Importance of Achieving a “Fit” Cardiorespiratory Fitness Level for Several Years on the Incidence of Type 2 Diabetes Mellitus: A Japanese Cohort Study

Haruki Momma1,2, Susumu S. Sawada2, Robert A. Sloan3, Yuko Gando2, Ryoko Kawakami4, Shin Terada5, Motohiko Miyachi2, Chihiro Kinugawa6, Takashi Okamoto6, Koji Tsukamoto6, Cong Huang1, Ryoichi Nagatomi1, and Steven N. Blair7,8

1Division of Biomedical Engineering for Health and Welfare, Tohoku University Graduate School of Biomedical Engineering, Sendai, Japan
2Department of Health Promotion and Exercise, National Institutes of Biomedical Innovation, Health and Nutrition, Tokyo, Japan
3Department of Psychosomatic Internal Medicine, Graduate Medical and Dental School, Kagoshima University, Kagoshima, Japan
4Faculty of Sport Sciences, Waseda University, Tokorozawa, Japan
5Department of Life Sciences, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo, Japan
6Tokyo Gas Health Promotion Center, Tokyo, Japan
7Department of Epidemiology and Biostatistics, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA
8Department of Exercise Science, Arnold School of Public Health, University of South Carolina, Columbia, SC, USA

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ABSTRACT

Background: The “Physical Activity Reference for Health Promotion 2013” provides “fit” reference values for cardiorespiratory fitness (CRF) for good health. The importance of achieving a fit CRF level for several years on the subsequent prevention of type 2 diabetes mellitus (T2DM) remains to be clarified.

Methods: This cohort study was conducted in 2,235 nondiabetic males aged 21 to 59 years, enrolled in April 1986 through March 1987. We calculated the ratio of the area under the curve (AUCratio) for actual measured values and the AUC for the reference values of CRF in each individual during an 8-year measurement period before the baseline. According to whether they met a fit CRF level or not, participants were categorized into groups based on the AUCratio (FitAUC or UnfitAUC) and initial CRF (Fitinitial or Unfitinitial), respectively. T2DM was evaluated on health checkups until March 2010.

Results: During the follow-up period, 400 men developed T2DM. After adjustment for confounders, as compared with those in the FitAUC group, the hazard ratio (HR) for those in the UnfitAUC group was 1.33 (95% confidence interval [CI], 1.06–1.65). A combined analysis of the categories of initial value and AUCratio showed that, compared with the Fitinitial and FitAUC group, the HRs of Fitinitial and UnfitAUC, Unfitinitial and FitAUC, and Unfitinitial and UnfitAUC groups were 1.41 (95% CI, 0.99–2.00), 1.18 (95% CI, 0.81–1.70), and 1.40 (95% CI, 1.08–1.83), respectively.

Conclusion: Achievement of a fit CRF level established in the Japan physical activity guideline for several years was associated with lower subsequent risk of T2DM.

Key words: Physical Activity Reference for Health Promotion 2013; maximal oxygen uptake; area under the curve; metabolic memory

INTRODUCTION

According to the International Diabetes Federation, the number of patients with diabetes has reached 415 million worldwide.1 Strategies to prevent or delay diabetes are needed to mitigate the projected increase to 642 million before 2040. Physical inactivity is widely known as one of the modifiable risk factors for the development of type 2 diabetes mellitus (T2DM).2 Cardiorespiratory fitness (CRF) is considered an objective measurement of physical activity level,3,5 and CRF level predicts the development of T2DM.6–12 Therefore, maintaining higher CRF is an important strategy to prevent T2DM.

