CARDIOVASCULAR HEALTH ACROSS THE GLYCEMIC SPECTRUM

KAN WANG

Cardiovascular Health across The Glycemic Spectrum

Kan Wang

The studies described in this thesis were performed within the Rotterdam Study, the PREVEND study, the China Health and Retirement Longitudinal Study, and the UK Biobank. We gratefully acknowledge the contributions of participants, research staff, data management, and health professionals of all studies.

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Part I

General Introduction

Diabetes mellitus, more simply known as diabetes, is one of the fastest-growing global health emergencies of the 21st century [1], with increasing prevalence worldwide since 1980 [2]. The landscape of diabetes and its complications has positively changed over the past few decades owing to the improvement in prevention, treatment, and management strategies of diabetes [3, 4]. Yet, diabetes remains a significant cause of mortality, morbidity, and health-system burden worldwide. According to the latest International Diabetes Federation report, the estimated global prevalence of diabetes among individuals between 20-79 years old was 10.5% (536.6 million people) in 2021, rising to 12.2% (783.2 million) in 2045. The related health expenditures were estimated at 966 billion USD in 2021 and are projected to reach 1,054 billion USD by 2045 [5]. Type 2 diabetes (T2D) accounts for over 90% of persons with diabetes. Despite being solely defined on the basis of persistently raised blood glucose, T2D is increasingly recognised as a complex entity driven by a chronic positive energy balance, with multiple metabolic and homoeostatic disturbances developing over the course of the disease [6]. T2D can affect multiple organ systems and is associated with a variety of vascular and nonvascular complications.

The development of T2D is a multistage process, with a precursor condition referred to as prediabetes, defined as glycemic parameters higher than normal but still below the diabetes thresholds. Prediabetes also is a serious global health burden affecting approximately 319 million adults in 2021, with a projection of 441 million in 2045 [1]. Prediabetes indicates a higher risk of developing T2D and its complications [7, 8]. Some have questioned this label's validity and usefulness, arguing that it may lead to overdiagnosis with unnecessary early treatment of T2D [9]. More interestingly, prediabetes is not a robust diagnostic entity, with 5-10% of prediabetes progressing to T2D each year and the same proportion converting back to normoglycemia [10]. Despite these controversies, prediabetes potentially represents a valuable window of opportunity to further curb the diabetes-related health burden.

Multistage model for the development of type 2 diabetes

T2D is a chronic condition with raised blood glucose levels resulting from insufficient production or ineffective use of the hormone insulin. According to Weir's multistage model [11], there are five stages in the progression of diabetes, each of which is characterised by different changes in β-cell mass, phenotype, and function. Specifically, stage 1 is compensation: insulin secretion increases to maintain normoglycemia in the face of insulin resistance or decreasing β-cell mass. Stage 2 is the stable adaptation period when β-cells are no longer fully compensating for increased insulin resistance; and thus, glucose values are not entirely maintained. This period probably starts when glucose levels are still within the normal range and is usually accompanied by loss of β-cell mass and disruption of function. Stage 3 is a transient, unstable period of early decompensation in which glucose levels rise relatively rapidly to the frank diabetes of stage 4, which is characterised as stable decompensation with more severe β-cell dedifferentiation. Finally, stage 5 is characterised by severe decompensation representing a profound reduction in β-cell mass with progression to ketosis. This theory is also supported by evidence from several longitudinal studies [12-14].

Cardiometabolic health and incident type 2 diabetes

Over the past few decades, advances in epidemiological research have profoundly improved

our understanding of the risk profile for T2D development, which consists of a matrix of environmental, genetic, and epigenetic factors that interact with one another and operate within the larger physical-sociocultural environment [15]. Despite increasing knowledge regarding risk factors for T2D, the prediction and prevention of T2D still need improvement [16, 17]. To fill these gaps, other possible mechanisms of diabetes development started to receive attention. For example, cardiac autonomic dysfunction has been reported to be associated with reduced insulin sensitivity and β-cell function and is suggested as an important mechanism of cardiovascular complications in T2D [18, 19]. However, the evidence for the association between heart rate variability (HRV), a marker of cardiac autonomic dysfunction, and T2D is inconclusive. Available studies used only single exposure measurements, cross-sectional designs, and short follow-up periods, which are prone to confounding and reverse causation. Other risk factors have also been proposed to explain the residual risk of traditional risk profiles for T2D development. For example, obesity, generally defined by an excess of body fat causing prejudice to health, is at the centre of diabetogenesis, but it can no longer be evaluated solely by the body mass index (BMI) because it represents a heterogeneous entity [20]. Given the closer association of visceral adiposity with the pathogenesis of insulin resistance and diabetes, more accurate anthropometric measures of adiposity, such as relative fat mass (RFM), have been developed to further assess future diabetes risk, even at normal BMI levels [21, 22]. In addition, some subclinical biomarkers, such as arterial stiffness, are evidently increased during the prediabetes stage [23], and may also be associated with incident T2D [24]. Findings in this regard, however, remain inconclusive.

Meanwhile, many T2D cases are preventable through appropriate lifestyle modifications, even in the case of a strong genetic predisposition. Multilevel intervention measures are warranted, and lifestyle change, such as weight control, healthy diet, physical activity, and smoking cessation, is recommended as a cornerstone for preventing T2D and its complications [25, 26]. So far, studies targeting diabetes prevention have mainly focused on one or certain risk factors and do not take the interactive nature of risk factors into account. In 2010, the American Heart Association introduced the concept of cardiovascular health (CVH) as part of the goals for reducing deaths from cardiovascular disease [27]. As many cardiovascular risk factors also confer a large risk for T2D, the concept of CVH is applicable to T2D [28]. Yet, data on the lifetime risk of incident T2D across different CVH categories are scarce.

Hypertension management for type 2 diabetes

Guidelines for CVD reduction in T2D are generally aligned across professional societies, but there remain some notable differences with regard to risk stratification, especially for blood pressure management [29]. Hypertension is a major preventable risk factor for cardiovascular disease and mortality among T2D [30, 31]. However, the treatment and control of hypertension are far from optimal, giving rise to a tremendous health burden worldwide [32]. In 2017, the American College of Cardiology / American Heart Association released a guideline that updated the definition and treatment of hypertension. The guideline defines hypertension as blood pressure ≥130/80 mmHg and recommends initiating antihypertensive drug treatment at a blood pressure of 130/80 mmHg or higher with a treatment goal of <130/80 mmHg in patients with T2D and hypertension [33]. Considering the importance of amendments in guideline recommendations for managing population health, studies from different countries are needed

to investigate the impact. The discrepancy in the management of hypertension between guidelines also indirectly underscores the debate about the optimal blood pressure levels, especially for those with diabetes, for whom the health benefit of intensive blood pressure control remains unclear [34-36].

In addition, as patients with T2D are currently living longer, multimorbidity is becoming a new norm. Around 80% of diabetic individuals have at least one other chronic disease [37]. Multimorbidity increases the complexity of T2D management through frailty, polypharmacy, and treatment burden. Available guidelines recommend more personalised blood pressure targets during T2D management based on the presence of comorbidities and individual care needs [38-40]. However, the methods to define multimorbidity are solely based on the number of chronic diseases. Considering the heterogeneity in multimorbidity patterns, this simple approach is an unspecific health indicator that does not always capture the complex underlying pathophysiologic mechanisms [41, 42]. As such, significant knowledge gaps remain.

Cardiovascular and other emerging complications of type 2 diabetes

As diabetes develops and progresses towards its complications, treatment becomes more challenging, and the costs dramatically rise [43]. The complications of T2D are usually divided into macrovascular conditions, such as coronary heart disease, stroke and peripheral arterial disease, and microvascular conditions, including diabetic kidney disease, retinopathy and peripheral neuropathy. Cardiovascular disease (CVD) is the leading cause of morbidity and mortality for T2D [30]. Independently from other conventional risk factors, diabetes alone confers about a twofold excess risk for CVD [44]. Notably, the relative excess risk of vascular events with T2D may be greater in women than men, calling for sex-specific prevention and treatment strategies [45].

Meanwhile, metabolic and physiological features are dysregulated in people with prediabetes, and traditional cardiovascular risk factors such as obesity and dyslipidemia also become more prevalent during this stage [46]. However, data on sex-specific lifetime risk of CVD across the glycemic spectrum, in particular in prediabetes state, are scarce. Also, as mentioned earlier, prediabetes is not a stable diagnostic entity. Few data are available that evaluate whether the observed increased risk is the result of prediabetes per se or the transition from prediabetes to diabetes during follow-up [8]. There is even less evidence regarding the role of a healthy lifestyle in the temporal cardiometabolic disease transition among people with prediabetes.

Advances in diabetes management have resulted in the emergence of a different set of diabetes complications that also warrant further investigation [47]. With a declining mortality rate, CVD no longer accounts for the majority of deaths among patients with diabetes. Population-based studies show that cancer is now the leading cause of death in people with diabetes in some countries or regions [48, 49], and the proportion of deaths due to dementia has also risen since the turn of the century [50]. In addition, disability is also highly prevalent in patients with diabetes, with prevalences reported between 47 and 84% [51]. Here, disability was defined as a difficulty in functioning in one or more life domains as experienced by an individual with a health condition in interaction with contextual factors [52]. Disability-related conditions, such as frailty and fracture, can complicate diabetes management in older adults

through hypoglycemia, polypharmacy and treatment burden [53]. However, compared to the traditional complications, the effects of hyperglycemia on these emerging complications, such as cancer, cognition, and physical functioning, have so far been less investigated. Moreover, as multimorbidity is becoming a new norm among patients with diabetes, whether the concept of CVH is also applicable to help prevent these prevailing non-communicable diseases and their multimorbidity is unclear.

THIS THESIS

Objective

The aims of this thesis were to investigate the role of cardiometabolic health in T2D development. Cardiometabolic health included emerging risk factors: HRV, RFM, arterial stiffness/remodelling, and composite CVH. I further aimed to investigate the implication of blood pressure management in T2D. Last, I sought to examine the burden of complications across the glycemic spectrum. The complications included CVD, cognitive decline, physical disability, and multimorbidity of non-communicable diseases.

Study design

The studies in this thesis were embedded in four population-based cohort studies: The Rotterdam Study, The PREVEND Study, The China Health and Retirement Longitudinal Study, and The UK Biobank.

The Rotterdam Study

The Rotterdam Study (RS) is an ongoing prospective cohort of the community-dwelling population aged 55 years and older in Rotterdam, the Netherlands. The detailed study design has been described elsewhere [54]. Briefly, the baseline examination for the first cohort of the RS was completed between 1990 and 1993 (RS-I). The study was extended in 2000 to include all inhabitants who became 55 years of age or moved into the research area after the start of the study (RS-II). In 2006, a further extension of the cohort was initiated (RS-III) that included participants aged 45 years and older. There were no eligibility criteria to enter the Rotterdam Study apart from the minimum age and residential area based on postal codes. The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare, and Sport (Population Screening Act WBO, license number 1071272-159521-PG). Written informed consent was obtained for all study participants.

The PREVEND Study

The PREVEND study is a prospective cohort study of 8592 community-dwelling adults living in Groningen, the Netherlands, designed to investigate whether increased urinary albumin excretion (UAE) was associated with the risk of future cardiovascular and renal disease in the community (1997-1998). The detailed study design has been described elsewhere [55]. Briefly, all inhabitants from the city of Groningen, aged 28 to 75 years, were asked to respond to a short questionnaire and provide early-morning urine samples (n=85,421), and 40,856 individuals (47.8%) responded. Responders with UAE greater than or equal to 10 mg/L (n=7786), and a randomly selected control group with UAE less than 10 mg/L (n=3395), were invited to the

outpatient clinic for a comprehensive health assessment. Insulin-treated individuals, pregnant women (self-reported), and unwilling subjects were excluded from the study. A final total of 6000 individuals with UAE greater than or equal to 10 mg/L and 2592 individuals with UAE less than 10 mg/L underwent further investigation and constituted the baseline PREVEND cohort (n=8592). The PREVEND study has been approved by the medical ethics committee of the University Medical Center Groningen (registration number MEC 96/01/022). Written informed consent was obtained for all study participants.

The China Health and Retirement Longitudinal Study (CHARLS)

The CHARLS is a nationally representative longitudinal survey of persons in China 45 years of age or older, including assessments of the social, economic, and health circumstances of community residents. The detailed study design has been described elsewhere [56]. Briefly, the national baseline survey for the study was conducted between June 2011 and March 2012 and involved 17,708 respondents selected through multistage probability sampling. CHARLS respondents are followed every two years, using a face-to-face computer-assisted personal interview. Physical measurements are made at every 2-year follow-up, and blood sample collection is done once every two follow-up periods. Three follow-up waves (2013, 2015, and 2018) were completed after the baseline survey. The CHARLS has been approved by the Peking University Institutional Review Board. Written informed consent was obtained for all study participants.

The UK Biobank

The UK Biobank is a population-based study with 502,415 individuals aged 40-69 years recruited between 2006 to 2010 from the general population across the U.K. At baseline, participants attended one of the 22 assessment centers where they completed a touch screen questionnaire to report their demographic information, lifestyle, medication use, and health status and provided biological samples, as described in detail elsewhere [57]. UK Biobank received ethical approval from the North West Multi-center Research Ethics Committee (MREC) covering the whole of the UK, and all participants provided electronic informed consent.

