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Cardiorespiratory fitness, genetic susceptibility, inflammation and risk of incident type 2 diabetes: A population-based longitudinal study

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ABSTRACT

Objective: To examine whether the association between cardiorespiratory fitness (CRF) and type 2 diabetes (T2D) is modified by genetic susceptibility and inflammation.
Participants: The prospective study included 57,185 participants (40–70 years) who were free from T2D and received the CRF assessment at enrollment (2006–2010) in the UK biobank. CRF was examined through a submaximal cycle ergometer test and expressed in metabolic equivalent of tasks (METs), genetic susceptibility was quantified using a genetic risk score, and inflammation was assessed according to the concentration of C-reactive protein. All these three factors were categorized into tertiles.
Results: During a median follow-up of 10.4 years, 5477 (7.0%) cases of T2D were ascertained. CRF was inversely associated with the risk of T2D in a dose-response manner. The hazard ratio (HR) was 0.85 (95% confidence interval [CI]: 0.79–0.92) per 1 MET increment of CRF. There was a significant interaction between CRF and genetic susceptibility to T2D in relation to the risk of T2D (P for interaction = 0.03). Compared with participants with high CRF and low genetic susceptibility, the HR was 4.98 (95% CI: 3.17–7.82) for those with low CRF and high genetic susceptibility. A similar pattern was observed in participants with low CRF and high inflammation compared with those who had high CRF and low inflammation (HR = 2.53; 95% CI: 1.83–3.48), though the interaction between CRF and inflammation did not reach statistical significance. T2D risk declined progressively with increased CRF among different inflammation categories.
Conclusion: Our study reveals that genetic susceptibility may modify the association between CRF and T2D, highlighting that risk of T2D associated with genetics could benefit most from interventions on improving CRF.

1. Introduction

Diabetes remains a critical public health concern globally. The prevalence of diabetes was estimated to be 10.5% in adults in 2021, affecting approximately 536.6 million people worldwide, and the prevalence is predicted to increase to 12.2% (783.2 million) by 2045 [1]. Accumulating evidence indicates that physical activity can act as a modifiable protective factor against the development of type 2 diabetes (T2D) [2]. However, the self-reported physical activity level in many related epidemiological studies might be inaccurate due to recall bias and can only provide a snapshot of behavior [3,4]. As an alternative, cardiorespiratory fitness (CRF), the ability of the circulatory and respiratory systems to supply oxygen during sustained physical activity, has been considered as an objective indicator of physical activity that can be estimated with standardized exercise tests [4,5]. CRF is an integrated reflection of the functional consequences of recent physical activity habits, diseases, and inherited factors [6,7]. Previous studies have reported an inverse association between CRF and incident T2D [8–11]. However, some studies failed to account for the effect of reverse causation and some potential confounders, such as diet habit and blood lipid. These limitations could have biased the true association between CRF and T2D. Moreover, very few prospective studies examined the association of CRF measured by submaximal exercise tests with risk of T2D in a large-scale population.

Genetic susceptibility plays an essential role in the risk of incident T2D. Since 2007, genome-wide association studies (GWASs) have identified more than 100 T2D-related loci [12–14]. Since 2007, GWASs

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Abbreviations

BMI	body mass index
CI	confidence interval
CRF	cardiorespiratory fitness
CRP	C-reactive protein
GWAS	genome-wide association studies
HR	hazard ratio
MET	metabolic equivalent of tasks
GRS	genetic risk score
SD	standard deviation
SNP	single nucleotide polymorphisms
T2D	type 2 diabetes

have identified more than 100 T2D-related loci [15], which can quantitatively evaluate the genetic predisposition and serve as potential predictors for T2D [16]. Likewise, one of the factors plausibly implicated in the association between CRF and T2D is inflammation. Systemic inflammation may induce insulin resistance and endothelial dysfunction [17], and is a critical risk factor for the development of T2D [18,19]. However, it is unclear whether genetic susceptibility and inflammation modify the association between CRF and risk of T2D.

Therefore, the purposes of this study were 1) to prospectively investigate the association between CRF, assessed objectively by using the submaximal bicycle ergometer test, and the risk of incident T2D using data from the UK Biobank, a very large population-based cohort study; 2) to determine whether the association of CRF with T2D was modulated by genetic susceptibility and inflammation.

2. Material and methods

2.1. Study design and population

This was a prospective, population-based cohort study derived from the UK Biobank cohort, where 502,528 adults (37–73 years old) were recruited from the general population between April 2006 and December 2010 and followed up to September 2020. Participants attended one of 22 assessment centers across England, Scotland, and Wales, where they completed nurse-led electronic questionnaires, physical examination, and biological sample collections [20]. In this study, incidence of T2D was the outcome; CRF, genetic risk and inflammation were the exposure variables.

We utilized a subsample of 77,953 individuals who underwent a submaximal cycle ergometer test at baseline. Of these, 67,273 participants generated usable measurements. Participants were further excluded if they have abnormal maximum heart rate (<40 or >220 bpm) (n = 48), prevalent T2D at baseline (n = 3401), missing information on genetic variants and inflammation (n = 6639), leaving 57,185 participants included in final study.

