched cookies were well accepted by the majority of the subjects. Of the 30 subjects enrolled in the study, only 2 did not finish. One woman failed to consume SPI cookies because of intestinal upset, and the other could finish only the first half of the trial because of personal reasons. The intakes of energy, proteins, fats and carbohydrates were similar at all periods of study: in the initial period, during the first and second months and at the wash-out period, which may also mean that the cookies were well accepted.

The third reason for the favorable results may be the level of hyperlipidemia in our subjects. As reviewed by Anderson et al. (*1*) the cholesterol-lowering effect of SPI was observed more clearly in subjects with high serum total- and LDL-cholesterol concentrations than in those with normal and low lipid concentrations. Our inclusion criteria were overweight (BMI 25–34 kg/m^2), fasting serum total cholesterol 240–330 mg/dL, non-HDL-cholesterol 150–280 mg/dL; HDL-cholesterol 40–70 mg/dL and triglycerides 100–280 mg/dL.

In conclusion, the present study showed that the administration of 30 g SPI for 2 mo has favorable effects on serum lipid concentrations in Russians with hyperlipidemia.

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HM initiated this study; SY and KT designed the study; EAB, MAS, HM, TY, SY and KT invented the formula for preparation of SPI and skimmed curd-enriched cookies; IGM and VAD coordinated the subject recruitment and data collection; NAF performed the nutritional survey and coordinated measurement of the physical activity; EAB and MAS supervised the laboratory analyses; EAB, VAD and SY performed the statistical analyses; EAB, TY, SY, KT and HM interpreted the results of the study; EAB wrote the manuscript; TY, SY, KT and HM assisted in the manuscript preparation. None of the authors had any personal or financial conflict of interest.

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Cardiorespiratory Fitness as a Quantitative Predictor of All-Cause Mortality and Cardiovascular Events in Healthy Men and Women

A Meta-analysis

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Context Epidemiological studies have indicated an inverse association between cardiorespiratory fitness (CRF) and coronary heart disease (CHD) or all-cause mortality in healthy participants.

Objective To define quantitative relationships between CRF and CHD events, cardiovascular disease (CVD) events, or all-cause mortality in healthy men and women.

Data Sources and Study Selection A systematic literature search was conducted for observational cohort studies using MEDLINE (1966 to December 31, 2008) and EMBASE (1980 to December 31, 2008). The Medical Subject Headings search terms used included *exercise tolerance, exercise test, exercise/physiology, physical fitness, oxygen consumption, cardiovascular diseases, myocardial ischemia, mortality, mortalities, death, fatality, fatal, incidence,* or *morbidity*. Studies reporting associations of baseline CRF with CHD events, CVD events, or all-cause mortality in healthy participants were included.

Data Extraction Two authors independently extracted relevant data. CRF was estimated as maximal aerobic capacity (MAC) expressed in metabolic equivalent (MET) units. Participants were categorized as low CRF (<7.9 METs), intermediate CRF (7.9-10.8 METs), or high CRF (≥10.9 METs). CHD and CVD were combined into 1 outcome (CHD/CVD). Risk ratios (RRs) for a 1-MET higher level of MAC and for participants with lower vs higher CRF were calculated with a random-effects model.

Data Synthesis Data were obtained from 33 eligible studies (all-cause mortality, 102 980 participants and 6910 cases; CHD/CVD, 84 323 participants and 4485 cases). Pooled RRs of all-cause mortality and CHD/CVD events per 1-MET higher level of MAC (corresponding to 1-km/h higher running/jogging speed) were 0.87 (95% confidence interval [CI], 0.84-0.90) and 0.85 (95% CI, 0.82-0.88), respectively. Compared with participants with high CRF, those with low CRF had an RR for all-cause mortality of 1.70 (95% CI, 1.51-1.92; $P<.001$) and for CHD/CVD events of 1.56 (95% CI, 1.39-1.75; $P<.001$), adjusting for heterogeneity of study design. Compared with participants with intermediate CRF, those with low CRF had an RR for all-cause mortality of 1.40 (95% CI, 1.32-1.48; $P<.001$) and for CHD/CVD events of 1.47 (95% CI, 1.35-1.61; $P<.001$), adjusting for heterogeneity of study design.

Conclusions Better CRF was associated with lower risk of all-cause mortality and CHD/CVD. Participants with a MAC of 7.9 METs or more had substantially lower rates of all-cause mortality and CHD/CVD events compared with those with a MAC of less 7.9 METs.

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CORONARY HEART DISEASE (CHD) is a major cause of disability and premature death throughout the world.[1] Epidemiological studies have demonstrated an inverse association between physical fitness and the incidence of CHD or all-cause mortality in healthy or asymptomatic participants. Physical fitness is typically expressed as cardiorespiratory fitness (CRF) and is assessed by exercise tolerance testing[2]; however, it is rare for clinicians to consider CRF when evaluating future risk of CHD.[3]

A major reason for lack of consideration of CRF as a marker of CHD risk may be that the quantitative association of CRF for cardiovascular risk is not well established. The degree of risk reduc-

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tion associated with each incremental higher level of CRF, the criteria for low CRF, and the magnitude of risk associated with low CRF have been inconsistent among studies. Our goal of this meta-analysis was to systematically review the quantitative relationship between CRF and all-cause mortality and CHD or cardiovascular disease (CVD) events in healthy individuals.