THESIS OUTLINE

After this general introduction (**Chapter 1**), **Chapter 2** of this thesis focuses on the role of cardiometabolic health in the development of T2D. **Chapters 2.1**, **2.2** and **2.3** discuss the role of HRV (**Chapter 2.1**), RFM (**Chapter 2.2**), and arterial stiffness/remodelling (**Chapter 2.3**) in T2D risk. **Chapter 2.4** evaluates the role of CVH and genetic variants on the lifetime risk of incident T2D. **Chapter 3** investigates the implication of hypertension management in T2D. **Chapter 3.1** estimates the concordance and discordance in the prevalence of hypertension and antihypertensive medication recommendations for Chinese adults with diabetes using definitions from different guidelines. **Chapter 3.2** demonstrates the associations of blood pressure with all-cause and cause-specific mortality within different multimorbidity patterns among patients with T2D. **Chapter 4** describes the burden of traditional and emerging complications across the glycemic spectrum. **Chapter 4.1** evaluates the differences in the first manifestations of CVD across different glycemic spectrums. **Chapter 4.2** investigates the role

of incident T2D and healthy lifestyle in the associations between prediabetes and the risk of CVD and mortality. **Chapter 4.3** assesses longitudinal changes in cognition and disability according to glycemic status and different glycemic transitions in middle-aged and older Chinese. **Chapter 4.4** estimates the lifetime risk for multimorbidity of non-communicable diseases across different CVH categories. **Chapter 5** provides an overview of the main findings from all studies in this thesis. In this chapter, major strengths and limitations, as well as implications and recommendations for future studies are discussed.

Part II

Cardiovascular health and incident type 2 diabetes

2.1

Chapter 2.1

Heart rate variability and type 2 diabetes

ABSTRACT

Background: Hyperglycemia and autonomic dysfunction are bidirectionally related. We investigated the association of longitudinal evolution of heart rate variability (HRV) with incident type 2 diabetes (T2D) among the general population.

Methods: We included 7630 participants (mean age 63.7 years, 58% women) from the population-based Rotterdam Study who had no history of T2D and atrial fibrillation at baseline and had repeated HRV assessments at baseline and during follow-up. We used joint models to assess the association between longitudinal evolution of heart rate and different HRV metrics (including the heart-rate corrected standard deviation of the normal-to-normal RR intervals (SDNNc), and root mean square of successive RR-interval differences (RMSSDc)) with incident T2D. Models were adjusted for cardiovascular risk factors. Bidirectional Mendelian randomization (MR) using summary-level data was also performed.

Results: During a median follow-up of 8.6 years, 871 individuals developed incident T2D. One standard deviation (SD) increase in heart rate (hazard ratio [HR], 1.20, 95% confidence interval (CI), 1.09-1.33), and log(RMSSDc) (1.16, 95% CI 1.01-1.33) were independently associated with incident T2D. The HRs were 1.54 (95% CI 1.08-2.06) for participants younger than 62 years and 1.15 (95% CI 1.01-1.31) for those older than 62 years for heart rate (*p* for interaction <0.001). Results from bidirectional MR analyses suggested that HRV and T2D were not significantly related to each other.

Conclusions: Autonomic dysfunction preceeds development of T2D, especially among younger individuals, while MR analysis suggests no causal relationship. More studies are needed to further validate our findings.

INTRODUCTION

Cardiac autonomic dysfunction due to hyperglycemia has been suggested as a mechanism of cardiovascular complications in type 2 diabetes mellitus (T2D) [18]. Major organs responsible for insulin secretion and sensitivity, glucose production, and metabolism, including the pancreas, liver, and skeletal muscle, are innervated by the autonomic nervous system [58]. Yet, there are numerous pathways whereby autonomic dysfunction could, in turn, affect glucose metabolism. Notably, an autonomic imbalance was already present in persons with prediabetes [59] and was associated with incident diabetes [60]. Autonomic dysfunction has also been related to reduced insulin sensitivity and beta-cell function in people without diabetes [19, 61].

Heart rate variability (HRV) is a marker of cardiac autonomic dysfunction, and a single assessment of HRV has been associated with changes in fasting glucose level [62] and incident T2D [63]. Taken together, alterations in autonomic function may contribute to the pathogenesis of T2D. However, HRV has a strong and inverse relationship with heart rate, so HRV parameters should be corrected for heart rate during analysis. Therefore, the results from previous studies using uncorrect HRV might be confounded. In addition, given the considerable impact of age on HRV [64] and the possible bidirectional association between hyperglycemia and autonomic dysfunction [59, 60], studies using only single HRV measurements, cross-sectional designs, and short follow-up periods are all prone to confounding and reverse causation. Joint model is a novel method which can perform simultaneous analyses of repeated exposure measurments and survival data, and its principal advantage is the correct treatment of noisy and incompletely observed time-varing exposure information. Thus, this approach is appropriate to estimate the hazard of T2D incident for the HRV metrics as time-varying covariates, which enables unbiased estimation of the relationship between the exposure and the outcome.

In the large prospective population-based Rotterdam Study with repeated measurements of HRV, we investigated the prospective association of evolution of HRV, as a proxy for autonomic function, with the incidence of T2D. In addition, we conducted a bidirectional Mendelian randomization (MR) analysis using summary-level data to explore the causality of the association between HRV and T2D.

METHODS

Study design and population

This study was embedded within the Rotterdam Study (RS), a prospective cohort study of community-dwelling persons in Ommoord, Rotterdam, The Netherlands. The detailed study design has been described elsewhere [54]. Briefly, the baseline examination of the first cohort was completed between 1990 and 1993 (RS-I, n=7983) with participants aged 55 years or over. The study was extended in 2000, with the second cohort of individuals who had reached 55 years or moved into the study area after 1990 (RS-II, n=3011). In 2006, a third cohort was enrolled, including inhabitants aged 45 years and older (RS-III, n=3932). The overall response rate for the Rotterdam Study was 72%. There were no eligibility criteria to enter the Rotterdam Study apart from the minimum age and residential area based on postal codes. Participants attended follow-up examinations every 3-5 years. All participants provided written informed consent to participate in the study and to have their information obtained from their treating

physicians.

The current study was based on the third visit of RS-I (RS-I-3) and the first visit of RS-II (RS-II-1) and RS-III (RS-III-1). Participants with no informed consent for follow-up data collection (n=137), prevalent T2D (n=2133) or prevalent atrial fibrillation (n=344) at baseline, or no available electrocardiogram (ECGs) measurements (n=1496) were excluded. Therefore, 7630 participants were included in the study (**Figure S1**).

Assessment of heart rate variability

A standard 10-second, 12-lead resting ECGs was recorded during each follow-up examination with an ACTA Gnosis electrocardiograph (Esaote Biomedica, Italy) at a sampling frequency of 500 Hz and stored digitally. ECGs were processed by the Modular ECG Analysis System (MEANS), an ECG computer program that has been validated extensively [65, 66]. HRV was calculated based on RR intervals between normal heartbeats; RR intervals were excluded if they immediately preceded or followed premature atrial complexes or premature ventricular complexes. The following HRV indices were used for the analyses: the heart rate-corrected standard deviation of the normal-to-normal RR intervals (SDNNc) and the heart rate-corrected root mean square of successive RR-interval differences (RMSSDc) [64].

Assessment of cardiovascular risk factors

Information on covariates was collected at baseline using a structured questionnaire. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Fasting blood glucose and insulin levels, and total and high-density lipoprotein cholesterol were measured using standard laboratory techniques. Homeostatic model assessment of insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) were calculated based on fasting blood glucose and serum insulin concentration to assess insulin resistance and β-cell function separately. Smoking status was categorized into never, former, and current smoking. Blood pressure was measured in the right upper arm with the participant in a sitting position, of which the mean of 2 consecutive measurements was used. Physical activity levels were assessed using validated questionnaires (the Zutphen Physical Activity Questionnaire for RS-I and RS-II [67], the LASA Physical Activity Questionnaire for RS-III [68]) and further quantified into metabolic equivalent task (MET) values per week doing moderate and vigorous-intensity activities classified according to the 2017 Dutch Physical Activity Guideline [69]. Medication use (blood pressure- and lipid-lowering drugs) was derived from baseline questionnaires, pharmacy data and was categorized and defined according to the World Health Organization Anatomical Therapeutic Chemical (WHO ATC) classifications. Specifically, antihypertensive medication, use of beta blockers, use of calcium blockers, and lipid-lowering medication were defined according to the WHO ATC categories c02, c07, c08, and c10 respectively. In addition, information about prevalent cardiovascular disease (including coronary heart disease, heart failure, and stroke) was also collected at baseline.

Assessment of type 2 diabetes

Participants were followed up from the date of attending the baseline visit onwards. At baseline and during follow-up, cases of T2D were ascertained by the use of general practitioners' records, hospital discharge letters, and serum glucose measurements collected from center visits, which

took place roughly every 4 years. T2D was defined as a fasting blood glucose concentration equal to or above 7.0 mmol/L, a non-fasting blood glucose concentration of 11.1 mmol/L or higher (when fasting samples were unavailable), or the use of blood glucose-lowering medications. Information about blood glucose-lowering medications was obtained from both structured home interviews and pharmacy dispensing records. Two study physicians independently adjudicated all potential events of T2D. In the case of disagreement, a consensus was sought from a diabetologist. Participants were followed until incident T2D, death, or the end of the study period (January 1st, 2015).

Statistical analyses

Descriptive statistics were performed by reporting mean (standard deviation (SD)) or median (interquartile range (IQR)) for continuous variables and number (percentages) for categorical variables. Two HRV metrics (SDNNc and RMSSDc) were log-transformed to fulfill the normality assumption. Heart rate and log-transformed HRV metrics were further standardized to allow for direct comparisons of effect sizes, per 1-SD increase. Linearity was explored with restricted cubic splines for each exposure, with no evidence of deviation from linearity (p for non-linear: 0.479 for HR; 0.865 for log[SDNNc]; 0.286 for log[RMSSDc]).

For the longitudinal analysis, joint models for longitudinal and time-to-event data were performed [70]. The joint model estimates continuous profiles of each HRV metric based on the repeated measured data collected during the whole follow-up period for each individual; therefore, it would consider individual variations and reduce the bias associated with missing data. In addition, joint models are more appropriate for estimating the hazard of incident T2D for the HRV metrics as time-varying covariates because they account for their endogenous nature. For the HRV metrics, we used linear mixed-effect models. When appropriate and judged by residuals plots, transformed HRV metrics were used as dependent variables. We included age (the time scale variable) and sex in the fixed-effects, with both the intercept and the slope fitted as random effects. Next, a joint model was implemented by combining the joint distribution of HRV metrics in the linear mixed-effects model with the Cox model. For the crude model, we included baseline age, sex, and cohort in the survival part of the models. The full model was fitted by further adjusting for BMI, smoking status, systolic blood pressure, total and high-density lipoprotein cholesterol, use of blood pressure-lowering or lipid-lowering medication, and prevalent cardiovascular disease. We also used Spearmen correlation to examine the cross-sectional associations between heart rate and different HRV metrics and glycemic traits (fasting blood glucose and insulin levels, HOMA-IR, and HOMA-β) at baseline.

To check for any possible effect modification by age, sex, BMI, or use of blood pressure-lowering medication, we separately added an interaction term between each variable; (age [continuous], sex [dichotomous], BMI [continuous], use of blood pressure-lowering medication [dichotomous]), and HRV metrics to the joint model and then further explored these by stratification. To ensure a sufficient sample size for subgroup analyses, age stratification was based on the median age (62 years), and BMI stratification was based on the cutoff point for overweight (25.0 kg/m^2). To test the robustness of the longitudinal findings, we performed the following sensitivity analyses: (1-3) excluded participants who had prediabetes (defined as a fasting blood glucose concentration >6.0 mmol/L and <7.0 mmol/L), had prevalent

cardiovascular disease, or were underweight (BMI ≤18.5 kg/m^2) at baseline; (4) further adjusted the model for fasting blood glucose and physical activity; (5) excluded participants used beta blockers or calcium blockers at baseline or during follow-up; (6) conducted a complete case analysis.

Additionally, we conducted two-sample bidirectional MR analyses to examine the association between heart rate-uncorrected HRV (SDNN and RMSSD) and T2D. The inverse variance weighted (IVW) method was the main method used in our analyses. MR estimates were presented as odds ratios (ORs) with corresponding 95% CIs. More details on the rationale, assumptions and sensitivity analyses of the MR analyses are shown in the **Methods S1**.

Information on covariables was missing for up to 2.5%. To deal with missing values, we used single imputation with the expectation-maximization method. Data were handled and analyzed with SPSS Statistics version 25.0.0.1 (IBM Corp., Armonk, NY) and R, CRAN version 4.0.5, with packages "JMbayes2" and "TwoSampleMR". All analyses were performed at the significance level of 0.05 (2-tailed).

RESULTS

Among 7630 included participants, the median age was 62.1 (IQR: 45.5 – 98.0) years, and 4444 (58%) were women (**Table 1**). During a median follow-up time of 8.6 years (IQR: 7.1-14.1), 871 individuals developed T2D (incidence rate: 11.4 cases per 1000 person-years).