2.2. Exposures

In this study, the exposures of interest were CRF, genetic susceptibility and inflammation. CRF was measured using a previously validated 6 min incremental ramp cycle ergometer test, which has been applied in previous studies [21–23]. Maximum oxygen consumption (VO_{2max}, in mL kg^{-1} min^{-1}), an indicator of CRF, was estimated from the following regression equation: 7 + (10.8 × maximal work rate (in watts)/body weight (in kg)) first and then transformed into maximal metabolic equivalent of tasks (METs, 1 MET = 3.5 mL kg^{-1} min^{-1}) [24]. The maximal work rate (in watts) was predicted from the participant's age-predicted maximum heart rate (208–7 × age) based on a linear regression model that was previously fitted using paired data of heart rate and workload monitored during the test [25]. CRF was categorized as low (first tertiles), moderate (middle tertiles) and high (high tertiles) group within sex and ten-year age groups (e.g. 50–60 years).

A total of 488,377 (97.2%) participants in the UK Biobank were genotyped using two arrays, UK BiLEVE and UK Biobank Axiom. Detail genotyping, quality control, and imputation procedures have been previously described elsewhere [26]. A weighted genetic risk score (GRS) for T2D was created based on 139 common single nucleotide polymorphisms (SNPs) selected from a GWAS meta-analysis, which has the largest sample size of individuals with European ancestry to date (62,892 T2D cases and 596,424 controls) (Supplemental Table S1) [27]. The included SNPs were demonstrated to be nearly independent by linkage disequilibrium. Each SNP was recoded as 0, 1, or 2 according to the number of risk alleles. The weighted GRS was calculated using the following equation: GRS = (β1 × SNP1 + β2 × SNP2 + … + β139 × SNP139) × (139/sum of the β coefficients), where β is the weighted risk estimate for T2D. Participants were then categorized into 3 groups with low, moderate, or high genetic susceptibility to T2D according to the GRS tertiles.

Blood samples were collected at baseline (2006–2010). C-reactive protein (CRP) (mg/L) was measured by immunoturbidimetric assay on a Beckman Coulter AU5800, and inflammation was categorized as low, moderate or high group by CRP tertiles. Further details of these measurements can be found at the UK Biobank website (https://biobank.ctsu.ox.ac.uk/showcase).

2.3. Outcome ascertainment

The primary outcome for this study was the incidence of T2D. All residents in England, Scotland, and Wales have a unique National Health Service identification number, which was used for linking all participants to electronic health records, where T2D cases were identified and the date of T2D diagnosis was extracted. T2D was defined by the International Classification of Diseases Tenth Revision (ICD-10) code E11. Information on timing of incident T2D was collected through cumulative medical records of hospital diagnoses (until 30 September 2020).

2.4. Covariates

Information of potential confounders were extracted from baseline questionnaires, including age, sex, ethnicity, employment status, educational attainment, Townsend deprivation index reflecting socioeconomic status, smoking status, alcohol intake, diet pattern, physical activity, body mass index (BMI), triglyceride, fasting glucose, hypertension, and family history of diabetes. Hypertension was defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mm Hg or use of antihypertensive medication. BMI was calculated as weight (in kilograms) divided by height (in meters) squared. Healthy dietary patterns were adapted from the American Heart Association Guidelines and defined as follows [28,29]: ≥4.5 servings total fruit and vegetable intake consumption per week, ≥2 fish intake per week, ≤2 times intake of processed meat per week and ≤5 times red meat intake per week. We defined a healthy diet as adherence to at least two of the healthy food items. The details of these measurements can be found in the study protocol (https://www.ukbiobank.ac.uk/).

2.5. Statistical analysis

We summarized baseline characteristics by tertiles of CRF using descriptive statistics, reporting the mean and standard deviation (SD) of continuous variables, and proportions for categorical variables. We compared the baseline characteristics by CRF tertiles using Chi-square test for categorical or One-Way ANOVA for continuous variables.

Missing information on covariates were coded as a missing indicator category for categorical variables or imputed with mean values for continuous variables. Cox proportional hazard regression models with

age as the time scale were utilized to estimate the hazard ratios (HRs) and confidence intervals (CIs) of T2D. The proportional hazards assumption was tested using Schoenfeld residuals. We conducted several main analyses. Firstly, separate associations of CRF, genetic susceptibility and inflammation with T2D were calculated. We ran 4 sets of multivariable-adjusted models: (1) adjusted for age, sex; (2) additionally adjusted for ethnicity, educational attainment, employment status, socioeconomic status, smoking status, alcohol intake, BMI, hypertension, physical activity, triglyceride, serum fasting glucose at baseline and family history of diabetes; (3) additionally adjusted for dietary pattern, and (4) additionally adjusted for CRF, genetic susceptibility and inflammation mutually.

Secondly, restricted cubic splines models with five knots were used to investigate the dose-response associations between CRF as a continuous variable and T2D, and HR per 1-MET increment of CRF was estimated. Thirdly, joint associations between tertiles of CRF and genetic susceptibility and between tertiles of CRF and inflammation in relation to the risk of T2D were calculated, with the referent category comprising individuals who were in both the highest tertile of CRF and the lowest tertile of genetic susceptibility/inflammation. To investigate whether genetic susceptibility and inflammation modified the association of CRF with T2D, we also tested the statistical significance of interaction terms by likelihood ratio test comparing models with and without cross-product interaction terms and conducted sub-group analyses where appropriate.

A post hoc exploratory subgroup analysis was also conducted by sex, age at recruitment (<60 years, ≥60 years), socioeconomic status (low, medium and high), BMI (<30 kg/m^2, ≥30 kg/m^2), smoking status (never, past, current), moderate alcohol intake (yes, no), physical activity (<7.5 MET/h/w, ≥7.5 MET/h/w), healthy diet (yes, no), triglyceride (<1.7 mmol/L, ≥1.7 mmol/L), prevalent hypertension (yes, no) and family history of diabetes (yes, no). To evaluate interactions between CRF and potential T2D risk factors, multiplicative interaction was assessed by adding interaction terms to the Cox models.