METHODS

Search Strategy

The meta-analysis was conducted according to the checklist of the Meta-analysis of Observational Studies in Epidemiology.[4] We performed a systematic literature search of MEDLINE (1966 to December 31, 2008) and EMBASE (1980 to December 31, 2008) for observational cohort studies. Three search themes were combined using the Boolean operator *and*. The first keywords were related to CRF (combined exploded versions of the Medical Subject Headings [MeSH] as follows: *exercise tolerance* OR *exercise test* OR *exercise/physiology* OR *physical fitness* OR *oxygen consumption*); the second keywords were related to the outcome of this meta-analysis (combined unexploded version of MeSH [*cardiovascular diseases*] or the exploded version of MeSH [*myocardial ischemia*]) or the following text words (*mortality* OR *mortalities* OR *death* OR *fatality* OR *fatal* OR *incidence** OR *event** OR *morbidity*); and the third keywords were related to risk estimates (combined text words as follows: *regression analysis* OR *regression model** OR *statistical regression** OR *logistic regression** OR *logit regression** OR *logistic model** OR *logit model** OR *Cox model* OR *hazard model* OR *odds ratio** OR *ORs* OR *relative odds* OR *risk ratio** OR *relative risk** OR *RRs*). We also included studies published in non-English language. In addition, we searched the reference lists of all identified relevant publications.

Inclusion and Exclusion Criteria

We included papers if (1) CRF was assessed by an exercise stress test; (2) the association of CRF with all-cause mortality and with CHD or CVD was evaluated; (3) CRF could be assessed as maximal aerobic capacity (MAC), expressed in units of metabolic equivalents (METs), which is defined as the ratio of intensity of physical activity to that of sitting at rest; and (4) risk ratios (RRs) and their corresponding 95% confidence intervals (CIs) relating to each category of MAC were reported or could be calculated. We excluded studies that were intended only for patients having a specific disease that presented a major risk factor, such as diabetes, hypertension, and familial hypercholesteremia, as well as studies that included patients with CHD or chronic heart failure.

To avoid double counting of a cohort, study selection was limited to a single set of results when multiple publications were available for a single observational study. The first priority for selection was the study with the longest follow-up and the second was the study with full cohort analysis covering the largest number of participants among articles from a single cohort. We conducted 2 separate meta-analyses for risk of all-cause mortality and CHD or CVD in relation to CRF. When an individual study provided data on both CHD or myocardial infarction (MI) and CVD,[5-7] priority for data abstraction was given to CVD because CVD is more comprehensive than CHD and MI. Similarly, if data on both events and deaths were provided,[6,8,9] priority was given to events.

We combined CHD and CVD into 1 outcome (CHD/CVD), which included studies whose outcome was a CVD event, CVD death, CHD event, or CHD death, because the number of eligible studies included was limited. Although criteria for the end point in CHD varied from study to study, the end points that we specified as CHD outcome in our meta-analysis were (1) death from MI; (2) death from CHD including MI; and (3) a CHD event, a term which meant either death from CHD, sudden cardiac death, occurrence of nonfatal CHD, or nonfatal MI. Additionally, we included studies whose outcome was either CVD death (ie, encompassing death from cardiovascular causes other than CHD) or CVD events (ie, lumping together fatal and nonfatal CVD).

Data Abstraction

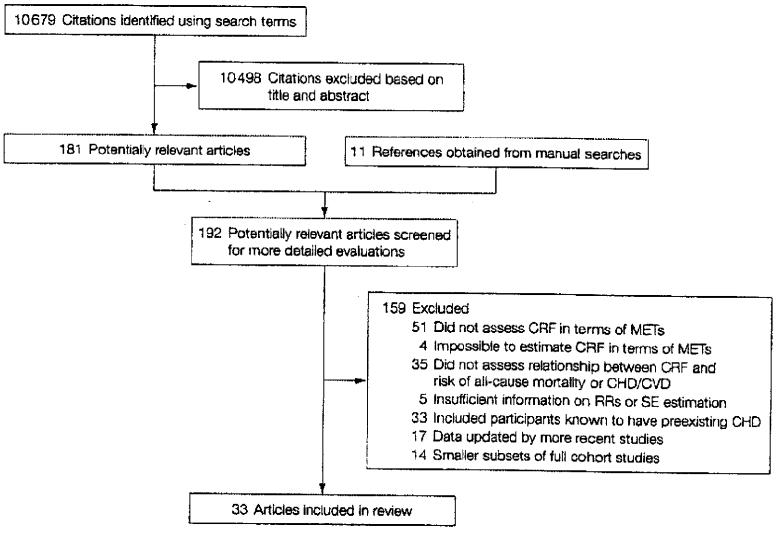
Data abstracted were the first author's name, year of publication, country of origin, specific outcomes, duration of follow-up, methods for outcome assessment, instrument or methods for measurement of CRF, whether maximal exercise testing (defined as instructing participants to continue exercise until their maximal workload) was conducted, mean of participants' age, proportion of men, number of participants and number of new cases (ie, deaths or events) during the observational periods, adjusted variables, and whether participants with abnormal electrocardiogram findings (ie, ST elevation/depression) during exercise testing were included. Two of our investigators (S. Kodama and H. Sone) independently reviewed each published paper and extracted relevant information. Any disagreement was resolved by consensus.