In Japan, the Ministry of Health, Labor and Welfare published the “Physical Activity Reference for Health Promotion 2013” in March 2013.13 It describes the reference values of physical activities and CRF to be achieved by individuals to reduce the risks of various diseases, including diabetes. The reference value for CRF is established according to sex and age. These reference values are based on the results of a systematic review and meta-analysis of findings obtained on the association between non-communicable diseases and CRF.13 However, few studies have verified their validity,14,15 and there has only been one validation study examining T2DM as an outcome.14 Previously, we examined the association between CRF level and development...
of T2DM among Japanese males.\textsuperscript{14} We reported that those with a “fit” CRF level (greater than or equal to the reference value) at baseline had a lower risk of T2DM compared to those with an “unfit” CRF level (less than the reference value) at baseline.\textsuperscript{14} Although this finding suggests that a fit CRF level has a beneficial influence on reducing the risk of T2DM, the importance of achieving a fit CRF level for a given time period on the subsequent development of T2DM remains to be clarified.

We hypothesized that those who did not achieve a fit CRF level for several years would have a higher subsequent risk of T2DM. We further hypothesized that, even if CRF level temporarily exceeds the fit reference value, the subsequent risk of T2DM may increase unless the level is maintained for several years. To verify our hypotheses, we examined the association between achievement of a fit CRF level for several years and subsequent risk of T2DM in a cohort study among Japanese males.

\section*{METHODS}

\textbf{Participants}

This cohort study investigated the relationship between CRF and health outcomes in Japanese men.\textsuperscript{16,17} Male employees of a company in the Tokyo area participated in this study. Annual medical examinations and exercise tests are conducted in the company to maintain the health of the employees in accordance with the Industrial Safety and Health Law and related laws in Japan.

A total of 9,221 men who underwent exercise tests and had annual medical examinations from April 1986 through March 1987 participated in this study. Of these, 2,488 males underwent a blood glucose test according to the Industrial Safety and Health Act and related laws in Japan. Moreover, to obtain the CRF levels during multiple measurement of CRF, men who performed four or more exercise tests during April 1979 through March 1987 were selected as follow-up participants. Men with fewer than four measurements were excluded ($n = 124$). Men whose accurate CRF values were impossible to estimate because of their inability to continue the exercise test for longer than 4 min due to abnormal electrocardiograms (ECGs) were also excluded ($n = 6$). Women were excluded from the study due to the small number of female employees in the company ($n = 25$). Men who had a cardiovascular disease ($n = 1$) or diabetes ($n = 35$) before 1986 were also excluded. All participants had no history of stroke before the baseline. Of the remaining 2,297 participants, 51 men who had left the company by the end of 1987, and 11 men with missing data from potential confounding factors were excluded. The final 2,235 men were selected as participants and were followed up until March 2010.

The ethics committee of the National Institutes of Biomedical Innovation, Health and Nutrition of Japan has approved this study.

\textbf{Medical examination}

The medical examination performed in 1986 measured the study participants’ height, weight, and resting blood pressure. We measured body weight using a weighing scale, which was calibrated and standardized as per the law, and the participants were dressed lightly without shoes. We calculated body mass index (BMI) using the height and weight measurements. An automated sphygmomanometer measured the resting blood pressure with the participant in a sitting position. Furthermore, we investigated the potential confounding factors related to CRF and T2DM development of the participants, including drinking habits, smoking habits, working conditions, and family history of diabetes, using a self-administered questionnaire.

\textbf{Cardiorespiratory fitness}

We used the estimated maximal oxygen uptake as an index of CRF. We measured the estimated maximal oxygen uptake with a submaximal exercise test using a bicycle ergometer. The exercise test was composed of a maximum of three stages, with each stage lasting 4 min, with the load gradually increasing with each stage. The load at the start of the test was 98 W, 86 W, 74 W, and 61 W for those 19 to 29, 30 to 39, 40 to 49, and 50 to 60 years old, respectively. We measured the heart rate from the R–R intervals on an ECG. We estimated the maximal heart rate based on the participant’s age and set the target heart rate at 85% of the maximal heart rate. The test increased the load by 37 W at each stage until the participant reached the target heart rate. If we detected an abnormal ECG, including an increased number of premature ventricular contractions during the exercise test, the test was terminated. We estimated the maximum oxygen uptake according to the Astrand and Ryhming nomogram\textsuperscript{18} and the Astrand age correction factors\textsuperscript{19} using the heart rate obtained in the final minute of the final stage for each participant. The method used to estimate the maximum oxygen uptake in this study was highly correlated with the direct method in a previous study ($r = 0.92$).\textsuperscript{20}