We undertook a series of sensitivity analyses to evaluate the robustness of our findings. First, we further adjusted for antihypertensive medication, lipid-lowering medication vitamin and mineral supplement use in the multivariable model. Second, we addressed the issues of potential undiagnosed diabetes at baseline by removing participants with fasting glucose levels ≥ 7.0 mmol/L and repeated the main analyses. Third, the possible influence of worse health condition on CRF-T2D associations was evaluated by excluding the participants with cancer or cardiovascular disease (CVD). Fourth, we repeated all analyses after excluding all participants who developed T2D during the first two years of follow up to reduce the possibility of spurious associations due to reverse causation. Finally, we used a multiple imputation approach to impute the missing values for the non-systematically missing covariables. Five imputed datasets were generated and estimates were combined using Rubin's rules. All analyses were performed using STATA 15 statistical software (StataCorp). All P values were two-sided, and $P <$ 0.05 was considered statistically significant.

3. Results

Of the 57,185 participants included in this study, the mean (SD) age was 56.4 (8.2) years and the proportion of women was 54.4%. Table 1 shows the participants' characteristics by tertiles of CRF. During a median follow-up of 10.4 years, a total of 5477 (7.0%) cases of T2D were identified.

Table 2 shows the separate associations of CRF, genetic susceptibility and inflammation with the risk of T2D. The incidence rates per 1000 person-year were 4.43 (95% CI, 4.14–4.73) in the low CRF group, 0.78 (95% CI, 0.66–0.91) in the high CRF group. Higher CRF was strongly associated with lower risk of T2D, and the association was attenuated after adjustment for potential confounders. The HR of the high CRF group compared with the low CRF group was 0.65 (95% CI: 0.53–0.79)

Table 1
Baseline characteristics of participants by cardiorespiratory fitness.

Characteristics	Total	CRF (MET)			*P* value
		Low	Moderate	High	
Total	57,185	19,087 (33.4)	19,083 (33.3)	19,015 (33.3)	
Sex					0.990
Male	26,030 (45.5)	8682 (45.5)	8685 (45.5)	8663 (45.6)	
Female	31,155 (54.5)	10,405 (54.5)	10,398 (54.5)	10,352 (54.4)	
Age (mean, SD)	56.4 (8.2)	56.9 (8.2)	56.6 (8.2)	55.6 (8.0)	<0.001
Townsend deprivation index	−1.20 (2.90)	−1.01 (3.0)	−1.30 (2.8)	−1.30 (2.8)	<0.001
Ethnicity					<0.001
White	52,346 (91.5)	17,148 (89.8)	17,572 (92.1)	17,626 (92.7)	
Black	1490 (2.6)	820 (4.3)	419 (2.2)	251 (1.3)	
South Asian	1718 (3.0)	537 (2.8)	547 (2.9)	634 (3.3)	
Mixed background	1273 (2.2)	447 (2.3)	415 (2.2)	411 (2.2)	
Missing	358 (0.6)	135 (0.7)	130 (0.7)	93 (0.5)	
Employment status					<0.001
Worked	33,900 (59.3)	10,653 (55.8)	11,304 (59.2)	11,943 (62.8)	
Retired	18,379 (32.1)	6563 (34.4)	6338 (33.2)	5478 (28.8)	
Unemployed	3733 (6.5)	1455 (7.6)	1072 (5.6)	1206 (6.3)	
Others	1173 (2.1)	416 (2.2)	369 (1.9)	388 (2.0)	
Education level					<0.001
College or university degree	20,930 (36.6)	5761 (20.2)	6841 (35.8)	8328 (43.8)	
Professional qualifications	28,458 (49.8)	10,037 (52.6)	9580 (50.2)	8841 (46.5)	
Others	7171 (12.5)	3021 (15.8)	2452 (12.8)	1698 (8.9)	
Missing	626 (1.1)	268 (1.4)	210 (1.1)	148 (0.8)	
Smoking status					<0.001
Never	32,151 (56.2)	10,571 (55.4)	10,365 (54.3)	11,215 (59.0)	
Former	19,624 (34.3)	6741 (35.3)	6901 (36.2)	5982 (31.5)	
Current	5110 (8.9)	1640 (8.6)	1726 (9.0)	1744 (9.2)	
Missing	300 (0.5)	135 (0.7)	91 (0.5)	74 (0.4)	
Alcohol intake (g/day; mean, SD)	14.4 (17.1)	14.8 (18.9)	14.7 (16.9)	13.8 (15.3)	<0.001
Healthy dietary pattern	24,461 (42.8)	7623 (39.9)	8005 (41.9)	8833 (46.4)	<0.001
Physical activity (MET/h/w)	45.8 (40.8)	43.0 (39.3)	45.2 (40.3)	49.3 (42.7)	<0.001
Hypertension	37,944 (66.4)	15,622 (81.8)	12,450 (65.2)	9872 (51.9)	<0.001
SBP (mm Hg; mean, SD)	136 (17)	145 (19)	134 (14)	130 (15)	<0.001
DBP (mm Hg; mean, SD)	82 (10)	87 (10)	81 (8)	78 (8)	<0.001
BMI (kg/m^2; mean, SD)	26.9 (4.4)	30.2 (4.8)	27.2 (2.5)	23.4 (2.3)	<0.001
Triglyceride (mmol/L)	1.65 (0.94)	1.87 (1.04)	1.72 (0.96)	1.36 (0.73)	<0.001
Fasting glucose (mmol/L)	5.05 (0.64)	5.13 (0.75)	5.03 (0.59)	4.98 (0.56)	<0.001
Family history of diabetes	12,548 (21.9)	4725 (24.8)	4272 (22.4)	3551 (18.8)	<0.001
Antihypertensive medication use	9426 (16.5)	4854 (25.4)	2951 (15.5)	1621 (8.5)	<0.001
Lipid-lowering medication use	7869 (13.8)	3465 (18.2)	2741 (14.4)	1663 (8.7)	<0.001