In studies using CRF as a categorical variable, we standardized all reported RRs into comparison of the risk of the lower CRF group with that in the higher CRF group. Therefore, when the lowest CRF group was referent, we converted the reported RR into its reciprocal. When a study provided several RRs, such as unadjusted and adjusted RRs, the most completely adjusted RR was used. The standard error (SE) of each RR was derived from 95% CIs or *P* values. If data related to RR and its corresponding SE were not provided, their value was directly calculated using data on the number of participants (P) and new cases (C) of risk and the reference (ref) groups in each comparison, using the equation:

$$RR = [(C_{risk}/P_{risk})/(C_{ref}/P_{ref})],\ SE^2 = [(1/C_{risk})-(1/P_{risk})]+[(1/C_{ref})-(1/P_{ref})].$$

The MAC was calculated from the exercise workload at the termination of exercise testing and relative exercise intensity (ie, proportion of the workload to MAC). The exercise workload was converted into MET units (1 MET corresponds to 3.5 mL/min/kg of oxygen consumption [$\dot{V}O_2$]), according to the Metabolic Calculation Handbook by

Figure 1. Selection of Articles for Meta-analysis



CHD indicates coronary heart disease; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; METs, metabolic equivalents; and RRs, risk ratios.

the American College of Sports Medicine.[10] Relative exercise intensity was estimated using a linear equation according to Swain et al[11]:

heart rate at exercise/maximal heart rate = 0.64 × ($\dot{V}O_2$ at exercise/maximal $\dot{V}O_2$).

For some specific exercise stress tests, the MAC was directly estimated using the prediction equation determined by a previous validation study for each protocol of the exercise test (the Balke treadmill test,[12,13] the modified Bruce test,[14] and the Canadian Home Fitness test[15]).

When exposure was expressed as a range, we converted it into point estimates expressed as average exposure using the midpoint of the range except for the lowest and highest fit group. If data on the average value were not available, it was estimated by the assumption that the MAC levels of the study population had a normal distribution using the mean value and its SD of each study sample. This assumption is consistent with a prior study.[16] However, if the SD was not available, we assumed that its value equaled 2 METs, according to the statement of the American Heart Association.[17]

After converting all exposures into MET units, we additionally adjusted MET units for age and sex. According to a Statement for Healthcare Professionals From the American Heart Association,[17] we assumed that the MAC is 2 METs lower in women than in men and that for each year of aging, it decreased by 0.1 MET based on a prior study.[18] Finally, we represented CRF as the adjusted MAC under the assumption that all participants were 50-year-old men in the analyses described below.

Dose-Response and Categorical Analyses

We first performed dose-response analyses by summarizing how much risk reduction could be predicted per incremental increase in CRF. The study-specific RR for each higher MET (corresponding to 1-km/h higher running/jogging speed) in MAC, if not reported, was estimated by regressing the natural logarithm of the RR (lnRR) according to each CRF category against its corresponding mean MAC value, using the method described by Greenland and Longnecker.[19]

We then performed categorical analyses to summarize the risk of all-cause mortality and CHD/CVD for low CRF. We assigned every RR reported in each study to 1 of the following 3 comparisons based on the CRF level of risk and reference group: (1) low vs high CRF, (2) low vs intermediate CRF, and (3) intermediate vs high CRF. This method is based on a previous meta-analysis of the relationship between activity level and stroke risk.[20] For studies that presented risk estimates for more than 2 CRF categories, the ranges of the adjusted MAC of the lowest, highest, and in-between categories defined by each study were 5.5 to 7.8, 11.0 to 15.2, and 7.9 to 10.7 METs, respectively; except that in 2 studies,[21,22] the second highest category of CRF was more than 11.0 METs and, in 1 study,[7] the highest category of CRF was 10.6 METs.

To avoid overlap of the CRF range of the 3 categories, we defined low, intermediate, and high CRF as less than 7.9 METs, 7.9 to 10.8 METs, and 10.9 METs or more, respectively. Consequently, we could assign every RR in each study to 1 of the 3 predefined subgroups with 2 exceptions. In 2 studies,[21,22] the mean MAC values for both the highest and the second highest category were the same as the high CRF category (defined by ≥10.9 METs). Therefore, RR data for comparison between 2 CRF categories could not be included in our categorical analysis for these 2 studies.

Statistical Analysis

The pooled RRs for a 1-MET higher level of MAC and the lower CRF in comparison with the higher CRF within each of the 3 comparisons were estimated by using a fixed-effects or random-effects model.[23] If significant heterogeneity of RRs that was tested by calculating the I^2 statistic[24] was present, we chose the pooled estimates from the random-effects model because it is better than the fixed-effects model and it explains between-study heterogeneity.