To determine integrated CRF level from April 1979 through March 1987, we calculated the area under the curve (AUC) in each individual during the 8-year measurement period (Figure\textsuperscript{1}).\textsuperscript{21} Next, we calculated the reference area (AUC\textsubscript{ref}) for each individual based on the guideline for fit reference values during the same period. The reference values were provided by the guideline as follows: 39 mL/kg/min for males aged 18 to 39 years, 35 mL/kg/min for males aged 40 to 59 years, and 32 mL/kg/min for males aged 60 to 69 years.\textsuperscript{13} After calculating the ratio of AUC to AUC\textsubscript{ref}, we multiplied the result by 100 (AUC\textsubscript{ratio}). We assumed that those with the AUC\textsubscript{ratio} above 100 achieved their CRF level above the reference values during the measurement period. If AUC\textsubscript{ratio} was less than 100, we assumed that the CRF level was below the reference values during the period. Participants with an AUC\textsubscript{ratio} of 100 or more were assigned to the FitAUC group, whereas participants with AUC\textsubscript{ratio} less than 100 were assigned to the UnfitAUC group. The number of CRF measurements during the 8-year measurement period differed for each participant; thus, we used the values of AUC and AUC\textsubscript{ref} divided by the measurement period to standardize the indices. In addition to the AUC\textsubscript{ratio} for CRF, the initial CRF measurement during the measurement period were also categorized into two groups: more than the reference values (Fit\textsubscript{initial}) or less than the reference values (Unfit\textsubscript{initial}) in accordance with the guideline.\textsuperscript{13}

\textbf{Type 2 diabetes mellitus}

During the medical examinations conducted from April 1986 through March 2010, we determined the year of T2DM development. From April 1986 and March 2010, participants with a fasting blood glucose level exceeding 7.0 mmol/L (126 mg/dL) were regarded as having diabetes. Furthermore, using a questionnaire administered during health checkups, we
asked whether a diagnosis of T2DM was made by a physician. If more than one of the above criteria was met, the year of the earliest episode was regarded as the year of T2DM development.

Statistical analysis
We compared baseline characteristics of participants based on CRF categories (FitAUC and UnfitAUC groups). We showed the median value (interquartile range) for continuous variables and the percentage for category variables.

We set T2DM and the CRF category (FitAUC and UnfitAUC groups) as the outcome and exposure variables, respectively. Cox proportional hazards regression analysis was used to compare the differences between T2DM incidence rates based on CRF categories, and calculated the age-adjusted hazard ratios (HRs) and 95% confidence intervals (CIs). Furthermore, we adjusted the following as potential confounding factors at baseline (1986): age (continuous variable), BMI (continuous variable), systolic blood pressure (continuous variable), smoking (never-smokers, past smokers, current smokers of 1–20 cigarettes/day, and ≥21 cigarettes/day), drinking habit (none, 1–40 g/day, and ≥41 g/day), desk work (yes or no), family history of diabetes (yes or no), fasting blood glucose (continuous variable), and frequency of measurements during the 8-year CRF measurement period (continuous variables). We also drew the adjusted cumulative incidence curve according to the category of the AUCratio for CRF using the above-mentioned multivariate model. We obtained the plot of one-minus-survival function by using the mean of each covariate as a constant value.

In addition, as the guideline described the CRF reference values according to age, we performed a stratified analysis, based on younger than 40 years and older than 40 years, by using the same model as the aforementioned covariates.

We also performed a sensitivity analysis, which excluded participants who had developed diabetes within 3 years of starting follow up, to investigate the effect of reverse causality.