(*continued on next page*)

Table 1 (*continued*)

Characteristics	Total	CRF (MET)			*P* value
		Low	Moderate	High	
Vitamin supplements use	18,767 (32.8)	5958 (31.2)	6234 (32.7)	6575 (34.6)	<0.001
Mineral supplements use	21,032 (36.8)	6675 (35.0)	7026 (36.8)	7331 (38.6)	<0.001
Inflammation level					
Low	19,257 (33.7)	3595 (18.8)	5790 (30.3)	9872 (51.9)	
Moderate	18,872 (33.0)	6075 (31.8)	7058 (37.0)	5739 (30.2)	
High	19,056 (33.3)	9417 (49.3)	6235 (32.7)	3404 (17.9)	
Genetic susceptibility					0.012
Low	19,063 (33.4)	6214 (32.6)	6386 (33.5)	6463 (34.0)	
Moderate	19,061 (33.3)	6406 (33.6)	6290 (33,0)	6365 (33.5)	
High	19,061 (33.3)	6467 (33.9)	6407 (33.6)	6187 (32.5)	

Data are n (%), unless otherwise specified. BMI, body mass index; SD, standard deviation; SBP, systolic blood pressure; DBP, diastolic blood pressure; MET, metabolic equivalent task.

in model 4. Additional adjustment for antihypertensive medication and lipid-lowering medication slightly attenuated the estimates (Supplemental Table S2). However, the main results were not notably changed after additional adjustment for vitamin supplements use and mineral supplements use (Supplemental Table S3). Moreover, restricted cubic spline regression indicated that CRF was associated with T2D in a dose-response manner, the HR was 0.85 (95% CI: 0.79–0.92) per 1-MET increment of CRF (Fig. 1).

Similar findings were observed in associations with genetic susceptibility and inflammation. Compared with participants who had low genetic susceptibility and inflammation, high genetic susceptibility and inflammation was separately associated with 120% and 42% higher risk of T2D (Table 2). The results were not appreciably altered if we further excluded participants with glucose level ≥ 7.0 mmol/L at baseline (Supplemental Table S4), or participants with prevalent CVD and cancer history (Supplemental Table S5), or participants diagnosed with T2D in the first 2 years of follow-up (Supplemental Table S6) or participants with imputed missing covariates (Supplemental Table S7).

To evaluate whether genetic susceptibility and inflammation modify the associations of CRF with the risk of T2D, we created two joint variables with 9 categories. The risk of T2D increased progressively with decreased CRF within each genetic susceptibility category. Compared with participants with high CRF and low genetic susceptibility, the adjusted HRs of T2D were 4.98 (95% CI: 3.17–7.82) for those with low CRF and high genetic susceptibility and 3.91 (95% CI: 2.43–6.29) for those with high CRF and high genetic susceptibility, suggesting there were moderately significant reduction of HR in the high genetic susceptibility group if they have high CRF compared with low CRF. Moreover, the interaction between CRF and genetic susceptibility in relation to T2D was statistically significant (*P* for interaction = 0.03) (Fig. 2a, Supplemental Table S8), indicating that the risk of T2D associated with high genetic susceptibility may be modulated by improved CRF. In joint analysis, however, we did not notably find significant interaction between CRF and inflammation on the risk of T2D (*P* for interaction = 0.09) (Fig. 2b, Supplemental Table S8), implying that risk of T2D increased with lower CRF and with higher inflammation, independent of the level of each other. Participants with low CRF and high inflammation group had 2.53 times higher risk of T2D compared with those who had high CRF and low inflammation (HR = 2.53, 95% CI: 1.83–3.48). In addition, we found that the inverse associations between CRF and risk of T2D were stronger in participants with lower genetic susceptibility than in those with the higher genetic susceptibility (Fig. 3). Specifically, high CRF was strongly associated with 61% (HR = 0.39, 95% CI: 0.23–0.65) and 45% (HR = 0.55, 95% CI: 0.38–0.80) lower risk of T2D among individuals with low genetic susceptibility and moderate genetic susceptibility, respectively. Among individuals with high genetic susceptibility, we found that high CRF was moderately associated with 23% (HR = 0.77, 95% CI: 0.58–1.01) lower risk of T2D.

Similar interaction patterns were observed if we further excluded participants with glucose level ≥ 7.0 mmol/L at baseline (Supplemental Fig. S1), or participants with CVD and cancer history (Supplemental Fig. S2), or participants diagnosed with T2D in the first 2 years of follow-up (Supplemental Fig. S3) or participants with imputing missing covariates (Supplemental Fig. S4).

Fig. 4 displayed the stratified analyses according to potential T2D risk factors, including sex, age, socioeconomic status, BMI, smoking status, alcohol intake, physical activity, healthy diet, hypertension, triglyceride, and family history of diabetes. We found consistent inverse dose-response associations between CRF and T2D across subgroups. But no interactions were observed between CRF and all these factors except

Table 2
The associations of cardiorespiratory fitness, genetic susceptibility and inflammation with risk of type 2 diabetes.