To examine the effect of study characteristics on risk reduction per 1-MET higher level of MAC, sensitivity analyses were conducted for the possible confounders (mean age [≥50 years or not], sex [only men or not], adjustment for smoking [yes or no], adjustment for multiple confounders, defined as adjustment

Table 1. Characteristics of Studies Included in the Meta-analysis

Source (Location)	No. of Participants	Men, %	Mean (or Midpoint) Age, y	Mean Follow-up, y	Methods for Outcome Measures	Specific Outcomes (CHD/CVD Criteria)	No. of Events for Each Outcome	Instrument for Assessing CRF	Whether Max or Sub Reached[a]
Aijaz et al,[29] 2008 (US)	8620	73	52	16	Registry	All-cause mortality	535	Treadmill	Max
Aktas et al,[30] 2004 (US)	3554	81	57	8	Registry	All-cause mortality	114	Treadmill	Sub
Allen et al,[31] 1980 (US)									
Men	350	100	NA	1.1	Questionnaire	CHD event (MI, sudden cardiac death)	34	Ergometer	Max
Women	302	0	NA				10		
Arraiz et al,[32] 2004 (Canada)	NA	NA	47	7	Registry	All-cause mortality; CVD death (NA)	55; 37	Canadian Home Fitness Test	Sub
Balady et al,[33] 2004 (US)									
Men	1431	100	45	18.2	Hospital record	CHD event (onset of AP, coronary insufficiency, MI)	224	Treadmill	Sub
Women	1612	0	45				81		
Bruce et al,[34] 1980 (US)	2365	100	45	5.6	Questionnaire	CHD event (NA)	47	Treadmill	Max
Cumming et al,[35] 1975 (Canada)	486[b]	100	53	3	Questionnaire	CHD event (NA)	26	Ergometer	Max
Erikssen et al,[36] 1998 (Norway)	1428	100	57	13	Registry	All-cause mortality; CVD death (CHD, stroke, the other CVD)	238; 120	Ergometer	Max
Erikssen et al,[37] 2004 (Norway)	2014	100	49	26	Questionnaire and registry	CHD death (CHD, sudden cardiac death)	300	Ergometer	Max
Farrell et al,[38] 2004 (US)	6925	0	43	11.4	Registry	All-cause mortality	195	Treadmill	Sub
Gulati et al,[16] 2003 (US)	5721	0	52	8.4	Registry	All-cause mortality	180	Treadmill	Max
Gulati et al,[39] 2005 (US)	5636	0	52	9	Registry	All-cause mortality; CVD death (*ICD-9, ICD-10*)	171; 52	Treadmill	Max
Gulati et al,[40] 2005 (US)	5721	0	52	8.4	Registry	CVD death (NA)	180	Treadmill	Max
Gyntelberg et al,[41] 1980 (Denmark)	5249	100	50	5	Registry	CHD event (MI, sudden cardiac death)	170	Ergometer	Sub
Hein et al,[42] 1992 (Denmark)	4999	100	48	17	Registry	All-cause mortality	941	Ergometer	Sub
Jouven et al,[43] 2005 (France)	5713[b]	100	48	23	Hospital record	CHD death (MI death)	210	Ergometer	Sub
Kampert et al,[44] 1996 (US)	25 341	100	43	8.4	Registry	All-cause mortality	601	Treadmill	Sub
Katzmarzyk et al,[45] 2005 (US)	19 173	100	43	10.2	Registry	All-cause mortality	477	Treadmill	Sub
Laukkanen et al,[5] 2007 (Finland)	1639	100	52	16.6	Registry	All-cause mortality; CVD event (*ICD-9, ICD-10*)	304; 340	Ergometer	Max
Laukkanen et al,[9] 2008 (Finland)	1639	100	52	16.6	Registry	All-cause mortality; CVD event (*ICD-9, ICD-10*)	304; 340	Ergometer	Max
Miller et al,[8] 2005 (UK)	578	100	52	7.3	Questionnaire, registry, and hospital record	All-cause mortality; CVD event (*ICD-9*)	68; 62	Ergometer	Sub
Mora et al,[46] 2003 (US)	2994	0	55	20.3	Questionnaire and registry	All-cause mortality; CVD death (NA)	427; 147	Treadmill	Sub
Myers et al,[47] 2002 (US)	2534[b]	100	56	6.2	Registry	All-cause mortality	288	Treadmill and ergometer	Sub
Peters et al,[48] 1983 (US)	2779	100	45	4.8	Hospital record	CHD event (MI, sudden cardiac death)	36	Ergometer	Sub
Rywik et al,[49] 2002 (US)	1083	57	52	6.8	Registry	CHD event (AP, MI, sudden cardiac death)	76	Treadmill	Max

(continued)

Table 1. Characteristics of Studies Included in the Meta-analysis (continued)