Finally, a combination analysis was conducted using the categories based on the initial CRF measurement value (Fitinitial and Unfitinitial) and the categories based on AUC for CRF (FitAUC and UnfitAUC groups) during the measurement period. The Fitinitial group × FitAUC group was used as the reference group, and we calculated HRs and 95% CIs using the same covariates mentioned previously.

The proportionality assumption of the models was tested using a log-minus-log plot; no evidence of violation was found. All analyses were done using SPSS version 22 (IBM Japan, Tokyo, Japan). A two-tailed P-value less than 0.05 was considered statistically significant.

RESULTS
Study participants
The median age of the participants at the start of follow-up was 43 years. The median and maximum follow-up periods (April 1987 through March 2010) were 15 and 23 years, respectively. During total follow-up of 31,408 person-years, 400 participants developed T2DM. During follow-up before March 2010, a total of 1,636 participants dropped out. Of these, 46 dropped out at 49 years of age or younger for reasons other than mandatory retirement. The other 1,590 participants dropped out at 50 years of age or older possibly due to mandatory retirement.

Table I shows the characteristics of the study participants at baseline according to the category of the AUCratio for CRF. The BMI, systolic blood pressure, diastolic blood pressure, and fasting blood glucose at baseline (1986) in the UnfitAUC group were higher than in the FitAUC group. In the UnfitAUC group, the proportions of smokers, alcohol drinkers, those with a family history of diabetes, and those doing desk work were also higher than in the FitAUC group. On the other hand, the value of AUCratio, initial CRF value between 1979 and 1986, and CRF at baseline (1986) in the UnfitAUC group were lower than in the FitAUC group.
in the UnfitAUC group to that in the FitAUC group was 1.72 (95% CI, 1.05–2.07). Figure 2 shows the cumulative incidence curve after adjustment with confounding factors for T2DM according to the category of the AUCratio for CRF. In the UnfitAUC group, the cumulative incidence of T2DM during the follow-up period was lower than in the FitAUC group. In addition, a sensitivity analysis excluding participants who developed diabetes within 3 years after the start of follow-up showed that the multivariable-adjusted HR of T2DM in the UnfitAUC group to that in the FitAUC group was higher (HR 1.28; 95% CI, 1.01–1.63).

As the reference values of CRF are established according to age, measurements during 1979–1986; CI, confidence interval; HR, hazard ratio. *Adjusted for age (continuous variable), body mass index (continuous variable), systolic blood pressure (continuous variable), smoking status (never-smokers, former smoker, 1–20 cigarettes/day, and ≥21 cigarettes/day), drinking status (none, 1–40 g/day, and ≥41 g/day), a family history of diabetes (yes or no), desk work (yes or no), fasting glucose (continuous variable) at baseline, and measurement times of cardiorespiratory fitness (continuous variable).

AUC, area under the curve for cardiorespiratory fitness based on individuals’ measurements during 1979–1986; AUCadj, area under the curve based on the reference value of cardiorespiratory fitness in the text; BMI, body mass index; CRF, cardiorespiratory fitness; DBP, diastolic blood pressure; SBP, systolic blood pressure.

Data are presented as median (interquartile range) or N (%).

The median frequency of CRF measurement between 1979 and 1986 was 7 times in the two groups.

Table 2 shows the hazard ratio of T2DM according to the category of the AUCratio for CRF. The age-adjusted HR of T2DM in the UnfitAUC group to that in the FitAUC group was 1.72 (95% CI, 1.39–2.12) (P < 0.001). Considering potential confounding factors, there was also a significant difference (HR 1.33; 95% CI, 1.06–1.65). Figure 2 shows the cumulative incidence curve after adjustment with confounding factors for T2DM according to the category of the AUCratio for CRF. In the UnfitAUC group, the cumulative incidence of T2DM during the follow-up period was always lower than in the FitAUC group. In addition, a sensitivity analysis excluding participants who developed diabetes within 3 years after the start of follow-up showed that the multivariable-adjusted HR of T2DM in the UnfitAUC group to that in the FitAUC group was higher (HR 1.28; 95% CI, 1.01–1.63).