	Events	Incidence rate per 1000 person-year	HR (95% CI)			
			Model 1	Model 2	Model 3	Model 4
Cardiorespiratory fitness						
Low	867	4.43 (4.14–4.73)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Moderate	404	2.04 (1.85–2.25)	0.47 (0.42–0.53)	0.82 (0.72–0.94)	0.82 (0.72–0.94)	0.84 (0.73–0.95)
High	154	0.78 (0.66–0.91)	0.19 (0.16–0.22)	0.61 (0.50–0.75)	0.61 (0.50–0.75)	0.65 (0.53–0.79)
P for trend			<0.001	<0.001	<0.001	<0.001
Genetic susceptibility						
Low	274	1.38 (1.23–1.56)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Moderate	458	2.32 (2.12–2.54)	1.69 (1.46–1.97)	1.59 (1.37–1.85)	1.59 (1.37–1.85)	1.58 (1.36–1.84)
High	693	3.53 (3.28–3.80)	2.64 (2.29–3.04)	2.21 (1.92–2.55)	2.21 (1.92–2.55)	2.20 (1.91–2.54)
P for trend			<0.001	<0.001	<0.001	<0.001
Inflammation						
Low	218	1.08 (0.95–1.24)	1 (Reference)	1 (Reference)	1 (Reference)	1 (Reference)
Moderate	397	2.03 (1.84–2.24)	1.69 (1.43–1.99)	1.11 (0.94–1.31)	1.11 (0.93–1.31)	1.08 (0.91–1.28)
High	810	4.14 (3.86–4.43)	3.49 (3.00–4.05)	1.49 (1.27–1.76)	1.48 (1.26–1.74)	1.42 (1.20–1.67)
P for trend			<0.001	<0.001	<0.001	<0.001

Model 1 was adjusted for age and sex;
Model 2 was additionally adjusted for ethnicity, educational attainment, employment status, Townsend Deprivation Index, smoking status, alcohol intake, body mass index, hypertension, physical activity, triglyceride and serum fasting glucose at baseline and family history of diabetes;
Model 3 was additionally adjusted for dietary pattern;
Model 4 was additionally adjusted for genetic susceptibility, inflammation and CRF mutually.

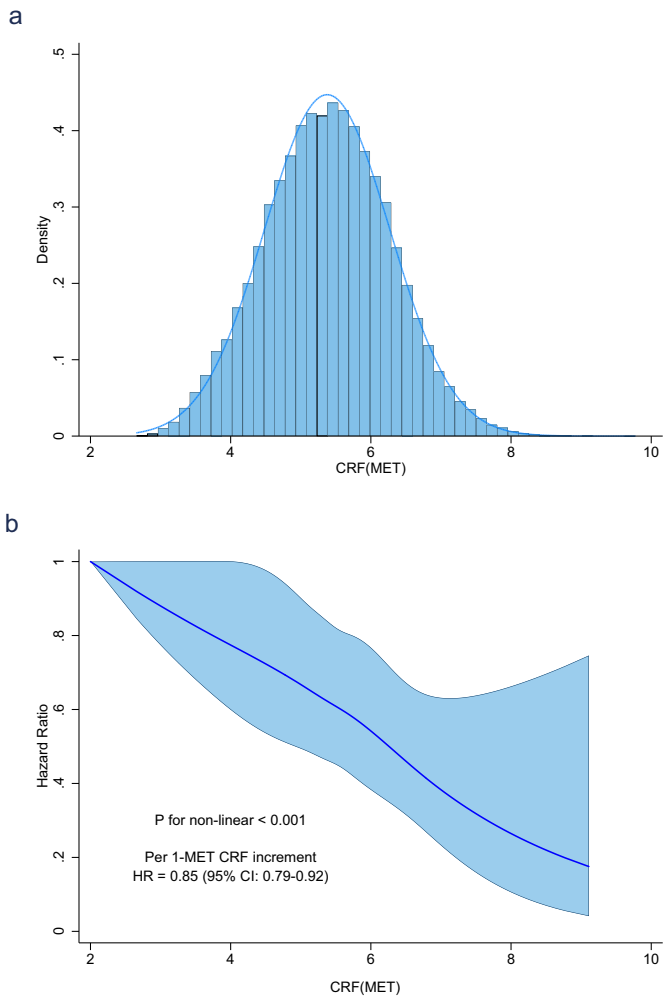


Fig. 1. The distribution of cardiorespiratory fitness (CRF) (a) and dose-response association with risk of T2D (b).
Restricted cubic spline regression was performed with 5 knots, and the lowest value of CRF was used as a reference value. HRs (95% CIs) were estimated after controlling for age, sex, ethnicity, educational attainment, employment status, Townsend Deprivation Index, smoking status, alcohol intake, body mass index, hypertension, physical activity, dietary pattern, triglyceride and fasting glucose at baseline and family history of diabetes.