In the joint model analysis, heart rate and HRV metrics were positively associated with incident T2D (**Table 2**). For heart rate, one SD increment was associated with the risk of developing T2D in the crude model (hazard ratio [HR], 1.18; 95% CI, 1.07-1.31)). After adjustments, the association remained significant (HR; 95% CI: 1.20; 1.09, 1.33). For HRV, both metrics showed positive associations with T2D development with statistically significant associations only found for RMSSDc. The HRs (95% CIs) of incident T2D per SD increment of log(RMSSDc) were 1.16 (1.02, 1.30) in the crude model and 1.16 (1.01, 1.33) in the fully adjusted model. The association of SDNNc with incident T2D was not statistically significant (HR 1.10 (0.94, 1.29) in the full model.

We observed a significant interaction between age (continuous) and heart rate (*p* for interaction <0.001). We stratified participants based on the median age (62 years), and found that the association between heart rate and incident T2D was relatively stronger among younger participants (**Figure 1, Table S1**). Although significant associations were restricted to men (**Figure 1, Table S1**), the interaction term for sex was not statistically significant. The HRs (95% CIs) of incident T2D per SD increment were 1.25 (1.09, 1.43) for men and 1.16 (0.99, 1.35) for women for heart rate, 1.23 (1.01, 1.51) for men and 0.97 (0.78, 1.20) for women for log(SDNNc), and 1.23 (1.04, 1.46) for men and 1.08 (0.89, 1.30) for women for log(RMSSDc). We also did not find a significant interaction for BMI or use of blood pressure-lowering medication (**Table S1**), although statistically significant associations were restricted to participants who were overweight or without use of blood pressure-lowering drugs, respectively.

Table 1. Baseline characteristics of the total study population

	Total population (n=7630)
Age, years	63.7 (9.5)
Women, n (%)	4444 (58%)
Education, n (%)	
Primary	859 (11%)
Lower/intermediate general or lower vocational	2989 (40%)
Intermediate vocational or higher general	2252 (30%)
Higher vocational or university	1466 (19%)
Height, cm	168.4 (9.5)
Weight, kg	76.7 (14.0)
Body mass index, kg/m^2	27.0 (4.1)
Smoking status, n (%)	
Current	1529 (20%)
Former	3590 (47%)
Never	2511 (33%)
Systolic blood pressure, mmHg	138.0 (20.6)
Use of blood pressure-lowering medication, n (%)	2183 (29%)
Total cholesterol, mmol/L	5.8 (1.0)
High-density lipoprotein, mmol/L	1.4 (0.4)
Use of lipid-lowering agents, n (%)	1359 (18%)
Fasting blood glucose, mmol/L	5.4 (0.6)
Fasting insulin level, mmol/L	71.0 (50.0, 99.0)
HOMA-IR	2.8 (1.9, 4.0)
HOMA-β	126.2 (89.6, 175.3)
History of cardiovascular disease, n (%)	639 (8%)
Metrics of heart rate variability	
Heart rate	67.7 (61.2, 74.9)
SDNNc	26.1 (16.4, 43.5)
RMSSDc	32.1 (19.8, 53.6)

Values are mean (standard deviation) or median (interquartile range) for continuous variables and number (percentages) for categorical variables.

In sensitivity analyses, similar associations between heart rate and different HRV metrics with incident T2D were observed after excluding participants who had prediabetes (n=1029) or prevalent cardiovascular disease (n=639) at baseline and also after excluding participants who were underweight (n=48) or used beta blockers or calcium blockers during follow-up (n=1236). In further analyses with additional adjustments for baseline measurement of fasting blood glucose and physical activity and in a complete case analysis, results remained consistent with our main results (**Table S2**).

Table 2. Joint model results for the association between longitudinal measures of heart rate and heart rate variability with incident type 2 diabetes

Model	Number of participants	Number of events	HR (95% CI)	*P*
Heart rate				
Model 1	7630	871	1.18 (1.07, 1.31)	0.0018
Model 2	7630	871	1.20 (1.09, 1.33)	0.0004
Log(SDNNc)				
Model 1	7630	871	1.05 (0.89, 1.21)	0.5538
Model 2	7630	871	1.10 (0.94, 1.29)	0.2256
Log(RMSSDc)				
Model 1	7630	871	1.16 (1.02, 1.30)	0.0227
Model 2	7630	871	1.16 (1.01, 1.33)	0.0438

Model 1 was adjusted for baseline age, sex, and cohort for relative risk model. Model 2 was further adjusted for body mass index, smoking status, systolic blood pressure, use of blood pressure-lowering medications, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and history of cardiovascular disease (coronary heart disease, heart failure, and stroke) at baseline. The hazard ratio for incident diabetes was calculated per 1-SD increase in heart rate or the log of HRV indices (SDNNc and RMSSDc).

The Spearmen correlation analyses indicated that heart rate was significantly associated with all glycemic traits, including fasting blood glucose, insulin, HOMA-IR, and HOMA-β, while RMSSDc was only related to fasting blood glucose (**Figure 2**). After excluding individuals with baseline prediabetes, the associations between HRV metrics and glycemic traits were not statistically significant anymore (**Figure S2**).

A total of 9 SNPs for SDNN and 9 SNPs for RMSSD were available in the T2D GWAS and were used for the MR analyses after removal of potential outliers (**Table S3**). As presented in **Table S4**, the results from the MR analyses suggested no causal association between HRV and incident T2D (OR (95% CIs) were 0.94 (0.75, 1.18) per one unit log increment for SDNN and 1.04 (0.82, 1.32) per one unit log increment for RMSSD. In addition, 156 SNPs for T2D were available in the HRV GWAS (**Table S5**), and the results from the MR analyses showed that genetically predicted T2D was not significantly associated with log(SDNN) or log(RMSSD) (**Table S6**). The WME and MR-Egger slope estimates were also insignificant, consistent with the IVW method after correcting for outliers using MR-PRESSO during the bidirectional MR analaysis, and we found no evidence for violation of the MR assumptions.

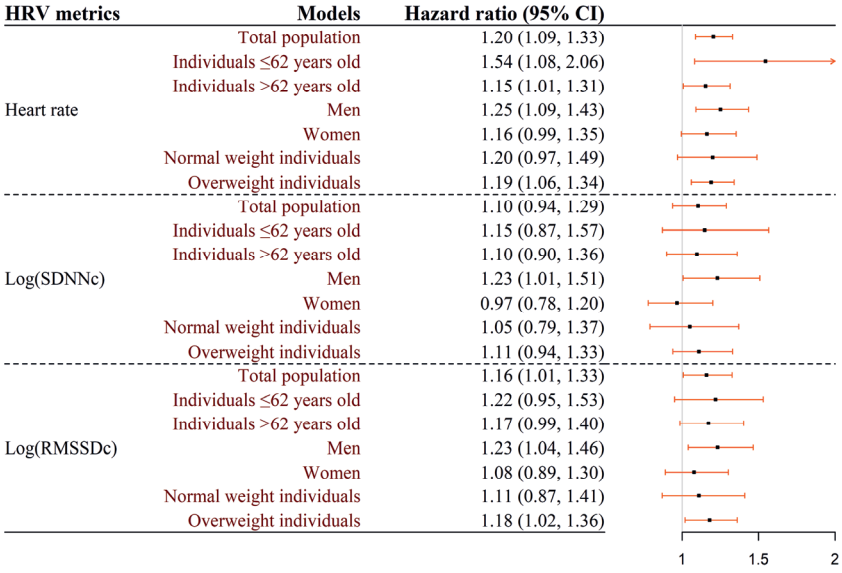

Figure 1. Forest plot summarizing the joint model results for the associations of heart rate and heart rate variability with incident type 2 diabetes.
Note: The hazard ratio for incident diabetes was calculated per 1-SD increase in heart rate or in the log of HRV indices (SDNNc and RMSSDc).

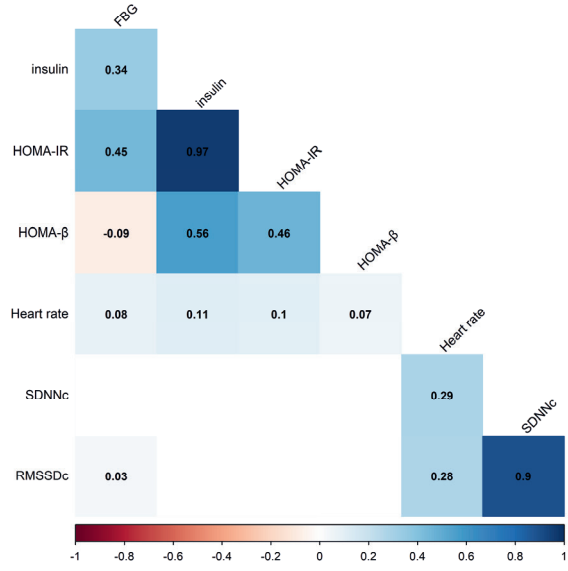

Figure 2. Correlation plot between heart rate and heart rate variability and glycemic traits.

DISCUSSION

In this large prospective population-based cohort study, longitudinal evolutions of both heart rate and different HRV metrics were significantly associated with new-onset T2D, independent of a vast number of other contributing factors. However, the effects were largely restricted to younger individuals. MR analyses suggested no causal association between HRV and incident T2D.

At first sight, our findings suggest an association between elevated heart rate and increased risk of developing T2D, consistent with previous studies [71, 72]. However, the effects were restricted to younger individuals. As a surrogate marker for autonomic activity, a high heart rate usually indicates increased sympathetic activity, potentially inducing insulin resistance. On the one hand, a more straightforward relationship between a fast heart rate and sympathetic predominance at a young age may explain the relatively strong association among the young participants [71, 72]. On the other hand, older participants tend to have worse health status and use more medication such as beta-blockers that reduce heart rate [73] and hyperglycemia [74]. Hence, the associations might be diluted at old age. Moreover, heart rate is an useful biomarker that is easily obtained with no need for specialized techniques. Therefore, awaiting further confirmation, it might have the potential to improve existing population-based diabetes risk scores.

Although diabetes is the leading cause of primary autonomic dysfunction, limited evidence exists regarding the relationship between autonomic dysfunction and incident diabetes [75]. Prior studies assessing the risk of developing T2D associated with HRV have mostly shown an association of autonomic dysfunction with incident T2D. However, the direction between various HRV metrics and glycemic traits is still inconsistent [19, 59, 60, 63]. For example, the Atherosclerosis Risk In Communities (ARIC) study found no significant association between SDNN and incident T2D [63], while the Kangbuk Samsung Health Cohort reported that as SDNN and RMSSD tertiles increased, the risk of diabetes decreased [60]. Our findings support an association between increased SDNNc and RMSSDc with incident T2D. Notably, these two heart rate-corrected HRV metrics have not been studied before concerning incident T2D, which limits the comparability with former studies. The participants in our study were also considerably older than Kangbuk Samsung Health study [60]. Age has an impact on both SDNNc and RMSSDc. The upper limit of their normal values decreases until the age of 60 and increases markedly afterward [64]. Besides the autonomic nervous system dysfunction, increased HRV may also be affected by the sinus node dysfunction [76]. With growing age, pathologic changes occur in the sinoatrial node, including increasing collagen and elastic fibers [77]. Intrinsic sinus node function tends to deteriorate with age, resulting in prolonged R-R intervals and increased, irregular HRV [78], which was also found in our study [79]. Therefore, the association between increased HRV and incident T2D could, at least partly, be explained by sinus node dysfunction.

Unlike results from our longitudinal analyses, only heart rate and not HRV metrics was associated with different glycemic traits at the baseline of our study. The HRV effect could be difficult to observe due to compensatory mechanisms preserving glucose homeostasis among a substantial number of non-diabetic participants. In line, we found that the effect of HRV disappeared after excluding persons with prediabetes. A prior study also reported that only heart rate, not HRV, is associated with changes in insulin sensitivity. This could imply that pathways other than autonomic dysfunction mediate the associations with diabetes or that heart rate is just a marker of other mechanisms [19]. These results regarding the correlations with glycemic traits should, however, be interpreted with caution since they were based on cross-sectional analyses and cannot address the temporal relationship of heart rate and HRV with glycemic

traits. More studies are needed to further delineate the underlying mechanisms.

However, inconsistent with the longitudinal findings, our bidirectional MR analysis showed no causal association between HRV and T2D. This may be due to limited power since only a few instrumental variables for SDNN and RMSSD were available to be used for the MR analyses. Furthermore, unlike longitudinal analysis using these novel heart rate-corrected HRV (SDNNc and RMSSDc), the MR analysis could only use heart rate-uncorrected HRV (SDNN and RMSSD) due to the lack of available SNPs, which may partly explain the heterogeneity we observed. Previous study reported substantial overlap of loci between HRV and heart rate, with SNPs in five of the 21 heart rate loci being associated with HRV at genome-wide significance level and six more attaining nominal significance [80]. This suggests that part of the HRV SNPs exert their effect on heart rate through oscillatory modulation of pacemaker activity by the vagal nerves. Therefore, the insignificant association between RMSSD and T2D in our MR analysis might be biased by heart rate. Future GWAS with a larger sample size and individual level data could identify more genetic variants that could be used to assess the association between the heart rate-corrected HRV and T2D.