Source (Location)	No. of Participants	Men, %	Mean (or Midpoint) Age, y	Mean Follow-up, y	Methods for Outcome Measures	Specific Outcomes (CHD/CVD Criteria)	No. of Events for Each Outcome	Instrument for Assessing CRF	Whether Max or Sub Reached[a]
Sandvik et al,[50] 1988 (Norway)	1960[b]	100	50	15.9	Registry	All-cause mortality; CVD death (NA)	271; 143	Ergometer	Max
Sawada and Muto,[51] 1999 (Japan)	9986[b]	100	37	14	Questionnaire	All-cause mortality; CHD death (*ICD-10*)	247; 72	Ergometer	Sub
Slattery and Jacobs,[5] 1988 (US)	2431	100	50	18.5	Registry	All-cause mortality; CHD death (*ICD-8*)	631; 258	Treadmill	Sub
Sobolski et al,[52] 1987 (Belgium)	1476	100	48	5	Registry	CHD event (MI, sudden cardiac death)	19	Ergometer	Sub
Stevens et al,[21] 2002 (US)									
Men	2860	100	45	26	Questionnaire and registry	All-cause mortality; CVD death (*ICD-9*)	682; 270	Treadmill	Sub
Women	2506	0	47				484; 179		
Stevens et al,[22] 2004 (US)	1359	100	49	19	Questionnaire and registry	All-cause mortality; CVD death (*ICD-9*)	211; 98	Treadmill	Sub
Sui et al,[7] 2007 (US)									
Men	20 278	100	44	10.4	Questionnaire	CVD event (MI, stroke, coronary revascularization)	1512	Treadmill	Sub
Women	5909	0	45				159		
Villeneuve et al,[53] 1998 (Canada)	7561	48	45	7	Registry	All-cause mortality	129	Canadian Home Fitness Test	Sub

Abbreviations: AP, angina pectoris; CHD, coronary heart disease; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; *ICD-8, International Classification of Diseases, Eighth Revision*; *ICD-9, International Classification of Diseases, Ninth Revision*; *ICD-10, International Statistical Classification of Diseases, 10th Revision*; MI, myocardial infarction; NA, not available.
[a] Max, workload testing was continued until maximal workload; Sub, maximal workload was predicted from findings of submaximal exercise workload.
[b] Including participants with abnormal exercise electrocardiogram (ie, ST elevation/depression).

for >3 factors among obesity, hypertension, total cholesterol or low-density lipoprotein cholesterol, high-density lipoprotein cholesterol and diabetes [yes or no], mean follow-up [≥12 years or <12 years], instrument for assessing CRF [ergometer or others], and maximal exercise testing [yes or no]). To examine the extent to which between-study heterogeneity was explained by these study characteristics, we additionally conducted linear multiple regression analyses by simultaneously entering these confounders as explanatory variables.

Categorical analyses were repeated with multiadjustment for the prespecified confounders to consider the potential heterogeneity of study characteristics among the subgroups (ie, low vs high CRF, low vs intermediate CRF, and intermediate vs high CRF). Tests of interaction were performed to assess whether the association between CRF and the study outcomes varied across these 3 subgroups.

The Begg and Egger tests[25,26] were used for assessment of publication bias (ie, the tendency for positive associations to be published and negative or null associations to be unpublished). We also followed the Duval and Tweedie "trim and fill" procedure[27] as a method of adjustment for suspected publication bias. This method considers the possibility of hypothetical "missing" studies that might exist, imputes their RRs, and recalculates a pooled RR that incorporates the hypothetical missing studies as though they actually existed.

Two-sided $P \leq .05$ was considered statistically significant, except for the test of publication bias for which the recommended levels are $P \leq .10$.[28] Data were analyzed using STATA version 10 (STATA Corp, College Station, Texas).

RESULTS

Literature Search and Study Characteristics

FIGURE 1 shows the number of studies that were identified and excluded at different stages of the selection process. A total of 33 studies[5-9,16,21,22,29-53] were included in our meta-analysis. Characteristics of the 33 selected studies comprising 102 980 participants (range, 486-25 341) and 6910 cases (range, 26-941) for all-cause mortality and 84 323 participants (range, 302-20 278) and 4485 cases (range, 10-1512) for CHD/CVD are shown in TABLE 1. Twenty-one studies* reported all-cause mortality and 24 studies† reported CVD/CHD. Mean age and follow-up duration ranged from 37 to 57 years and 1.1 to 26 years, respectively. Eight studies[8,33,37,39,45,46,49,52] were used for the dose-response analyses only and 4 studies[9,16,40,44] were used for the categorical analyses only. In 20 studies,‡ RRs were adjusted for smoking and in 9 stud-

*References 5, 6, 8, 9, 16, 21, 22, 29, 30, 32, 36, 38, 39, 42, 44-47, 50, 51, 53.
†References 5-9, 21, 22, 31-37, 39-41, 43, 46, 48-52.
‡References 5, 7-9, 16, 21, 22, 30, 32, 33, 37-39, 44-46, 48, 50, 52, 53.

ies,[7-9,16,33,39,46,50,52] there were multiple study confounders (available in an eTable [http://www.jama.com]).

Dose-response Analyses

FIGURE 2 shows the pooled estimates for the reduction in risk of all-cause mortality and CHD/CVD per higher MET of exercise capacity. Pooled RRs of all-cause mortality and CHD/CVD per 1-MET higher level of MAC were 0.87 (95% CI, 0.84-0.90) and 0.85 (95% CI, 0.82-0.88), respectively. There was evidence of statistical heterogeneity of RRs across studies (I^2=82.3%; $P<.001$ for all-cause mortality; I^2=74.7%; $P<.001$ for CHD/CVD).