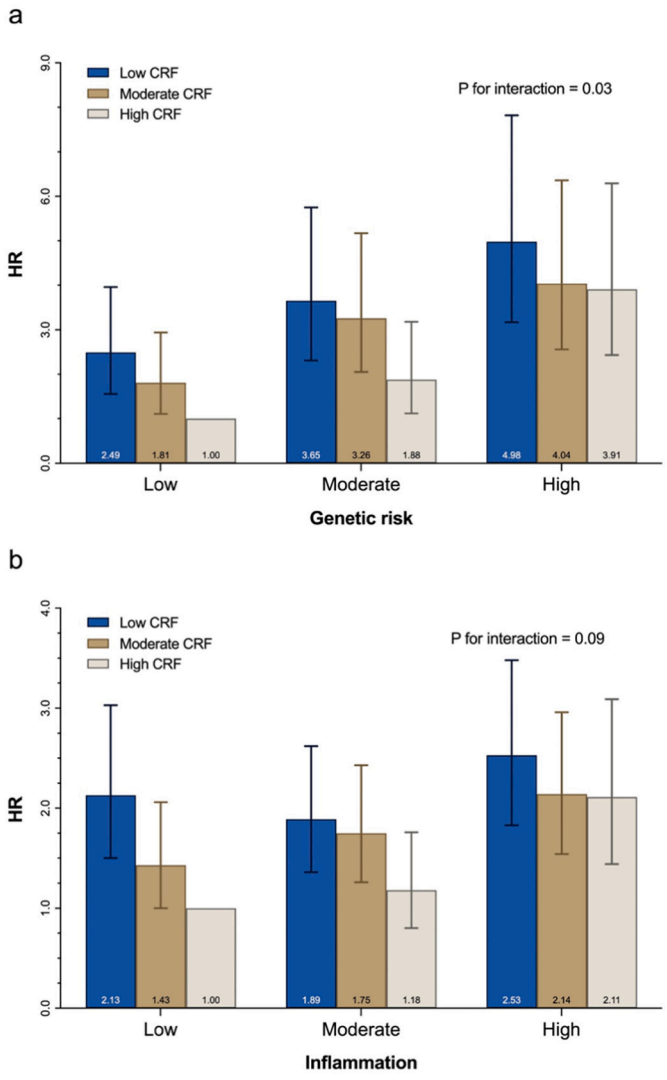


Fig. 2. The joint associations of cardiorespiratory fitness (CRF) and quintiles of genetic susceptibility (a) and inflammation (b) in relation to risk of T2D.
Results were adjusted for age, sex, ethnicity, educational attainment, employment status, Townsend Deprivation Index, smoking status, alcohol intake, body mass index, hypertension, physical activity, dietary pattern, triglyceride and serum glucose at baseline and family history of diabetes.

for family history of diabetes (*P* for interaction = 0.03).

4. Discussion

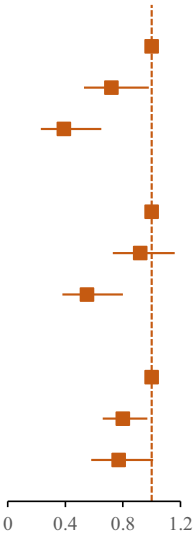
This study represents one of the largest longitudinal studies on the association between CRF and risk of incident T2D. Our results showed that the risk of T2D decreased by 15% for each 1-MET increment of CRF. The inverse association between CRF and T2D was stronger in those with low genetic predisposition to T2D. Besides, the association between CRF and T2D is modulated by genetic susceptibility, indicating that maintaining good fitness might moderately compensate for the detrimental effect of hereditation on risk of T2D.

Our finding of inverse associations between CRF and the risk of incident T2D was in agreement with previous studies. An observational cohort of 6249 women suggested that the upper third of CRF, measured using a maximal treadmill exercise test was associated with 39% (HR = 0.61, 95% CI: 0.38–0.96) lower risk of incident T2D compared with the least CRF third [30]. Copenhagen Male Study including 4988 Caucasian with a follow-up period up to 44 years revealed an inverse dose-response association between CRF and the risk of diabetes [8]. A meta-analysis conducted on 15 studies reported that each 1-MET increment of CRF was associated with 10% (HR = 0.90, 95% CI: 0.86–0.94) lower risk of T2D [10]. Of note, a large prospective study including 46,979 participants demonstrated that higher CRF was associated with a lower risk of incident diabetes regardless of demographic characteristics and baseline risk factors, but the follow-up time was relatively short (median 5.2 years) [31]. Our study additionally examined the hypotheses that the association of CRF with T2D is modulated by genetic susceptibility.

To the best of our knowledge, this is the first study investigating the interaction between genetic susceptibility, inflammation and CRF for incident T2D. The risk of T2D associated with high genetic susceptibility may be attenuated by improved CRF. Similarly, the risk of T2D decreased progressively as the CRF increased within each category of inflammation, though the interaction between CRF and inflammation was not statistically significant. A prospective cohort study from Atherosclerosis Risk in Communities study suggested that physical activity modified the association between genetic susceptibility and T2D, but the protective effect of physical activity was weakest among individuals with high genetic risk for T2D [32]. In line with our study, we

a Genetic susceptibility

Cardiorespiratory fitness (CRF)	Events	Incidence rate per 1000 person-year	HR (95% CI)	*P* value
Low genetic risk				
Low CRF	180	2.80 (2.42-3.25)	1 (Reference)	
Moderate CRF	73	1.10 (0.87-1.38)	0.72 (0.53-0.98)	0.035
High CRF	21	0.31 (0.20-0.48)	0.39 (0.23-0.65)	<0.001
Moderate genetic risk				
Low CRF	278	4.23 (3.76-4.76)	1 (Reference)	
Moderate CRF	138	2.12 (1.79-2.50)	0.92 (0.73-1.16)	0.475
High CRF	42	0.63 (0.47-0.86)	0.55 (0.38-0.80)	0.002
High genetic risk				
Low CRF	409	6.20 (5.63-6.83)	1 (Reference)	
Moderate CRF	193	2.92 (2.53-3.36)	0.80 (0.66-0.97)	0.022
High CRF	91	1.41 (1.15-1.74)	0.77 (0.58-1.01)	0.056



b Inflammation

Cardiorespiratory fitness (CRF)	Events	Incidence rate per 1000 person-year	HR (95% CI)	*P* value
Low inflammation				
Low CRF	95	2.56 (2.09-3.13)	1 (Reference)	
Moderate CRF	73	1.21 (0.96-1.53)	0.75 (0.54-1.03)	0.074
High CRF	50	0.48 (0.37-0.64)	0.57 (0.38-0.87)	0.010
Moderate inflammation				
Low CRF	201	3.21 (2.79-3.68)	1 (Reference)	
Moderate CRF	148	2.02 (1.72-2.37)	0.95 (0.75-1.21)	0.690
High CRF	48	0.80 (0.60-1.07)	0.67 (0.46-0.87)	0.038
High inflammation				
Low CRF	571	5.94 (5.48-6.45)	1 (Reference)	
Moderate CRF	183	2.84 (2.46-3.28)	0.80 (0.67-0.96)	0.020
High CRF	56	1.58 (1.22-2.06)	0.75 (0.55-1.02)	0.069