The strengths of this population-based study include the prospective cohort design, long follow-up time, and meticulous assessment of incident T2D. We also had detailed information regarding possible confounders. Another strength is using joint models, which enables the analysis of individual heart rate and HRV values, including those with missing data. It generates the most likely continuous exposure profile for each individual while simultaneously accounting for exposure and survival processes. Also, we are the first study to report the health effect of heart rate-corrected HRV metrics, which are more appropriate to allow meaningful comparison of different HRV measurements and their association with adverse outcomes. However, our study mainly included older individuals of European ancestry, limiting our findings' generalizability to younger populations and other ethnicities. In addition, although the moderately nonlinear change of HRV was reported by former studies, we found no evidence of deviation from linearity, which might be due to the different outcomes we used. The additional MR analysis we used also assumes linearity. Given that the more novel MR approaches can check the potential nonlinear association between exposure and outcomes using individual-level data, future studies with more detailed data and using comprehensive methods are needed to validate our findings.

CONCLUSIONS

Our results suggest that high heart rate and HRV were significantly associated with an increased risk of developing T2D, especially among younger individuals. To our knowledge, this is the only prospective investigation using repeated measurements of heart rate and HRV to investigate the role of autonomic dysfunction in the development of T2D. More studies are needed to validate our findings and to elucidate further the underlying mechanisms.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://academic.oup.com/jcem/advance-article/doi/10.1210/clinem/dgad200/7110036?login=false

2.2

Chapter 2.2

Relative fat mass and type 2 diabetes

ABSTRACT

Background: Relative fat mass (RFM) is a novel sex-specific anthropometric equation (based on height and waist measurements) to estimate whole-body fat percentage. We aim to examine associations of RFM with incident type 2 diabetes (T2D), and to benchmark its performance against body-mass index (BMI), waist circumference (WC) and waist-to-hip ratio (WHR).

Methods: This prospective longitudinal study included data from three Dutch community-based cohorts free of baseline diabetes. First, we examined data from the PREVEND cohort (median age and follow-up duration: 48.0 and 12.5 years respectively) using Cox regression models. Validation was performed in the Lifelines (median age and follow-up duration: 45.5 and 3.8 years respectively) and Rotterdam (median age and follow-up duration: 68.0 and 13.9 years respectively) cohorts.

Results: Among 7961 PREVEND participants, 537 (6.6%) developed T2D. In a multivariable model, all adiposity indices were significantly associated with incident T2D (P_{all}<0.001). While 1 SD increase in BMI, WC and WHR were associated with 68%, 77% and 61% increased risk of developing T2D [Hazard ratio $(HR)_{BMI}$: 1.68 (95%CI: 1.57-1.80), HR_{WC}: 1.77 (95% CI: 1.63-1.92) and HR_{WHR}: 1.61 (95%CI: 1.48-1.75)], an equivalent increase in RFM was associated with 119% increased risk [HR: 2.19 (95%CI: 1.96-2.44)]. RFM was associated with incident T2D across all age groups, with the largest effect sizes in the youngest (<40 years) age category [HR: 2.90 (95%CI: 2.15-3.92)]. Results were broadly similar in Lifelines (n=93,870) and Rotterdam (n=5279) cohorts.

Conclusions: RFM is strongly associated with new-onset T2D and displays the potential to be used in the general practice setting to estimate the risk of future diabetes.

INTRODUCTION

The worldwide prevalence of obesity has increased dramatically during the last fifty years, and excess body weight is currently recognized as a major global public health challenge [81]. A recent report on trends in adult body-mass index (BMI) in 200 countries from 1975 to 2014 showed that average BMI in men increased the most in English-speaking countries and average BMI in women increased the most in central Latin America [81]. In the United States (US), age-adjusted prevalence of obesity in adults was around 42% in 2017-2018 [82], and it is estimated that over half of the adult US population will be obese within 2030 [83]. Besides direct socioeconomic consequences, rising obesity rates will also result in increased incidence of several chronic diseases, particularly type 2 diabetes (T2D) [84].

The most common method for obesity screening is BMI measurement [81], and obesity is defined as a BMI greater than or equal to 30 kg/m^2. However, BMI is a non-specific marker of body mass and does not discern between fat mass, muscle mass and bone mass [85, 86]. Given the closer association of visceral adiposity with the pathogenesis of insulin resistance and diabetes [87, 88], alternative screening tools such as waist circumference (WC) and waist-hip ratio (WHR), that better reflect abdominal fat distribution, have also been included in diabetes risk prediction models [89]. Nevertheless, aggregate data suggest that the overall performance of BMI, WC and WHR is comparable while estimating future diabetes risk in the community [90].

Over the last 10 years, more accurate anthropometric measures of adiposity such as relative fat mass (RFM) [22], body shape index [91], body roundness index [92] and weight-adjusted waist index [93] have been developed. We recently showed that among novel and established anthropometric measures of adiposity, the RFM, which is calculated from WC and height, was the strongest predictor of heart failure risk in the general population [94]. In the current study, we postulate that RFM would be a stronger predictor of new-onset T2D than currently used measures of adiposity. Accordingly, we assessed associations of RFM, BMI, WC and WHR with incident T2D in the PREVEND cohort, and compared the results with those from two other general population-based cohorts: the LifeLines study and the Rotterdam study.

METHODS

Study design and population

The PREVEND study is a prospective cohort study of 8592 community-dwelling adults living in the city of Groningen, the Netherlands, designed to investigate whether increased urinary albumin excretion (UAE) was associated with the risk of future cardiovascular and renal disease in the community (year: 1997-1998). The detailed study design has been described elsewhere [55, 95]. Briefly, all inhabitants from the city of Groningen, aged 28 to 75 years, were asked to respond to a short questionnaire and provide early-morning urine samples (N=85,421), and 40,856 individuals (47.8%) responded. Responders with UAE greater than or equal to 10 mg/L (n=7786) as well as a randomly selected control group with UAE less than 10 mg/L (n=3395) were invited to the outpatient clinic for a comprehensive health assessment. Insulin-treated individuals, pregnant women (self-reported), and unwilling subjects were excluded from the study. A final total of 6000 individuals with UAE greater than or equal to 10 mg/L and 2592 individuals with UAE less than 10 mg/L underwent further investigation and constituted the baseline PREVEND cohort (N=8592) [55, 95]. From this sample, 631 participants were excluded for the following reasons: i) prevalent diabetes (n=324), ii) unavailable data on baseline diabetes status (n=88), iii) missing anthropometric data (n=107) iv) BMI<18.5 (n=71), v) WC<40 cm (n=1), and vi) missing covariates (n=40), resulting in a final total of 7961 participants available for analysis.

LifeLines is a prospective cohort study of 167,729 community-dwelling adults living in northern Netherlands (2006-2013). The detailed study design has been described elsewhwhere [96, 97]. For the current study, we included 99,147 participants with available data at baseline and follow-up (ie, second) visit. From this sample, 5277 participants were excluded for the following reasons: i) prevalent diabetes (n=3065), ii) unavailable data on baseline diabetes status (n=115), iii) missing anthropometric data (n=30), iv) BMI<18.5 (n=679), and v) missing covariates (n=1388), resulting in a final total of 93,870 participants left for analysis.

The Rotterdam study is a prospective cohort study of community-dwelling adults aged 55 years and older in Rotterdam, the Netherlands. The detailed study design has been described elsewhere [54, 98]. Briefly, the baseline examination for the first cohort was completed between 1990 and 1993 (RS-I) with 10,215 participants aged 55 years or over; the response rate was 78%. The Rotterdam study was extended in 2000 to include all inhabitants who became 55 years of age or moved into the research area after the start of the study (RS-II). For the current study, we used the third visit of RS-I (1997-1998; n=4797) and first visit of RS-II (2000-2001; n=3011). Among 7808 participants recruited, 2529 were excluded for the following reasons: i) no informed consent to access medical records (n=82), ii) prevalent diabetes or unavailable data on baseline diabetes status (n=1733), iii) missing anthropometric data (n=568), iv) BMI <18.5 kg/m^2 or WC<40 cm (n=42), and v) missing covariates (n=104), resulting in a final total of 5279 participants.

Clinical assessment

All participants had detailed medical history, physical examination and fasting laboratory assessment at the baseline examination. Family history of diabetes was defined as self-reported diabetes among parents and siblings. Smoking behaviour was self-reported, and was classified as currently smoking, quit smoking (<1 year or ≥1 year) or never smoked. Smoking variable for the current study was defined as "currently smoking" or "smoking cessation within the previous year." Baseline body weight, height, WC and hip circumference (HC) were measured in a standing position. WC was measured midway between the lowest rib and the iliac crest at the end of expiration. HC was measured at the widest portion at the level of greater trochanters. RFM was calculated as 64–[20×Height (m)/WC (m)] in men and 76–[20×Height (m)/WC (m)] in women [i.e., 64–(20×Height/WC)+(12×sex), with sex=0 (men), and sex=1 (women)] [22]. BMI was calculated as the ratio between weight and height-squared, and expressed as kg/m^2. WHR was calculated as the the ratio between WC and HC. Blood pressure was taken as the average of 2 seated measurements. Hypertension was defined as systolic BP (SBP)≥140 mm Hg, diastolic BP (DBP)≥90 mm Hg or self-reported antihypertensive medication usage. Homeostatic Model Assessment for Insulin Resistance (HOMA-IR) was calculated as glucose (mmol/L)×insulin (mIU/L)]/22.5. We defined insulin resistance as HOMA-IR>2.9 based on a previous study [99]. Elevated CRP was defined as hs-CRP>2mg/L [100]. Details on relevant assays are provided in the Supplementary Material.

Ascertainment of incident type 2 diabetes

Incident T2D was considered present when participants without prevalent diabetes had any of the following during any of the follow-up visits: i) fasting plasma glucose≥7.0 mmol/L (126 mg/dL) [all three cohorts], ii) random plasma glucose≥11.1 mmol/L (200 mg/dL) [PREVEND and Rotterdam cohorts], iii) HbA1c ≥ 6.5% [LifeLines cohort], iv) self-reporting of a physician diagnosis [PREVEND and LifeLines cohorts] or v) information about glucose-lowering medication use obtained from questionnaires, home interviews or central pharmacy registry [PREVEND and Rotterdam cohorts]. In the Rotterdam Study, two study physicians independently adjudicated all potential events of T2D; in the case of disagreement, a consensus

was sought from an endocrinologist.

PREVEND participants were followed until incident T2D occurrence, death, or until 1 January 2011, whichever came first; participants were invited for physical follow-up visits roughly every four years. LifeLines participants were followed until incident T2D occurrence, death or the first physical follow-up visit (2014-2017), which was roughly after five years from the baseline visit; participants were additionally followed with two questionnaires between the baseline and the first follow-up visit. Rotterdam Study participants were followed until incident T2D occurrence, death, or until 1 January 2015, whichever came first; participants were invited for physical follow-up visits roughly every four years.

Statistical analyses

Continuous data are presented as medians, Q1-Q3 (50^{th} percentile, 25^{th}-75^{th} percentile) and categorical variables are represented as percentages. We first explored the association of adiposity indices i.e., RFM, BMI, WC and WHR with prevalent insulin resistance and other components of the metabolic syndrome using age and sex adjusted logistic regression models.

In primary analyses, we examined associations of continuous adiposity indices with incident T2D in the PREVEND cohort using Cox regression models adjusting initially for age and sex, and subsequently for smoking status, prevalent hypertension and family history of diabetes [95]. We calculated hazard ratios in the total population, and in women and men separately. We examined whether additional adjustment for continuous HOMA-IR score, hs-CRP or UAE in multivariable models materially affected the interpretation of our results [101]. Next, we examined the incremental discriminatory value of individual adiposity indices for T2D risk prediction beyond clinical covariates using C-statistic. We also quantified the extent to which adiposity indices improved model fit based on Akaike information criteria (AIC) [102, 103], and according to P-values based on Likelihood ratio (LHR) test. A $P_{LHR}<0.01$ was considered as strong evidence against the null hypothesis [104]. Additionally, we calculated sex-specific hazard ratios of developing T2D across quintiles of RFM, BMI, WC and WHR after multivariable adjustment.

In secondary analyses, we evaluated associations of continuous adiposity indices with incident T2D across pre-specified age categories (<40, 40-50, 50-60, 60-70 and ≥70 years). We also examined associations of adiposity indices with incident T2D after adjusting for BMI in the total population, and across BMI categories (<25 kg/m^2, 25-30 kg/m^2 and ≥30 kg/m^2). Finally, we compared the main results from the PREVEND study with that from two other general population-based cohorts: the LifeLines study and the Rotterdam study.

Results of the Cox regression models show mean hazard ratios with 95% confidence intervals (CI), and effect sizes are presented per one standard deviation (SD) increase in adiposity index; standardization was done separately for men and women. Multiple testing corrected P-value of 0.0125 (0.05/4) denoted statistical significance. All statistical analyses were performed using STATA version-14.

RESULTS

We included 7961 individuals from the PREVEND study cohort without prevalent diabetes, of which 3990 (50.1%) were women. PREVEND participant characteristics are summarized in **Table 1**. Participant characteristics according to insulin resistance at baseline are shown in **Table S1**. In age and sex adjusted logistic regression models, all adiposity indices were significantly associated with prevalent insulin resistance in the total population, and RFM displayed the largest effect sizes (**Table 2**). Specifically, 1 SD increase in BMI was associated with 218% increased odds of being insulin resistant (Odds ratio [OR]: 3.18; 95% CI: 2.97-3.42).