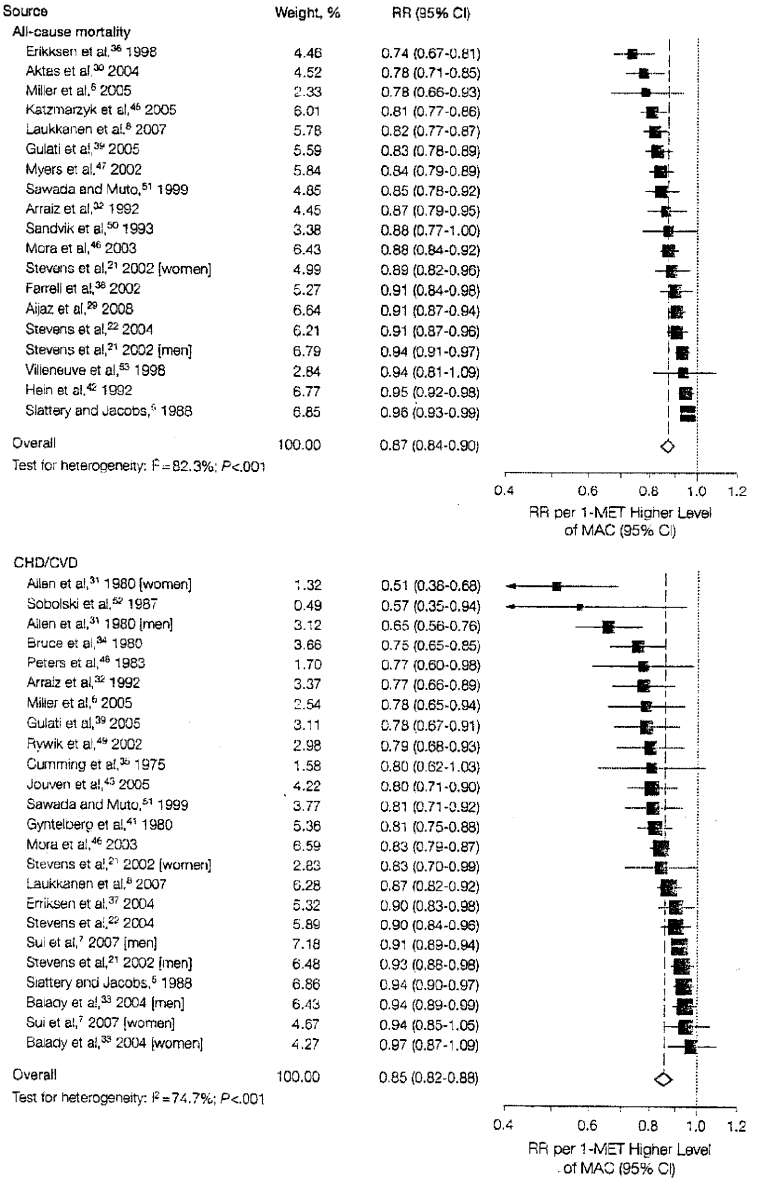
TABLE 2 shows the results of analyses investigating the associations of study characteristics on each outcome. The finding of risk reduction per higher MET for all-cause mortality and CHD/CVD was consistently significant in all of the stratified analyses. However, studies with a follow-up of at least 12 years had weaker associations with study outcomes compared with those that had follow-up of less than 12 years for all-cause mortality ($P=.08$) and CHD/CVD events ($P=.004$). The associations between CRF and risk of CHD/CVD events were stronger in studies that used an ergometer for assessing CRF ($P=.009$) or conducted maximal exercise testing ($P=.02$) and were weaker in studies that were adjusted for smoking ($P=.006$) or multiple metabolic factors ($P=.06$). However, these study characteristics did not influence the associations between MAC and risk of all-cause mortality.

Multiple regression analyses in which all the study characteristics listed in Table 2 were entered as independent variables indicated that study characteristics significantly explained the heterogeneity of the RRs per 1-MET higher level of MAC (all-cause mortality, 79% of total variance; $P=.01$; and CHD/CVD, 67% of total variance; $P=.01$). After adjustment for these study characteristics, there were neither significant differences in risk estimates of CHD/CVD between CHD and CVD (0.89; 95% CI, 0.86-0.92 and 0.89; 95% CI, 0.87-0.90, respectively; $P=.99$) nor between CHD or CVD death and CHD or CVD events (0.88; 95% CI, 0.86-0.90 and 0.90; 95% CI, 0.88-0.91, respectively; $P=.27$).

Categorical Analyses

We performed categorical analyses to summarize the risk of all-cause mortality and CHD/CVD for 3 subgroups (low vs high CRF [FIGURE 3], low vs inter-

Figure 2. Meta-analysis of All-Cause Mortality and CHD/CVD per 1-MET Higher Level of MAC



CHD indicates coronary heart disease; CI, confidence interval; CVD, cardiovascular disease; MAC, maximal aerobic capacity; MET, metabolic equivalent; RR, risk ratio. Area of each square is proportional to study weight.

mediate CRF [FIGURE 4], and intermediate vs high CRF [FIGURE 5]). After adjustment for heterogeneity of study characteristics and compared with high and intermediate CRF, respectively, the pooled RRs for the association of low CRF with all-cause mortality were 1.70 (95% CI, 1.51-1.92) and 1.56 (95% CI, 1.39-1.75), respectively. After adjustment for heterogeneity and compared with high and intermediate CRF, respectively, the pooled RRs for the association of low CRF with CHD/CVD events were 1.40 (95% CI, 1.32-1.48) and 1.47 (95% CI, 1.35-1.61), respectively. The pooled RRs for the associations of intermediate CRF with all-cause mortality and CHD/CVD events compared with high CRF were 1.13 (95% CI, 1.04-1.22) and 1.07 (95% CI, 1.01-1.13), respectively. However, tests of the interaction indicated that these estimates for comparisons between intermediate and high risk were significantly lower than for those between low vs high CRF and low vs intermediate CRF ($P<.001$ for any comparisons). Tests of interaction also indicated that there were no significant differences in risk estimates for low vs high CRF compared with low vs intermediate CRF (all-cause mortality, $P=.28$; CHD/CVD, $P=.33$).