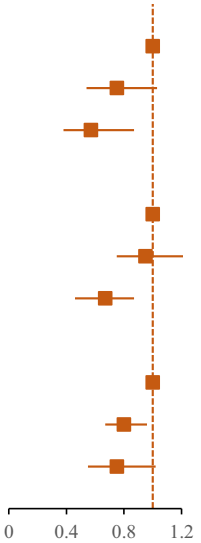


Fig. 3. The associations of cardiorespiratory fitness (CRF) with risk of T2D within genetic risk (a) and inflammation (b).
Results were adjusted for age, sex, ethnicity, educational attainment, employment status, Townsend Deprivation Index, smoking status, alcohol intake, body mass index, hypertension, physical activity, dietary pattern, triglyceride and serum glucose at baseline and family history of diabetes.

found that the beneficial effect of high CRF was nearly 50% lower in participants with both high genetic susceptibility and high inflammation than in those with both low genetic susceptibility and low inflammation, although the statistical power in the high genetic susceptibility and high inflammation group was modest due to relatively low number of individuals and events. Of note, the risks of T2D by different level of CRF among the high genetic susceptibility group were somewhat attenuated after excluding those with prevalent CVD and cancer, indicating that the difference between the low and high CRF groups might be partially attributed to these conditions. Overall, improved CRF has a significant protective effect on the risk of T2D no matter in general population or in population with major chronic diseases.

There are some biological mechanisms that may help to explain this inverse association. First, it is thought that high CRF has a close