An equivalent increase in RFM was associated with 313% increased odds of being insulin resistant (OR: 4.13; 95% CI: 3.78-4.51). All adiposity indices were also significantly associated with components of metabolic syndrome and inflammation, and RFM displayed the largest effect sizes (**Table 2**).

Table 1. PREVEND participant characteristics

	Women (n = 3990)	Men (n = 3971)
Clinical characteristics		
Age, years	46.9 (38.1, 57.0)	49.2 (39.9, 61.7)
White individuals, n (%)	3772 (95.7)	3799 (95.9)
Smoking, n (%)	1504 (37.7)	1508 (38.0)
Hypertension, n (%)	1071 (26.8)	1534 (38.6)
SBP, mm Hg	119 (109, 135)	131 (120, 143)
DBP, mm Hg	70 (65, 77)	76 (70, 83)
Diabetes (family history), n (%)	629 (15.8)	568 (14.3)
HOMA-IR > 2.9, n (%)	624 (16.0)	915 (23.5)
HOMA-IR (continuous)	1.5 (1.0, 2.3)	1.8 (1.2, 2.8)
Glucose, mmol/L	4.6 (4.2, 4.9)	4.8 (4.4, 5.2)
Insulin, mU/L	7.6 (5.4, 10.9)	8.4 (5.7, 12.6)
Total cholesterol, mmol/L	5.5 (4.8, 6.3)	5.6 (4.9, 6.3)
HDL-cholesterol, mmol/L	1.5 (1.2, 1.7)	1.1 (0.9, 1.4)
Triglycerides, mmol/L	1.1 (0.8, 1.5)	1.3 (0.9, 1.9)
CRP > 2 mg/L, n (%)	1438 (37.6)	1245 (33.1)
CRP, mg/L	1.3 (0.6, 3.1)	1.2 (0.5, 2.6)
UAE, mg/24h	8.4 (5.8, 14.0)	10.3 (6.8, 20.7)
Anthropometric measures		
RFM	34.8 (30.2, 39.4)	25.5 (22.1, 28.5)
BMI, kg/m^2	25.1 (22.5, 28.2)	25.9 (23.8, 28.3)
WC, cm	81.0 (74.0, 90.0)	93.0 (86.0, 100.5)
WHR	0.81 (0.77, 0.87)	0.94 (0.89, 0.98)
Incident outcome		
Type 2 diabetes, n (%)	202 (5.1)	320 (8.1)

Continuous variables are presented as median (interquartile range) and categorical variables as n (percentages).

During a median follow-up of 12.5 (11.7-12.9) years, 522 individuals (6.6%) developed T2D, of which 202 (38.7%) were women. The incidence rate of T2D was 4.5 per 1000 person-years in women and 7.4 per 1000 person-years in men. Participant characteristics according to incident T2D are shown in **Table S2**. In multivariable Cox regression models, all adiposity indices were significantly associated with outcome (P<0.001) (**Table 3**). While one SD increase in BMI, WC and WHR were associated with 68%, 77% and 61% increased risk of developing T2D in the total population, an equivalent change in RFM was associated with 119% increased risk of developing T2D (HR: 2.19, 95%CI: 1.96-2.44). We observed a statistically significant (sex×covariate) interaction in the direction of women for RFM, BMI and WC (P-value for interaction 0.001, 0.029 and 0.008 respectively), and additionally presented sex-specific coefficients (**Table 3**).

Additional adjustment for HOMA-IR reduced effect sizes in general but did not affect the interpretation of results. Adjustment for hs-CRP and UAE did not materially change the results (**Table S3**).

Table 2. Associations of standardized adiposity indices with prevalent insulin resistance, components of the metabolic syndrome and inflammation

	Prevalent insulin resistance		Prevalent hypertension		Low HDL-C		High triglycerides		High CRP levels	
	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI)	P-value
RFM	4.13 (3.78, 4.51)	<0.001	1.91 (1.79, 2.04)	<0.001	2.05 (1.93, 2.18)	<0.001	2.36 (2.20, 2.54)	<0.001	2.02 (1.90, 2.15)	<0.001
BMI	3.18 (2.97, 3.42)	<0.001	1.79 (1.69, 1.90)	<0.001	1.73 (1.65, 1.82)	<0.001	1.86 (1.76, 1.97)	<0.001	1.79 (1.69, 1.89)	<0.001
WC	3.38 (3.14, 3.64)	<0.001	1.77 (1.67, 1.88)	<0.001	1.85 (1.76, 1.96)	<0.001	1.96 (1.85, 2.08)	<0.001	1.87 (1.77, 1.98)	<0.001
WHR	2.25 (2.11, 2.41)	<0.001	1.54 (1.45, 1.63)	<0.001	1.72 (1.63, 1.81)	<0.001	1.90 (1.79, 2.02)	<0.001	1.61 (1.52, 1.70)	<0.001

Definitions: insulin resistance if homeostatic model assessment for insulin resistance (HOMA-IR) score > 2.9; hypertension if systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg or self-reported antihypertensive medication usage; low HDL-C if < 1.03 mmol/L in men and < 1.29 mmol/L in women; high triglycerides if > 1.7 mmol/L; high CRP levels if > 2 mg/L. Models were adjusted for age and sex.

Table 4. Associations of standardized adiposity indices with incident type 2 diabetes across age categories in the total population

	Age < 40 years		Age: 40-50 years		Age: 50-60 years		Age: 60-70 years		Age ≥ 70 years	
	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value	HR (95% CI)	P-value
RFM	2.90 (2.15, 3.92)	<0.001	2.27 (1.83, 2.80)	<0.001	1.97 (1.64, 2.37)	<0.001	2.00 (1.57, 2.54)	<0.001	1.65 (1.03, 2.65)	0.038
BMI	1.93 (1.63, 2.29)	<0.001	1.75 (1.52, 2.01)	<0.001	1.61 (1.42, 1.82)	<0.001	1.49 (1.27, 1.75)	<0.001	1.54 (1.13, 2.13	0.007
WC	2.23 (1.77, 2.80)	<0.001	1.86 (1.60, 2.17)	<0.001	1.64 (1.43, 1.89)	<0.001	1.61 (1.34, 1.92)	<0.001	1.48 (1.06, 2.08)	0.022
WHR	2.01 (1.56, 2.60)	<0.001	1.79 (1.49, 2.16)	<0.001	1.42 (1.23, 1.64)	<0.001	1.58 (1.32, 1.88)	<0.001	1.30 (0.93, 1.81)	0.120

Multivariable models were adjusted for age, sex, smoking, prevalent hypertension and family history of diabetes

Table 3. Associations of standardized adiposity indices with incident type 2 diabetes

	Age-sex adjusted		Multivariable adjusted	
	HR (95% CI)	P-value	HR (95% CI)	P-value
TOTAL				
RFM	2.38 (2.14, 2.64)	<0.001	2.19 (1.96, 2.44)	<0.001
BMI	1.77 (1.66, 1.89)	<0.001	1.68 (1.57, 1.80)	<0.001
WC	1.89 (1.75, 2.04)	<0.001	1.77 (1.63, 1.92)	<.0.001
WHR	1.71 (1.58, 1.86)	<0.001	1.61 (1.48, 1.75)	<0.001
WOMEN				
RFM	2.84 (2.41, 3.35)	<0.001	2.65 (2.23, 3.14)	<0.001
BMI	1.88 (1.71, 2.06)	<0.001	1.81 (1.63, 2.00)	<0.001
WC	2.06 (1.84, 2.30)	<0.001	1.95 (1.73, 2.19)	<0.001
WHR	1.70 (1.51, 1.91)	<0.001	1.63 (1.45, 1.85)	<0.001
MEN				
RFM	2.11 (1.84, 2.41)	<0.001	1.92 (1.67, 2.21)	<0.001
BMI	1.69 (1.54, 1.85)	<0.001	1.58 (1.44, 1.74)	<0.001
WC	1.76 (1.59, 1.96)	<0.001	1.63 (1.47, 1.82)	<0.001
WHR	1.74 (1.55, 1.96)	<0.001	1.61 (1.42, 1.81)	<0.001

Multivariable models were adjusted for age, sex, smoking, prevalent hypertension and family history of diabetes.

All measures of adiposity modestly improved model discrimination when added to the multivariable risk prediction model i.e. age, sex, smoking status, prevalent hypertension, and family history of diabetes (**Table S4**). The greatest improvement was observed after adding RFM and BMI (ΔC-statistic: 0.064 and 0.061 respectively). All measures of adiposity also strongly improved model fit: again, the greatest improvement was observed after adding RFM and BMI (ΔAIC -206 and -176 respectively) (**Table S4**). When we also included HOMA-IR, hs-CRP and UAE in the multivariable model, trends were generally similar although improvement in discrimination was nominal (**Table S5**).

Additional adjustment for HOMA-IR reduced effect sizes in general but did not affect the interpretation of results. Adjustment for hs-CRP and UAE did not materially change the results (**Table S3**).

All measures of adiposity modestly improved model discrimination when added to the multivariable risk prediction model i.e. age, sex, smoking status, prevalent hypertension, and family history of diabetes (**Table S4**). The greatest improvement was observed after adding RFM and BMI (ΔC-statistic: 0.064 and 0.061 respectively). All measures of adiposity also strongly improved model fit: again, the greatest improvement was observed after adding RFM and BMI (ΔAIC -206 and -176 respectively) (**Table S4**). When we also included HOMA-IR, hs-CRP and UAE in the multivariable model, trends were generally similar although improvement in discrimination was nominal (**Table S5**).

When multivariable models were adjusted for BMI, associations of RFM, WC and WHR with incident T2D were partially attenuated, but remained statistically significant. When directly compared, the effect size of RFM was significantly larger than BMI ($P_{difference}$=0.009), which was not the case for WC or WHR (**Table S6**). Across BMI categories, RFM was strongly associated with incident T2D in lean, overweight, and obese categories (**Table S7**).

We also examined the risk of incident T2D across sex-specific quintiles of adiposity indices (**Table S8**). Compared to men in the first quintile of RFM, men in the fifth quintile had 838% increased risk of developing T2D (HR: 9.38, 95% CI: 4.94-17.82). Compared to women

in the first quintile of RFM, women in the fifth quintile had a 2128% increased risk of developing T2D (HR: 22.28, 95% CI: 8.05-61.66).

Next, we summarized participant characteristics according to pre-specified age categories (**Tables S9** and **S10**), and examined associations of adiposity indices with incident T2D in each age category. Again, RFM displayed the strongest associations across all age categories (**Table 4**), with the largest effect sizes in the youngest (<40 years old) age category (HR: 2.90, 95% CI: 2.15-3.92).

Finally, we compared the main results from the PREVEND cohort with the results from two other Dutch general population cohorts. Participant characteristics of Lifelines and Rotterdam Study cohorts are provided in **Table S11**. The median duration of follow-up in the Lifelines cohort was 3.8 (3.2-4.6) years; the incidence rate of T2D was 4.2 events per 1000 person-years in women and 6.5 events per 1000 person-years in men. The median duration of follow-up in the Rotterdam cohort was 13.9 (8.6-15.4) years; the incidence rate of T2D was 11.7 events per 1000 person-years in women and 12.8 events per 1000 person-years in men. While RFM displayed the largest effect sizes amongst all indices of adiposity in the Lifelines cohort (HR: 2.49, 95% CI: 2.30-2.56) (**Table 5**), both RFM and BMI displayed strong associations with incident T2D in the Rotterdam cohort (HR: 1.44, 95% CI: 1.34-1.56 and HR: 1.38, 95% CI: 1.29-1.47, respectively) (**Table 5**). No significant effect modification by sex was observed in both cohorts i.e., the adiposity index×sex term was not significant with a P-value for interaction>0.1. Similar to the results from the PREVEND cohort, effect sizes for all adiposity indices were generally largest in the younger age categories in both LifeLines and Rotterdam Study cohorts (**Table S12**).

Table 5. Associations of standardized adiposity indices with incident type 2 diabetes in Lifelines and Rotterdam cohorts

	LIFELINES COHORT (n = 93870)		ROTTERDAM COHORT (n = 5279)	
	HR (95% CI)	P-value	HR (95% CI)	P-value
TOTAL				
RFM	2.49 (2.30-2.56)	<0.001	1.44 (1.34, 1.56)	<0.001
BMI	1.71 (1.67-1.76)	<0.001	1.38 (1.29, 1.47)	<0.001
WC	1.94 (1.86-2.01)	<0.001	1.32 (1.24, 1.39)	<0.001
WHR	1.65 (1.58-1.71)	<0.001	1.18 (1.12, 1.25)	<0.001
WOMEN				
RFM	2.51 (2.33-2.71)	<0.001	1.36 (1.22, 1.52)	<0.001
BMI	1.75 (1.68-1.83)	<0.001	1.32 (1.19, 1.47)	<0.001
WC	1.97 (1.86-2.07)	<0.001	1.24 (1.14, 1.36)	<0.001
WHR	1.63 (1.54-1.73)	<0.001	1.17 (1.08, 1.26)	<0.001
MEN				
RFM	2.36 (2.19-2.54)	<0.001	1.52 (1.37, 1.69)	<0.001
BMI	1.70 (1.63-1.76)	<0.001	1.42 (1.30, 1.54)	<0.001
WC	1.91 (1.81-2.01)	<0.001	1.42 (1.30, 1.56)	<0.001
WHR	1.67 (1.58-1.77)	<0.001	1.21 (1.11, 1.32)	<0.001

Multivariable models were adjusted for age, sex, smoking, prevalent hypertension and family history of diabetes in the Lifelines cohort and for age, sex, smoking and prevalent hypertension in the Rotterdam cohort.