Publication Bias

In risk estimates per 1-MET higher level of MAC, there was a statistically significant publication bias according to Egger test (all-cause mortality, $P=.002$; CHD/CVD, $P=.02$). However, adjustment for publication bias by the trim and fill procedure could not detect hypothetical negative unpublished studies that could influence the study. In some of the categorical analyses, statistically significant publication bias was also observed in risk estimates after adjustment for heterogeneity of study characteristics (pooled RR of all-cause mortality for low vs high CRF and low vs intermediate CRF, $P=.03$ by Egger test and $P=.03$ by Begg test, respectively; pooled RR of CHD/CVD for low vs intermediate CRF, $P<.001$ by Egger test). After incorporating the hypothetical studies using trim and fill methods, the risk estimates were attenuated in risk of all-cause mortality for low vs high CRF (RR, 1.48; 95% CI, 1.31-1.68) and low vs intermediate CRF (RR, 1.35; 95% CI, 1.18-1.54), and CHD/CVD for low vs high CRF (RR, 1.38; 95% CI, 1.30-1.45), which suggested the existence of potentially negative studies. Nevertheless, these biases did not change the general conclusions.

COMMENT

Our meta-analysis is the first to our knowledge to quantify CRF as measured by METs, which is a standard scale for expressing exercise workload, and its relationship to all-cause mortality and CHD or CVD events in healthy men and women. According to the dose-response analyses, a 1-MET higher level of MAC was as-

Table 2. Stratified Analyses of Pooled RR of All-Cause Mortality and CVD/CHD for Each MET Higher Level of Maximal Aerobic Capacity

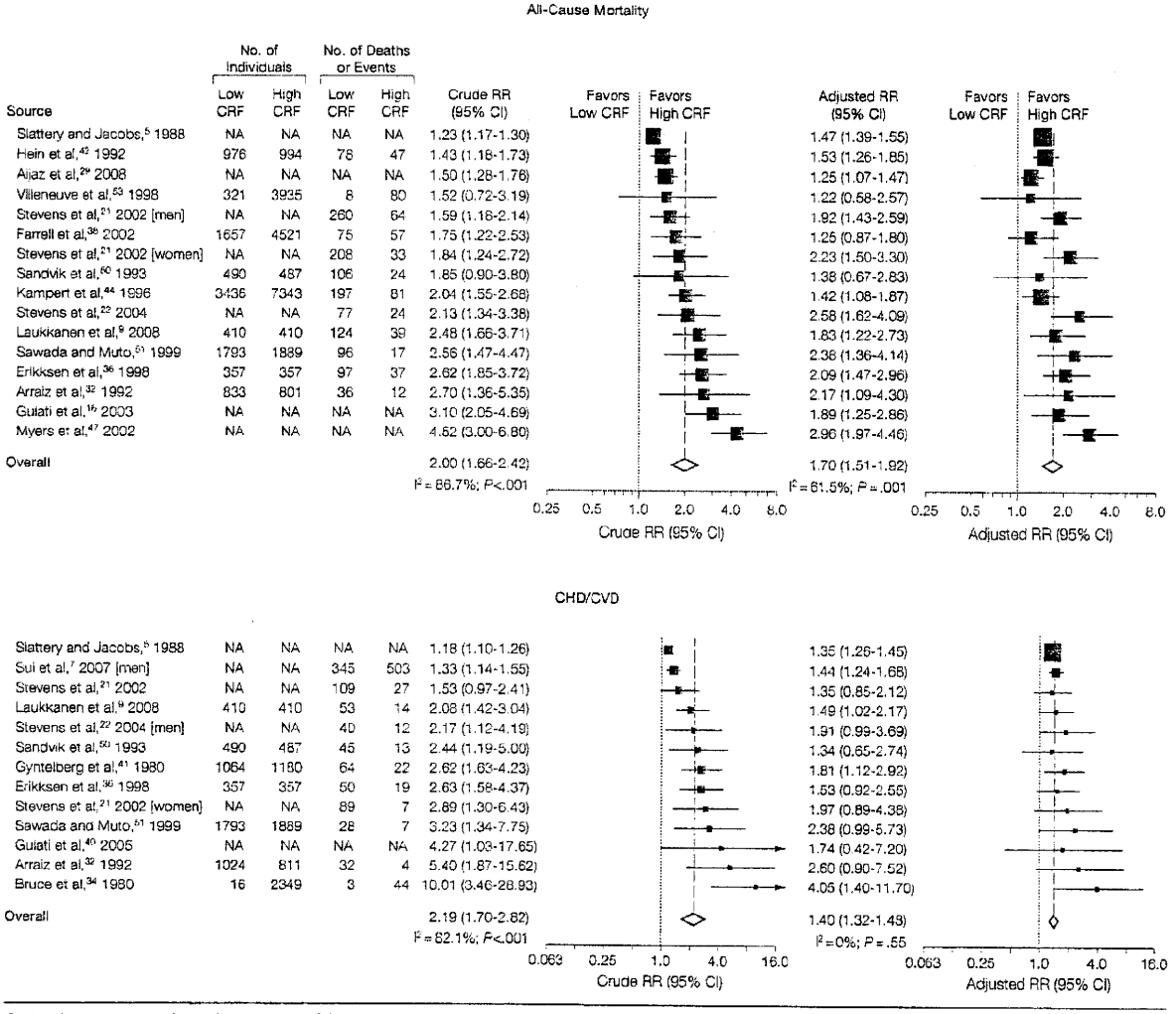
Characteristics	All-Cause Mortality			CHD/CVD		
	No. of Cohorts	RR (95% CI)	*P* Value[a]	No. of Cohorts	RR (95% CI)	*P* Value[a]
Mean age, ≥50 y						
No	10	0.90 (0.86-0.93)	.10	16	0.89 (0.88-0.91)	.80
Yes	9	0.84 (0.80-0.89)		8	0.84 (0.79-0.90)	
Only men						
No	8	0.87 (0.84-0.91)	.88	8	0.84 (0.81-0.87)	.60
Yes	11	0.87 (0.83-0.91)		16	0.86 (0.83-0.89)	
Adjustment for confounders, smoking						
No	7	0.87 (0.83-0.93)	.82	10	0.77 (0.70-0.85)	.006
Yes	12	0.87 (0.84-0.90)		14	0.89 (0.86-0.92)	
>3 Metabolic factors[b]						
No	14	0.86 (0.84-0.89)	.67	16	0.81 (0.77-0.86)	.06
Yes	5	0.86 (0.83-0.89)		8	0.89 (0.85-0.93)	
Patients with abnormal exercise electrocardiogram						
No	10	0.85 (0.81-0.90)	.20	16	0.83 (0.79-0.88)	.40
Yes	9	0.90 (0.86-0.93)		8	0.90 (0.88-0.92)	
Mean follow-up, 12 y						
No	8	0.84 (0.82-0.86)	.08	13	0.78 (0.72-0.84)	.004
Yes	11	0.91 (0.9-0.93)		11	0.89 (0.86-0.92)	
Ergometer used to assess CRF						
No	13	0.90 (0.89-0.92)	.82	13	0.89 (0.86-0.92)	.009
Yes	6	0.88 (0.84-0.91)		11	0.78 (0.73-0.84)	
Whether workload testing was continued until maximal workload						
No	15	0.88 (0.85-0.91)	.24	16	0.88 (0.85-0.91)	.02
Yes	4	0.84 (0.76-0.92)		8	0.77 (0.70-0.85)	