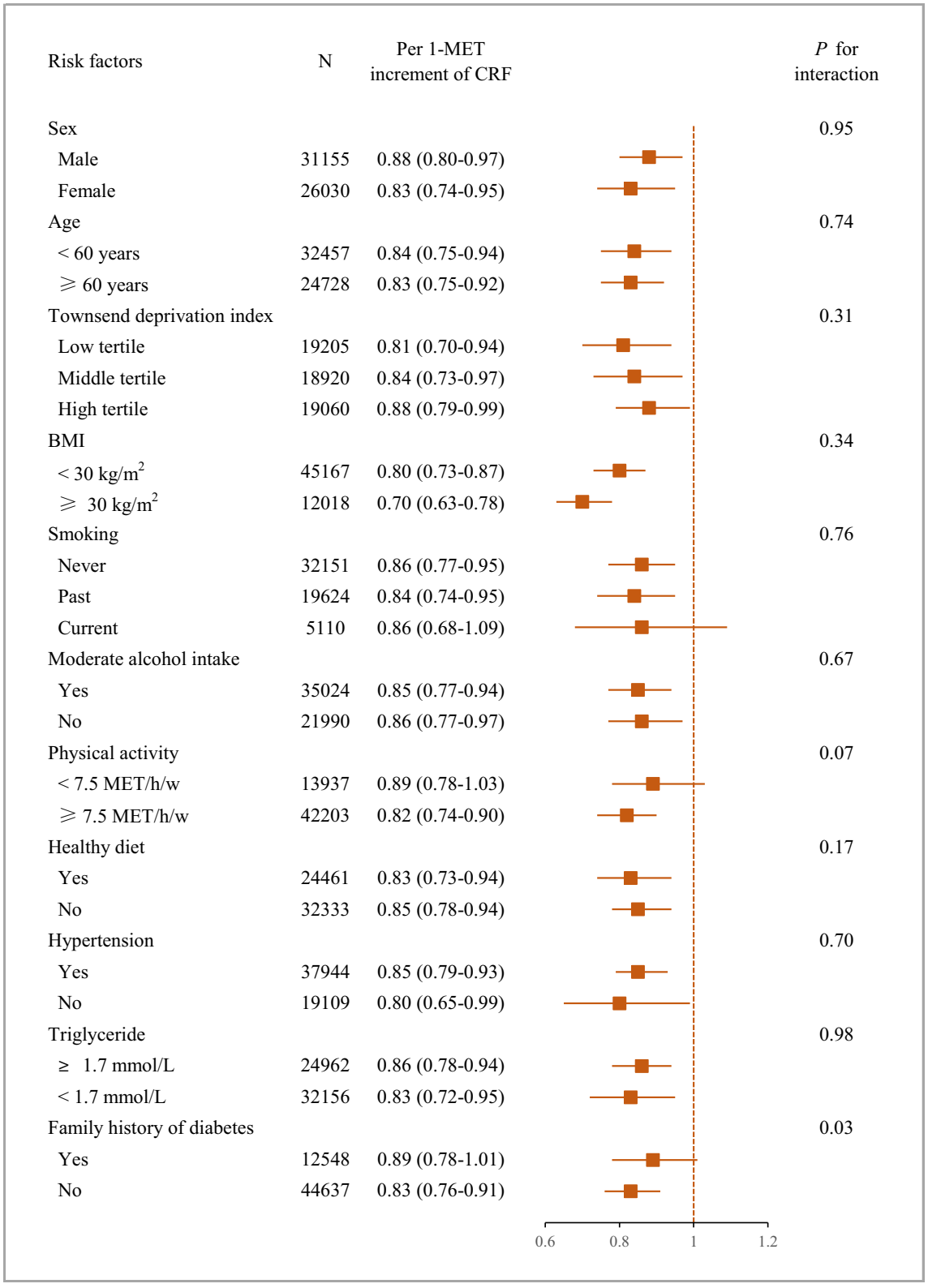


Fig. 4. Adjusted HR (95% CI) for per 1-MET cardiorespiratory fitness (CRF) increment and risk of T2D stratified by potential risk factors.
All analyses were adjusted for age, sex, ethnicity, educational attainment, employment status, Townsend Deprivation Index, smoking status, alcohol intake, body mass index, hypertension, physical activity, dietary pattern, triglyceride and serum fasting glucose at baseline and family history of diabetes. Moderate alcohol intake: women: >0 and ≤14 g/day, men: >0 and ≤28 g/day. Healthy dietary patterns were adapted from the American Heart Association Guidelines and defined as follows: Total fruit and vegetable intake: >4.5 pieces or servings a week, 3 tablespoons of vegetables were considered one serving; Total fish intake: >2 per week; Processed and red meat intake: 2 or fewer times intake of processed meat per week & 5 or fewer times intake of red meat per week. The healthy diet score was dichotomised as 1 = at least two of the healthy food items, 0 = fewer than 2 of the healthy food items. Hypertension was defined as systolic blood pressure ≥ 140 and/or diastolic blood pressure ≥ 90 mm Hg and/or use of antihypertensive medication at baseline.

relationship with increased physical activity [5], which may protect against hypertension and fatty liver disease by lessening adiposity, a critical risk factor of diabetes [33,34]. Our study showed that the effect of CRF on the risk of T2D was independent of physical activity, as the analytical models controlled for physical activity. Second, higher CRF can increase skeletal muscle, an important tissue for postprandial glucose uptake [35]. It improves autonomic function, decreases secretions of pro-inflammatory cytokines, and modifies metabolic risk factors for T2D [36]. Third, higher CRF has been shown to directly increase insulin sensitivity and glucose disposal [31,37]. Finally, people with higher CRF are more likely to adopt a healthier lifestyle that is beneficial for preventing the development of T2D [38]. Intervention studies to examine the benefits of aerobic exercise to strengthen CRF and resistance training to increase muscle strength and the resultant effects on T2D risk are warranted.

The major strengths of this study include the large sample size, the objective measures of CRF, and the robust findings in several sensitivity analyses. Despite these strengths, several limitations of the current study need to be considered. First, exercise testing was a late addition to Biobank data collection protocols in 2009 and relevant data were only available for 14% of participants. However, these participants are representative of the wider UK Biobank sample in terms of sociodemographic and biological characteristics [39]. In addition, we should be cautious in generalizing summary statistics to the general population since only 5.5% of UK residents participated in the UK Biobank cohort. However, this cohort can be used to provide valid estimates of exposure-disease relationships due to its large sample size and multitude of exposures [40,41]. Estimated relative risks derived from UK Biobank are consistent with more representative population cohorts [41]. Second, it is possible that CRF might be altered during the follow-up, and the measure of CRF at baseline could not fully capture its sustained effects over time on the risk of T2D. Further evaluations on persistent levels of inflammation during follow-up time are needed to confirm our findings. Third, the stratified analyses should be interpreted with caution due to the non-statistically significant interaction, which is also reflected in the wide and overlapping confidence intervals. Notwithstanding, effect modification can be present in the absence of a statistical interaction [42], as witnessed by our findings suggesting that the interaction between CRF and inflammation was modest. Moreover, our study did not adjust for multiple comparisons, which might cause false discoveries. Fourth, individuals with high CRF might eat more healthily, which are factors that could affect T2D risk. Thus, we carefully adjusted for diet pattern in our analyses. The association between CRF and T2D was consistent among participants with healthier or unhealthier diet pattern, suggesting that the observed association was not likely due to the correlation with healthier diet. Fifth, although our analyses were adjusted for known potential confounders and participants were followed up for a median of 11.4 years, it is possible that unmeasured confounding (such as specific diet habit, sleep duration, social connection, etc.) and reverse causation remained. However, several sensitivity analyses conducted in our study supported the robustness of our finding. Sixth, diagnoses of T2D were only obtained from electronic health records, which may lead to misclassification since not all T2D patients would have a diagnosis in the healthy records. Finally, given the observational study design, the exact conclusions of causality should be made with caution as residual confounding cannot be ruled out.

5. Conclusions

Overall, we found that there is an inverse and dose-response relationship between CRF and the risk of T2D in the general population. The inverse association between CRF and T2D was stronger in those with low genetic predisposition to T2D, indicating that maintaining good fitness may moderately offset the risk of incident T2D despite high genetic risk. These findings highlight the importance and necessity of improving CRF in public health and clinical practice.

CRediT authorship contribution statement

Chenjie Xu: Data curation, Methodology, Software, Visualization, Writing – original draft. **Yabing Hou:** Software, Methodology, Visualization, Writing – review & editing. **Keyi Si:** Writing – review & editing. **Zhi Cao:** Conceptualization, Investigation, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no competing interests.

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Availability of data and materials

The data that support the findings of this study are available from UK Biobank project site, subject to registration and application process. Further details can be found at https://www.ukbiobank.ac.uk.

Consent to publication

Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.metabol.2022.155215.

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