DISCUSSION

In the current study enrolling individuals from the Dutch general population, we examined associations of RFM, BMI, WC and WHR with incident T2D. We found that RFM was more strongly associated with incident T2D than commonly used measures of obesity. These associations were present across all age categories, and they were most pronounced in younger individuals.

BMI, initially called the Quetelet index, was developed approximately 200 years ago by a Belgian mathematician to characterize the "average" man [105, 106]. Currently, BMI is the most commonly used marker of obesity – not just on a population level but also on an individual level. However, BMI does not distinguish between fat mass and fat-free mass, and between subcutaneous and visceral fat deposition [85, 86]. These limitations and the understanding that visceral adipose tissue is more closely related to the pathogenesis of diabetes, resulted in the inclusion of WC or WHR – as an alternative to BMI in several diabetes risk prediction algorithms [89]. Nevertheless, aggregate data from meta-analyses show that associations of WC and WHR with incident diabetes are not substantially stronger than that of BMI [90].

RFM is a newly developed anthropometric index that more accurately estimates whole-body fat percentage compared to traditional equations such as BMI and WHR [22]. The RFM algorithm is easy to calculate, is derived from WC and height, and is sex-specific. In a large multi-ethnic cohort from the US including Mexican-Americans, European-Americans, and African-Americans, RFM displayed stronger correlations with dual-energy X-ray absorptiometry (DEXA)-obtained fat mass than BMI [22]. These results were also reproduced in a smaller external validation study enrolling 61 individuals from the Mexican population [107].

Previously, we examined associations of adiposity with new-onset heart failure in the PREVEND cohort and found that among multiple anthropometric indices of adiposity, RFM was the strongest predictor of heart failure risk [94]. Now, we report that association of RFM with new-onset T2D was also stronger than that of BMI, WC and WHR in the PREVEND cohort. Findings were similar in the more contemporary and substantially larger LifeLines cohort enrolling participants from the northern provinces of the Netherlands. In the Rotterdam Study cohort both RFM and BMI were strongly associated with incident T2D (**Table 5**).

Additionally, in the PREVEND cohort, we observed some sex-related differences in associations of RFM, BMI and WC with incident T2D on a relative scale i.e., women had higher hazard ratios than men. While effect sizes were also numerically larger in women in the LifeLines cohort (particularly for RFM), opposite trends were observed in the Rotterdam Study cohort i.e., larger effect sizes in men. This could, at least in part, be explained by the differences in the age range of the Rotterdam Study compared with the other two cohorts: the Rotterdam study enrolled older individuals, where the absolute risk of developing T2D was comparable among women and men.

Previous studies have demonstrated that lifestyle changes are effective in preventing both diabetes and obesity in high-risk individuals [108]. In the PREVEND cohort, we found that all measures of adiposity strongly related with the risk of developing T2D across all age categories, and these associations were strongest in participants younger than 40 years (**Table 5**). Similar trends were found across age categories in the LifeLines and Rotterdam Study cohorts (Table S12), and have also been observed in associations of risk factors with incident HF [109]. Although an inflated relative risk in younger participants may be attributed to their lower baseline risk of disease [110], our results highlight the need for adequate obesity control to prevent T2D development - not just in middle-aged and older individuals but also in younger

individuals with a relatively low risk factor burden.

We report for the first time, the association between RFM and incident T2D in the general population. The long term follow-up of participants and a 1:1 sex ratio further strengthen our analyses. As the PREVEND study, by design, included a higher proportion of individuals with UAE>10 mg/L, we also validated these results using data from two other general population-based cohorts. A more general limitation includes the unavailability of HbA1c measurements in the PREVEND and Rotterdam Study cohorts, and the unavailability of data on prescribed medication in the LifeLines cohort. Finally, although the current study included participants from three large cohorts, participants were almost exclusively Dutch and predominantly White, warranting validation of our findings in cohorts from other geographical locations and ethnicities.

CONCLUSIONS

RFM strongly predicts new-onset T2D in the Dutch population and displays the potential to be routinely used in the general practice setting to estimate future risk of diabetes. Our findings also highlight that adequate obesity control, particularly in young individuals, would substantially reduce the risk of developing T2D in the community.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://www.ejinme.com/article/S0953-6205(22)00453-8/fulltext

2.3

Chapter 2.3

Arterial stiffness/remodeling and type 2 diabetes

ABSTRACT

Background: We studied if large artery stiffness is involved in type 2 diabetes pathogenesis. We also investigated the effect of genetic risk for type 2 diabetes in these associations and the causality.

Methods: In the prospective population-based Rotterdam Study (n=3,055; mean age, 67.2 years), markers of aortic and carotid stiffnesses and measures of arterial remodeling were assessed. Cox proportional hazard regression analysis estimated the associations between arterial stiffness measures with incident type 2 diabetes. We used 403 single nucleotide polymorphisms to calculate the genetic risk score (GRS) for type 2 diabetes. We adopted Mendelian randomization (MR) analysis to evaluate the causal associations.

Results: Over a median follow-up of 14.0 years, higher carotid-femoral pulse wave velocity (hazard ratio,1.18; 95%CI: 1.04-1.35), carotid distensibility coefficient (1.17; 1.04-1.32), and carotid intima-media thickness (1.15; 1.01-1.32) were independently associated with incident diabetes. The associations were stronger among individuals with a higher GRS for type 2 diabetes. MR analysis did not support the causality of the observed associations.

Conclusions: Elevated arterial stiffness is independently associated with incident type 2 diabetes. For most arterial stiffness markers, the associations with incident type 2 diabetes were more robust in individuals with a higher GRS for diabetes.

INTRODUCTION

Type 2 diabetes mellitus has become one of the major challenges to human health in the 21st century. The number of individuals with diabetes is projected to rise from 415 million in 2015 to 700 million by 2045 [111]. Arterial stiffness is a subclinical measurement of cardiovascular diseases (CVD) and an independent predictor of vascular dysfunction that leads to altered central hemodynamics [112, 113]. A sustained increase in blood pressure due to increased arterial stiffness may induce structural changes in the arteries, known as arterial remodeling, leading to atherosclerotic plaques [114, 115].

Although evidence suggests that arterial stiffness increases in patients with type 2 diabetes and is closely associated with type 2 diabetes complications [116], knowledge regarding arterial stiffness before developing type 2 diabetes is limited [117-120]. Recent evidence suggests that increased arterial stiffness could be evident before the onset of type 2 diabetes and among individuals in a prediabetes state [23]. Findings in this regard, however, remain inconclusive. Notably, abnormal glucose metabolism is the key factor driving increased arterial stiffness stepwise from normal to prediabetes to type 2 diabetes [121]. Increased pulse pressure has been shown to independently identify subjects at risk for developing type 2 diabetes in a study that included 2,685 Japanese hypertensive patients [122]. However, it remains unclear whether large artery stiffness and its associated hemodynamic changes are involved in the pathogenesis of type 2 diabetes.

Type 2 diabetes is a multifactorial disease resulting from multiple genetic and environmental risk factors. A recent study included 152,611 participants in the UK Biobank and showed that the association between arterial stiffness index (ASI) and type 2 diabetes was partially modified by genetic susceptibility to type 2 diabetes [117]. However, this study was limited by using a few arterial stiffness measurements, i.e., ASI as a proxy of pulse wave velocity (PWV)/arterial stiffness.

Using data from the large population-based Rotterdam Study, we examined the association of markers of arterial stiffness and remodeling with new-onset type 2 diabetes. We also studied whether associations were modified by age, sex, blood glucose levels, or mean arterial pressure (MAP). As the association between vascular dysfunction and incident type 2 diabetes might be driven by changes in distinct metabolic parameters, insulin resistance, and β-cell function, we tested the associations only among the population with prediabetes. Our study investigated whether the associations might be modified by type 2 diabetes genetic susceptibility. Complementary to our genetic approach, we studied the associations between genetic variants for arterial stiffness and risk of type 2 diabetes by performing i) a Mendelian randomization (MR) analysis, using summary statistics from large-scale genome-wide association studies and ii) a weighted genetic risk score (GRS) analysis.

METHODS

Study design and population

This study is embedded within the Rotterdam Study (RS), a prospective cohort study of the community-dwelling population aged 55 years and older in Rotterdam, the Netherlands. Briefly, in 1990 all inhabitants (n=10,215) aged 55 years or over were invited; 7,983 invitees agreed to participate (RS-I). In 2000, 3,011 participants who had reached the age of 55 years (out of 4,472 invitees) were invited to participate in the second cohort (RS-II). There were no eligibility criteria to enter the Rotterdam Study apart from the minimum age and residential area based on postal codes. The complete design and rationale behind the Rotterdam Study have been described previously [54].

We included 3,055 participants with available data for carotid assessment and type 2 diabetes from the third examination of the first cohort (RS-I-3: 1997-1999) and the first examination of the second cohort (RS-II-1: 2000-2001). We included participants with information on prevalent and incident type 2 diabetes status with at least one baseline interview or clinical examination. We excluded those who did not provide or withdrew informed consent for the collection of follow-up data (n=313), participants with a history of type 2 diabetes (n=501) or insufficient baseline screening for type 2 diabetes /non-fasting glucose (n=1,232), and participants with a history of cardiovascular disease (n=492). **Figure 1** shows the flowchart of the study population.

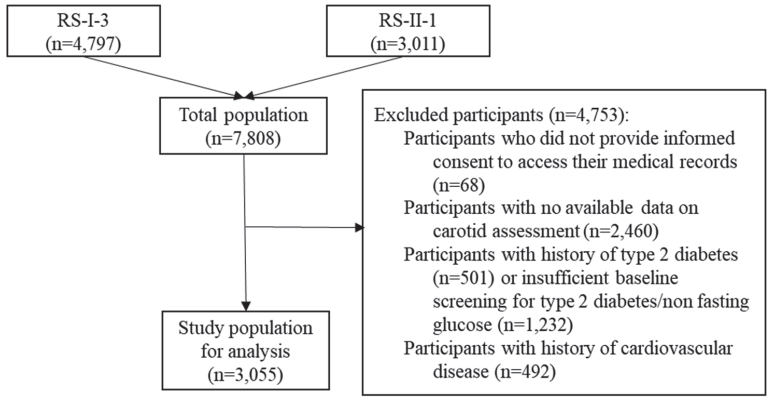

Figure 1. Flow-chart of the study population.

Baseline measurements

At baseline, information was obtained on individuals' characteristics, health status, medical and medication history, and lifestyle factors.

Measures of Arterial Stiffness and Arterial Remodeling

Functional arterial stiffness and remodeling measures were measured with subjects in the supine position [123]. Carotid-femoral pulse wave velocity (cf_PWV) is a non-invasive gold standard of arterial stiffness. Cf_PWV was assessed with an automatic device (Complior, Colson) by measuring the time delay between the rapid upstroke of the feet of simultaneously recorded pulse waves in the carotid and the femoral arteries [124]. PWV index was calculated as the ratio between the distance and the foot-to-foot time delay expressed in meters per second. Carotid distensibility coefficient (carDC) as a measure of carotid artery elasticity and was assessed with the subject's head tilted slightly to the contralateral side. The vessel wall motion of the right common carotid artery was measured through a duplex scanner (ATL Ultramark IV, operating frequency 7.5 MHz) connected to a vessel wall movement detector system. CarDC was calculated according to the following equation: $(2\Delta D \times D + \Delta D^2)/(\text{pulse pressure (PP)} \times D^2)$, 10^{-3}/kPa, where D is arterial diameter, ΔD is distension or the absolute stroke change in diameter during systole, and PP is brachial PP (calculated as systolic minus diastolic blood pressure). Lower carotid distensibility represents greater carotid stiffness. cf_PWV and carDC, are pressure-dependent and require blood pressure adjustment. Arterial remodeling refers to the structural and functional changes of the vessel wall and reflects an adaptation of the vessel wall to biochemical or biomechanical causes. Carotid intima-media thickness (cIMT) measures carotid atherosclerotic vascular disease that shows the thickness of the inner two layers of the carotid artery—the intima and media. cIMT was calculated as the average of left

and right common carotid IMT [125]. Carotid artery lumen diameter (carDi) was calculated as D−(2×cIMT), mm. Mean (CWS_{mean}) circumferential wall stress was calculated as mean arterial pressure×([lumen diameter/2]/IMT), kPa [126]. Pulsatile (CWS_{puls}) circumferential wall stress calculated as PP×(lumen diameter/2/cIMT), kPa.

Follow-up measurements and type 2 diabetes assessment

Follow-up data on vital status and incident type 2 diabetes for all individuals included in the study were available. Outpatient clinic reports and hospital discharge letters were collected from general practitioners and hospital records. Information on vital status was obtained from the central registry of the municipality of the city of Rotterdam.