Abbreviations: CI, confidence interval; CHD, coronary heart disease; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; RR, risk ratio.
[a] Represents meta-regression for differences across strata.
[b] Means of adjustment for more than 3 coronary risk factors among obesity (or body mass index or waist-to-hip ratio), systolic blood pressure (or hypertension), total cholesterol (or low-density lipoprotein cholesterol or hyperlipidemia), high-density lipoprotein cholesterol, and diabetes.

sociated with 13% and 15% decrements in risk of all-cause mortality and CHD/CVD, respectively. From the clinical viewpoint, these values may be considerable. For example, based on risk estimates of the components of metabolic syndrome according to the National Cholesterol Education Program,[54] these findings suggest that a 1-MET higher level of MAC is comparable to a 7-cm, 5-mm Hg, 1-mmol/L, and 1-mmol/L decrement in waist circumference,[55] systolic blood pressure,[56] triglyceride level (in men),[57] and fasting plasma glucose,[58] respectively, and a 0.2-mmol/L increment in high-density lipoprotein cholesterol.[59] It is possible that prediction of CHD risk could be improved by including CRF with already established risk factors for CHD.

In categorical analyses, individuals with low CRF (<7.9 METs in MAC) had a substantially higher risk of all-cause mortality and CHD/CVD compared with those with intermediate and high CRF (7.9-10.8 and ≥10.9 METs in MAC, respectively). These risk estimates were higher than those for individuals with intermediate CRF compared with those with high CRF, according to the test of interaction. These analyses suggest that a minimal CRF of 7.9 METs may be important for significant prevention of all-cause mortality and CHD/CVD. A previous review suggested that physical activity yielding 1000 kcal energy expenditure per week is needed for signifi-

Figure 3. Meta-analysis of All-Cause Mortality and CHD/CVD for Individuals With Low vs High CRF



CHD indicates coronary heart disease; CI, confidence interval; CRF, cardiorespiratory fitness; CVD, cardiovascular disease; MET, metabolic equivalent; NA, not available; RR, risk ratio. Low and high CRF categories were defined as less than 7.9 METs and 10.9 METs or more of maximal aerobic capacity, respectively, under the assumption that all participants were 50-year-old men. Crude and adjusted RR indicate RRs before and after adjustment for study heterogeneity among the subgroups, respectively.