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Data are presented as median (interquartile range) or N (%).
Influence of Achieving Fit CRF Level on Diabetes

Table 3. Hazard ratios for the incidence of type 2 diabetes according to the category of cardiorespiratory fitness stratified by age

| Category | Age range | Person-years of follow-up | Number of cases | Multivariate Adjusted HR (95% CI) | P value |
|----------|-----------|---------------------------|-----------------|----------------------------------|---------|
| FitAUC   | 21–39 years | 318                        | 6,059           | 53                               | 1.00 (Reference) | 0.00 (Reference) |
| FitAUC   | 21–39 years | 433                        | 7,902           | 112                             | 1.28 (0.90–1.83) | 0.18  |
| FitAUC   | 40–59 years | 653                        | 7,921           | 75                              | 1.00 (Reference) | 0.00 (Reference) |
| UnfitAUC | 40–59 years | 831                        | 9,526           | 160                             | 1.40 (1.05–1.86) | 0.023 |

In this study, we examined the influence of achieving a fit CRF level (according to the Ministry of Health, Labour and Welfare guideline) for several years on the subsequent risk of T2DM among Japanese men. The results showed that those with an unfit CRF level during the measurement period (UnfitCRF) had a higher risk of T2DM compared with those with a fit CRF level during the period (FitAUC). We also performed the combined analysis using the categories of initial CRF value and AUC ratio for CRF during the period based on the reference values. As a result, even when the initial CRF level exceeded the fit reference value, the risk of T2DM in those who did not maintain a fit CRF level during the period was higher, but not significantly (P = 0.057), than in those who maintained their CRF level over the fit reference values during the period. These results suggest that the achievement of a fit CRF level recommended in the “Physical Activity Reference for Health Promotion 2013” in Japan for several years contributes to a lower subsequent risk of T2DM.

Many previous studies have reported that a lower CRF level was associated with higher incidence of T2DM. This association has also been confirmed among Japanese males. For example, among Japanese males aged 20 to 40 years, the CRF level was negatively associated with the development of T2DM after a follow-up period of 14 years. Another study reported a negative association between physical performance in college and subsequent risk of T2DM. In line with these findings, we showed that, compared with those with an unfit CRF level according to the “Physical Activity Reference for Health Promotion 2013” in Japan at baseline, Japanese males with a fit CRF level had a lower risk of T2DM. This finding leads to the hypothesis that the risk of T2DM is reduced by achieving a fit CRF level recommended in the Japanese guideline over time. Based on this assumption, we examined the influence of achieving a fit CRF level for several years on the development of T2DM by calculating the ratio of the AUC for CRF to the reference area based on the reference values of the guideline during the 8-year measurement period for CRF. As a result, those with an unfit CRF level during the measurement period (FitAUC) had a higher risk of T2DM compared to those with a fit CRF level (UnfitAUC). The risk of incidence for T2DM in the UnfitAUC category was 33% higher than that in the FitAUC category. This association was observed particular in participants aged more than 40 years. Therefore, these results suggested that the risk of T2DM could be reduced by achieving a fit CRF level recommended in the Japanese guideline for several years. In addition, even when the CRF level at baseline was above the fit reference values, the risk of T2DM was higher in participants with an unfit CRF level during the measurement period.

As a mechanism underling the association between achievement of a fit CRF level for several years and lower incidence of T2DM, the cumulative effects of regular physical activities or exercise on glucose metabolism may be plausible. For example, animal studies showed that exercise during pregnancy in females enhanced insulin sensitivity and improved glucose homeostasis in mature offsprings. In addition, Shindo et al reported that rats that engaged in exercise during childhood had lower levels of blood glucose, insulin, and HOMA-IR at middle age after cessation of the exercise interventions compared with sedentary control rats. These findings may reflect the concept of “metabolic memory” or “cellular memory”, and changes in epigenetic modifications, including DNA demethylation, may be involved in its mechanism. In fact, previous human studies reported that acute exercise or exercise training for 6 months affected the methylation pattern of DNA or its promoter involved in glucose metabolism.