Incident type 2 diabetes was defined based on the World Health Organization (WHO) guideline as a fasting blood glucose concentration of 7.0 mmol/L or higher, a non-fasting blood glucose concentration of 11.1 mmol/L or higher (when fasting samples were unavailable), or the use of blood glucose-lowering medications [127]. Type 2 diabetes cases were ascertained at baseline and follow-up using general practitioners' records, hospital discharge letters, medication data, and serum glucose measurements collected from center visits every 3-5 years. Blood glucose-lowering medications were obtained from structured home interviews and pharmacy dispensing records (95%). Two physicians independently adjudicated all potential events of type 2 diabetes. In the case of disagreement, a consensus was achieved by a diabetologist. Follow-up started at baseline, and individuals were followed until the incident type 2 diabetes or death or the end of follow-up, January 1st , 2015.

Genotyping

Genotyping in Rotterdam Study has been performed using the Illumina 550K and 610K quad array (Illumina Inc., San Diego, CA, USA) and was imputed to the Haplotype Reference Consortium reference panel (version 1.0) with Minimac 3.

Covariables

Body mass index (BMI) was calculated as body weight (in kg) divided by the square of length (in meters). Mean arterial pressure (MAP) was defined as 1/3 systolic blood pressure plus 2/3 diastolic blood pressure. All biochemical variables were assessed in serum samples taken after overnight fasting. Serum total cholesterol (TC) (mmol/L) and high-density lipoprotein-cholesterol (HDL-c) (mmol/L) were both measured on the COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH, Germany). Non-HDL cholesterol was defined as total cholesterol minus HDL cholesterol. Smoking behavior was assessed using a computerized questionnaire and categorized into three groups: current, former (former smoker, or stopped cigarettes ≤ 12 months), and never (never smoker, or stopped cigarettes > 12 months).

Statistical analysis

Data were assessed visually for normality. We performed descriptive statistics by reporting mean (standard deviation [SD]) or median (interquartile range [IQR]) for continuous variables and numbers (percentage) for categorical variables. We first investigated the multicollinearity between different arterial stiffness/remodeling markers by calculating the variance inflation factor (VIF). The association between markers of arterial stiffness and remodeling at baseline with incident type 2 diabetes was evaluated using Cox proportional hazard regression models. The associations were adjusted for age, sex, and cohort (Model 1) and additionally adjusted for BMI, MAP, antihypertensive medications, heart rate, non-HDL-cholesterol [128], lipid-lowering medications, and smoking (Model 2). To test the proportional hazards assumption, the Schoenfeld residuals method was applied. We modeled all arterial stiffness and remodeling

measurements on a continuous scale (per SD). To detect possible non-linear associations between arterial stiffness and remodeling measurements with incident type 2 diabetes, we performed a non-linear spline analysis. We applied P-Splines (penalized cubic B-Splines) in the Cox models [129]. Many central knots were taken, followed by a penalty term optimized via generalized cross-validation to avoid over-fitting. This is a data-driven and explorative approach to detecting any non-linear relationship. We also included interaction terms in model 2 to study whether any significant associations were modified by age, sex, or MAP [130]. In a linear regression analysis, we also examined the associations between arterial stiffness/remodeling markers at baseline with follow-up measurements of fasting blood glucose.

In a series of sensitivity analyses, to further study the role of glycemic traits, we evaluated the associations between measurements of arterial stiffness and remodeling at baseline with incident type 2 diabetes by i) adding baseline fasting glucose level to model 2, ii) adding baseline fasting insulin level and homeostatic model assessment for insulin resistance (HOMA-IR) to model 2, iii) excluding individuals with prediabetes at baseline, and iv) testing the associations between various markers of arterial stiffness and remodeling with incident type 2 diabetes among the population with prediabetes at baseline. To account for reverse causality bias, we excluded incident type 2 diabetes cases (n=109) in the first 5 years of follow-up [131].

We also examined cross-sectional associations between arterial stiffness and remodeling with fasting serum glucose, HOMA-IR (a proxy of insulin resistance), and HOMA-β-cell function (methods and the corresponding results and discussion are shown in supplementary materials).

All measures of association are presented with 95% confidence intervals. We used $P<0.05$ as the significance level. Missing values on covariates were imputed using single imputation, the expectation-maximization method. All analyses were conducted in SPSS version 26 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) and R statistical software version 3.6.3.

Genetic studies

Association of type 2 diabetes genetic variants and arterial stiffness: In a set of genetic analyses, we further evaluated whether genetic predisposition to type 2 diabetes modifies the associations between arterial stiffness/remodeling and the risk of type 2 diabetes. We explored this effect modification through stratification by GRS tertiles. For type 2 diabetes GRS analysis, we included 403 independent type 2 diabetes genetic variants in 243 loci reported by a recent genome-wide association study (GWAS) in European ancestry; a meta-analysis of 32 GWAS included almost 900,000 individuals in the DIAMANTE Consortium [132]. We calculated the weighted GRS as b1 × SNP1 + b2 × SNP2 + .. + bn x SNPn, where b is the beta coefficient for each SNP, and n is the number of risk alleles (0, 1, 2). We rescaled the weighted score using the following equation to reflect the number of type 2 diabetes risk alleles: weighted GRS = weighted score × (total number of SNPs/sum of the b coefficients). In our analysis, and among 2,647 individuals with available genetic data, the GRS for type 2 diabetes ranged from 24.24 to 29.41, with higher GRS indicating a higher genetic risk of type 2 diabetes. We divided individuals into three groups, including low (24.24–26.43), intermediate (26.44–27.03), and high (27.04–29.41) GRS according to the GRS tertile.

Association of arterial stiffness genetic variants and type 2 diabetes:

Mendelian Randomization (MR): We first performed an MR analysis using publicly available summary-level data [133] from a GWAS for ASI in 127,121 UK Biobank participants of European ancestry that identified three genome-wide significant loci. This study showed three

SNPs associated with ASI (**Table S1**). The calculated estimates were expressed as odds ratios (ORs) on type 2 diabetes per unit difference in an ASI. Inverse-variance weighted (IVW) regression was used, which assumes no invalid genetic instruments, such as pleiotropic (affecting multiple exposures) SNPs[134]. When the intercept of this regression deviates from zero, this indicates a bias in the IVW estimates. MR Egger regression was further used to ensure that the IVW estimates were not biased by directional pleiotropy [134].

Weighted genetic risk score for arterial stiffness index: We calculated the weighted GRS based on the three SNPs for ASI from the UK Biobank study [133], as described earlier. We studied the associations of GRS for ASI (continuous variable) and fasting glucose, insulin and HOMA-IR at baseline (linear regression analyses) and incident type 2 diabetes (Cox regression analysis).

All analyses were performed using the R-based package" TwoSampleMR" (https://mrcieu.github.io/TwoSampleMR/).

RESULTS

We used data from 3,055 participants of the Rotterdam Study. The baseline characteristics of the total population are shown in **Table 1**. The population's mean age was 67.2 years (SD 7.9), and 1,816 (59.4%) participants were women. During a median follow-up of 14.0 (IQR 10.1-14.9) years, 395 (12.9) type 2 diabetes cases were identified (incidence rate: 10.5 per 1000 person-years).

Table 1. Baseline Characteristics of the study population

	Total (n=3,055)
Age, years	67.2 (7.9)
Sex, Female, n (%)	1,816 (59.4%)
BMI, kg/m^2	26.6 (3.8)
MAP	98.5 (12.6)
Antihypertensive medication, n (%)	835 (27.3%)
Heart rate bpm	70.4 (10.8)
Total cholesterol, mmol/L	5.9 (0.96)
HDL-cholesterol, mmol/L	1.4 (0.38)
Non-HDL-cholesterol, mmol/L	4.5 (0.98)
Lipid-lowering medication, n (%)	311 (10.2%)
Smoking (ever), n (%)	2,023 (66.2%)
[q1]	7.1 (0.86-15.1)
Physical activity, median, MET hour	88.3 (43.6)
Prevalent prediabetes, n (%)	513 (16.8%)
Fasting glucose levels, mmol/L	5.5 (0.54)
Fasting insulin levels, mmol/L	66.0 (47.0-92.0)
HOMA-IR	2.3 (1.6-3.3)
HOMA-B	95.6 (69.1-130.9)
cf_PWV, m/s	12.6 (2.8)
carDC, 10–3/kPa	12.2 (4.8)
carDi, mm	7.6 (0.93)
cIMT, mm	0.82 (0.14)
CWS_{mean}, kPa	44.6 (10.1)
CWS_{puls}, kPa	29.5 (8.2)

Values are mean (standard deviation) or median (interquartile range) for continuous variables and number (percentages) for categorical variables.

Figure 2A shows the association between arterial stiffness and remodeling measurements with incident type 2 diabetes. Increased (per SD) arterial stiffness and remodeling were associated with an incident type 2 diabetes after additional adjustment in model 2; hazard ratios (HR) and 95% confidence intervals (CI) were 1.18 (1.04-1.35) for cf_PWV, 1.17 (1.04-1.32) for carDi, 1.15 (1.01-1.32) for cIMT, and 1.28 (1.12-1.47) for CWS_{puls}. The association between CWS_{mean} and new-onset type 2 diabetes did not remain statistically significant after further adjustment in model 2. An increase in carDC (lower carotid stiffness) was associated with a lower risk of incident type 2 diabetes (0.96; 0.93-0.99) in model 2. Spline analyses for model 2 did not show any non-linear relationship between arterial stiffness/remodeling markers with incident type 2 diabetes (**Figure S1**). Age, sex, and MAP did not modify the associations between arterial stiffness/remodeling measurements and incident type 2 diabetes; the *p*-values for interaction were not statistically significant. Our results investigating the associations between markers of arterial stiffness/remodeling with follow-up measurements of fasting blood glucose showed statistically significant associations even after adjusting for confounders in all except for cf_PWV, carotid artery lumen diameter and CWS_{mean} in model 2 (**Table S2**).

As shown in **Table S3**, additional adjustments for baseline blood glucose attenuated the associations in a sensitivity analysis. However, the associations remained statistically significant except for cf_PWV (1.11; 0.97-1.28). We further adjusted the associations by adding fasting insulin and HOMA-IR to model 2, and the results did not substantially change (**Table S3**). Besides, after excluding individuals with prediabetes at baseline, results did remain statistically significant except for carDC (0.97; 0.93-1.00) and cIMT (1.09; 0.92-1.29) (**Figure 2B**). As shown in **Figure 2C** and **Table S4**, when we further studied the longitudinal associations between markers of arterial stiffness and remodeling with incident type 2 diabetes among the population with prediabetes at baseline (n=513), our results did not change substantially. However, it remained statistically significant only for the associations of carDC (0.96; 0.92-0.99) and cIMT (1.39; 1.12-1.73). Excluding incident cases during the first five years of follow-up did not significantly change the associations observed in model 2.

Our results showed multicollinearity (VIF around 5 or below) between arterial stiffness/remodeling markers. Still, it was not strong enough to warrant further adjustments in our statistical models (data not shown).

Among 2,647 individuals with genetic data (87% of the total population), in the multivariable-adjusted model, the associations between cf_PWV (1.34 (1.08-1.66)), carDC (0.93 (0.89-0.98)), CWS_{mean} (1.27 (1.01-1.58)) and CWS_{puls} (1.30 (1.04-1.63)) with type 2 diabetes remained statistically significant only among individuals with a high GRS for type 2 diabetes (**Table 2**).

Table 2. The association between arterial stiffness and incident type 2 diabetes, stratified by GRS for type 2 diabetes

	Low GRS	Intermediate GRS	High GRS
	HR (95%CI)	HR (95%CI)	HR (95%CI)
Carotid-femoral pulse wave velocity	0.86 (0.67-1.10)	0.99 (0.72-1.36)	**1.34 (1.08-1.66)**
Carotid distensibility	1.01 (0.95-1.08)	0.97 (0.92-1.01)	**0.93 (0.89-0.98)**
Carotid diameter	1.11 (0.84-1.47)	1.18 (0.94-1.48)	1.21 (1.00-1.47)
Carotid intima-media thickness	1.05 (0.77-1.44)	**1.41 (1.04-1.65)**	1.12 (0.90-1.40)
Mean carotid wall stress	1.14 (0.83-1.56)	0.91 (0.73-1.13)	**1.27 (1.01-1.58)**
Pulsatile carotid wall stress	1.22 (0.88-1.68)	**1.33 (1.03-1.72)**	**1.30 (1.04-1.63)**

The models were adjusted for age, sex, cohort, body mass index, mean arterial pressure, heart rate, non-HDL-cholesterol, blood pressure- and lipid-lowering medications, and smoking.