DISCUSSION

In this study, we examined the influence of achieving a fit CRF level (according to the Ministry of Health, Labour and Welfare guideline) for several years on the subsequent risk of T2DM among Japanese men. The results showed that those with an unfit CRF level during the measurement period (UnfitAUC) had a higher risk of T2DM compared with those with a fit CRF level during the period (FitAUC). We also performed the combined analysis using the categories of initial CRF value and AUC ratio for CRF during the period based on the reference values. As a result, even when the initial CRF level exceeded the fit reference value, the risk of T2DM in those who did not maintain a fit CRF level during the period was higher, but not significantly (P = 0.057), than in those who maintained their CRF level over the fit reference values during the period. These results suggest that the achievement of a fit CRF level recommended in the “Physical Activity Reference for Health Promotion 2013” in Japan for several years contributes to a lower subsequent risk of T2DM.

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Interestingly, compared with the $\text{Fit}_{\text{initial}} \times \text{Fit}_{AUC}$ group, the risk of T2DM in the $\text{Fit}_{\text{initial}} \times \text{Fit}_{AUC}$ group was higher, but not significantly, while the hazard ratio of the $\text{Fit}_{\text{initial}} \times \text{Fit}_{AUC}$ group was lower than that of the $\text{Fit}_{\text{initial}} \times \text{Fit}_{AUC}$ group. This finding suggests the possibility that disadvantage of initial unfit CRF level that led to the development of T2DM was attenuated via the improvement of a fit CRF level recommended by the Japanese guideline after the initial CRF measurement. Therefore, health care professionals may thereby encourage those with an unfit CRF level to engage in physical activity for the prevention of T2DM. Further studies are necessary to examine the influence of duration or timing of meeting the reference values in CRF on T2DM.

This study has several limitations. First, the participants of this study were limited to males aged 21 to 59 years. Because previous studies reported that there was a negative association between CRF and T2DM even in a population including females and 79-year-old or younger males, whether or not the results of this study may be applied to females or elderly persons needs to be further examined. Second, we had small number of obese participants in the study population; those with BMI of $\geq 30$ at baseline accounted only for 1% of the population. Therefore, the results of this study may be limited to a population with a relatively normal weight. Third, in this study, some potential confounding factors were considered, but the influence of unmeasured confounding factors, such as diet, was not ruled out. For example, it is possible that highly fit persons may have had more healthy dietary habits than low-fit persons. Therefore, the relationship between CRF level and the incidence of T2DM may be attenuated by considering the influence of diet. Fourth, we could not precisely explain the reasons why individuals who did not achieve the fit CRF level at the initial measurement subsequently achieved the fit level, because we did not have any data of medical checkups and lifestyles at the initial measurement of CRF. Generally, it is well-known that moderate-vigorous physical activity or relatively high intensity exercise, such as endurance training, can improve the level of CRF. Lastly, whether individuals met a fit CRF level or not was considered only from April 1979 through March 1987, but not during the entire follow-up period from April 1987 through March 2010. Therefore, the influence of changes of CRF during the follow-up periods on our findings was not considered. The favorable influence of meeting a fit CRF level on the incidence of T2DM would be attenuated when secular change of CRF was considered.

In conclusion, our findings showed that the achievement of a fit CRF level recommended in the “Physical Activity Reference for Health Promotion 2013” in Japan was associated with lower subsequent risk of T2DM. These results suggested that the reference CRF values established in the Japanese guideline appear to be reasonably valid for prevention of T2DM among middle-aged Japanese males.

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