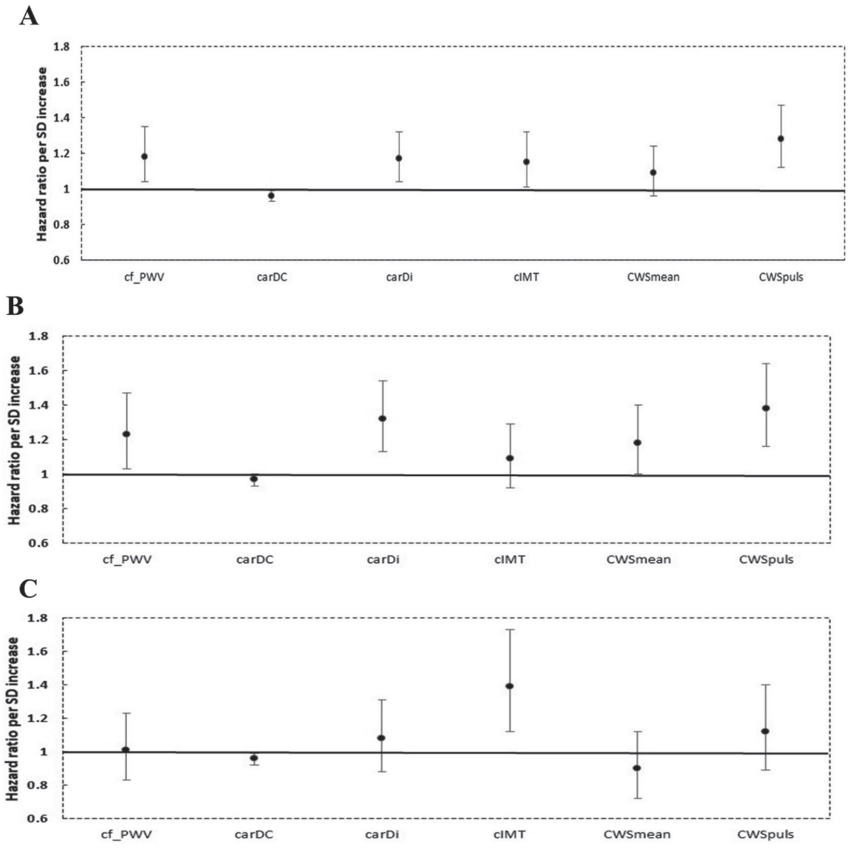

Figure 2. Association between markers of arterial stiffness and arterial remodeling with incident type 2 diabetes among the general population free of diabetes (A), among the general population free of diabetes and prediabetes (B), and among high-risk individuals with prediabetes at baseline (C).

Note: The models were adjusted for age, sex, cohort, body mass index, smoking, mean arterial pressure, antihypertensive medications, heart rate, non-HDL-cholesterol, and lipid-lowering medications.

The results of the IVW analysis showed no causal association between ASI and type 2 diabetes (OR: 1.37; 95%CI: 0.42-2.31). There was no evidence for horizontal pleiotropy (p-value for intercept: 0.60). We tested the associations between GRS for ASI and glycemic traits (baseline fasting blood glucose and insulin levels and HOMA-IR) and incident type 2 diabetes among 2,647 individuals with genetic data. Our finding showed statistically significant associations between GRS for ASI and fasting insulin (β: 0.001; p value=0.01) and HOMA-IR (β: 0.001; p value=0.03) at baseline, even after adjusting for potential confounders (model 2 of adjustment) but not with incident type 2 diabetes.

DISCUSSION

Our study showed that arterial stiffness and remodeling markers were associated with new-onset type 2 diabetes among women and men from the general population, free of cardiovascular disease and diabetes at baseline. The associations were not due to reverse causation. The associations were independent of established diabetes risk factors and baseline

blood glucose levels. Besides, the associations between markers of arterial stiffness and remodeling with type 2 diabetes were not modified by age, sex, and hypertension status. We also investigated the associations between arterial stiffness/remodeling markers with follow-up measurements of fasting blood glucose. Our results showed statistically significant associations even after adjusting for confounders for most markers. In addition, we found stronger associations between arterial stiffness and remodeling markers and type 2 diabetes in individuals with a higher GRS for type 2 diabetes. Our MR approach indicated that the relationship between arterial stiffness and type 2 diabetes is not causal. However, GRS for arterial stiffness index showed significant associations with fasting insulin and HOMA-IR as a proxy for insulin resistance.

We showed that increased aortic and carotid stiffnesses are associated with an increased risk of incident type 2 diabetes. Higher aortic stiffness is an independent predictor of incident type 2 diabetes in the general population [117-120, 135] or high-risk hypertensive individuals [122, 136]. A recent study evaluated the association between large artery stiffness (LAS) and the risk of type 2 diabetes in 5,676 participants of the Framingham Heart Study (FHS) over 7 years of follow-up. Applying the MR approach in the UK Biobank, this study found evidence supporting that greater LAS is associated with an increased risk of type 2 diabetes. This study showed that cf_PWV (HR: 1.36) and central pulse pressure (HR:1.26) were associated with an increased risk of incident diabetes [137]. We proved that increased carotid stiffness is associated with an increased risk of incident type 2 diabetes. An increase in the markers of arterial remodeling, including carDi, cIMT, and CWS_{puls} was associated with a greater risk for incident type 2 diabetes. Arterial remodeling is a homeostatic response to changes in the flow and circumferential stretch to restore normal shear stress and wall tension within certain operation limits [114], which means remodeling is closely related to hemodynamic stimuli. All these changes in the arterial structure suggest potential preclinical vascular dysfunction, which in turn may relate to future events related to diabetes [138].

Besides considering arterial stiffness as a marker of hypertension end-organ damage, arterial stiffness can directly induce metabolic dysregulations by dramatically slowing blood flow that accelerates hyperglycemia [138]. Blood flow is an essential factor that regulates the metabolic function of muscles. It is speculated that enhancing blood flow may induce insulin and glucose delivery to peripheral tissues and contribute to overall glucose disposal [138]. It has been suggested that, even before the onset of type 2 diabetes, altered arterial stiffness are evident in individuals with prediabetes [116]. Hyperinsulinemia induced by insulin resistance and impaired fasting glucose causes vascular dysfunction, leading to the increased renin-angiotensin-aldosterone system, impaired vascular reactivity/resistance, and abnormal glucose metabolism [139]. This suggests that the link between arterial stiffness and remodeling with type 2 diabetes can be through hyperglycemia. To examine this, we studied the associations between arterial stiffness/remodeling and incident type 2 diabetes in the presence of hyperglycemia. Our stratified analyses among individuals with prediabetes remained the same as the general population for most markers except for cIMT. The involvement of cIMT, a marker of arterial remodeling and a measure of atherosclerosis, in developing type 2 diabetes was stronger in the prediabetes stage than in the normoglycemic general population. Our result may suggest that early insulin resistance and impaired fasting glucose may enhance the impact of atherosclerosis on type 2 diabetes development. In a previous study, Ronald et al. [23] concluded that arterial stiffness and remodeling are increased with deteriorating glucose tolerance [140]. In this concept, an increase in cIMT could, at least partially, be viewed as a compensatory response to counteract the increased wall stress induced by the diameter enlargement.

Increased vascular *stiffness* is associated with increased MAP [130, 141] as a potential reciprocal risk factor for type 2 diabetes. Type 2 diabetes and hypertension are closely linked due to shared risk factors, e.g., endothelial dysfunction, vascular inflammation, and obesity [142]. Therefore, we hypothesized that the associations between arterial stiffness and remodeling might be modified by MAP. However, our study did not provide evidence for effect modification by MAP values on the associations between arterial stiffness and remodeling with new-onset type 2 diabetes. These together suggest that additional mechanisms such as oxidative stress, inflammation, or endothelial dysfunction might play a role in this association [143].

A major contributor to arterial stiffening is ageing, a dominant risk factor for type 2 diabetes and cardiovascular diseases, decreasing vascular elasticity [144]. So far, several studies have investigated the effect of age on arterial stiffness [144]. The age-associated increased stiffness of the aorta is greater than the carotid artery [145]. However, in our study, the associations between aortic and carotid stiffness and remodeling markers with new-onset type 2 diabetes were independent of age.

It is known that type 2 diabetes is often diagnosed with a delay of several years [146]. Hence, a degree of asymptomatic type 2 diabetes sufficient to cause vascular damage may be present long before the clinical diagnosis of diabetes. Therefore, diabetes-associated increased arterial stiffness and remodeling could occur long before the clinical diagnosis of diabetes. The associations were not due to the reverse causation as we had excluded incident cases of diabetes during the first five years of follow-up, and the results did not change. However, the mechanisms through which arterial stiffness and remodeling affect type 2 diabetes require additional investigation.

We examined the role of GRS for type 2 diabetes in the associations between markers of arterial stiffness and remodeling and incident type 2 diabetes. The results showed that type 2 diabetes genetic variations might modify the associations, in line with the previous study [117]. This might be explained by overlapped biological mechanisms involved in diabetes-related traits, e.g., obesity and arterial stiffness/remodeling, or similar genetic backgrounds between arterial stiffness/remodeling and type 2 diabetes. Although our MR analysis did not support the causality for diabetes, we showed that genetic variants of ASI are associated with insulin resistance in our population. Insulin resistance has been proposed as a pathway interacting with an individual's genetic background to cause type 2 diabetes [147]. However, our study looked at continuous measures of HOMA-IR as a proxy for insulin resistance and showed a causal association. There is great variability in the HOMA-IR threshold levels to define insulin resistance which might explain why the causal association observed for insulin resistance did not translate into the same causality for incident type 2 diabetes in our study.

Strengths of this study include the prospective cohort design, relatively long follow-up time, meticulous adjudication of incident diabetes, availability of several measures of arterial stiffness and remodeling within the same population, and access to a wide range of cardiovascular and diabetes risk factors, genetic information, and MR analysis. Some limitations, however, also need to be considered. Our dataset only includes baseline measurements of arterial stiffness, and we could not investigate the changes in arterial stiffness/remodeling markers over time concerning diabetes incidence. Our study mainly included individuals of European ancestry, limiting the generalizability of our findings to other populations. As pertinent to all prospective cohort studies with long follow-up times, loss of follow-up could have underestimated the observed effect. In MR analysis, we only had summary statistics available for ASI (a marker of arterial stiffness) with few SNPs.

An essential point regarding arterial stiffness (cf_PWV) is that strategies that may lead to

aortic de-stiffening still need to be demonstrated in future interventions and prospective studies. Over the last two decades, there has been increasing knowledge of the importance of arterial stiffness for the pathogenesis of age-related cardiovascular diseases. In the last decade, it demonstrated its predictive importance for cardiovascular outcomes in various clinical conditions, including type 2 diabetes. Up to now, most prospective studies have evaluated the effects of pharmacological or non-pharmacological (lifestyle) interventions in hypertension in the short term of a few months up to a year. The most potent therapy for reducing arterial stiffness is vigorously treating hypertension using pharmacological agents. Though, new pharmacological strategies to reduce arterial stiffness are still warranted.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://www.diabetesresearchclinicalpractice.com/article/S0168-8227(23)00002-5/fulltext

2.4

Chapter 2.4

Cardiovascular health and lifetime risk of type 2 diabetes

ABSTRACT

Background: Data on the lifetime risk of type 2 diabetes (T2D) incidence across different cardiovascular health (CVH) categories are scarce. Moreover, it remains unclear whether a genetic predisposition modifies this association.

Methods: Using data from the prospective population-based Rotterdam Study, a CVH score (body mass index, blood pressure, total cholesterol, smoking status, diet, and physical activity) was calculated and further categorized at baseline. Genetic predisposition to T2D was assessed and divided into tertiles by creating a genetic risk score (GRS). We estimated the lifetime risk for T2D within different CVH and GRS categories.

Results: Among 5993 individuals free of T2D at baseline (mean (standard deviation) age, 69.1 (8.5) years; 58% female), 869 individuals developed T2D during follow-up. At age 55 years, the remaining lifetime risk of T2D was 22.6% (95%CI: 19.4-25.8) for ideal, 28.3% (25.8-30.8) for intermediate, and 32.6% (29.0-36.2) for poor CVH. After further stratification by GRS tertiles, the lifetime risk for T2D was still the lowest for ideal CVH in the lowest GRS tertiles (21.5% (13.7-29.3)), in the second GRS tertile (20.8% (15.9-25.8)), and in the highest tertile (23.5% (18.5-28.6)) when compared to poor and intermediate CVH.

Conclusions: Our results highlight the importance of favorable CVH in preventing T2D among middle-aged individuals regardless of their genetic predisposition.

INTRODUCTION

Type 2 diabetes mellitus (T2D) is a common metabolic disease, characterized by disturbances in glucose and insulin metabolism. The number of adults who have diabetes has been 463 million in 2019 and is expected to increase to 700 million by 2045 [148]. The pathogenesis of T2D is driven by genetic and non-genetic factors, such as obesity, an unhealthy diet, and physical inactivity [149]. Therefore, multilevel intervention measures to improve T2D prevention are warranted. So far, studies targeting diabetes prevention have primarily focused on one [150, 151] or certain [152, 153] risk factors and do not take into account the interactive nature of risk factors.

The concept of cardiovascular health (CVH) was introduced by the American Heart Association (AHA) in 2010 as part of the AHA impact goals for promoting cardiovascular health and reducing deaths from cardiovascular disease (CVD) [27]. The CVH includes seven health factors and health behaviors associated with CVD and aging. As many of the cardiovascular risk factors also confer a larger risk for T2D, previous studies have shown that the concept of CVH is also applicable to T2D [28, 154, 155]. Yet, data on the lifetime risk of incident T2D across different CVH categories are scarce [148, 156]. Moreover, whether the impact of CVH on lifetime risk of incident T2D is affected by genetic predisposition remains unknown.

In this study, we used data from the large prospective population-based Rotterdam Study to evaluate the lifetime risk for incident T2D across different CVH categories. We further investigated the role of T2D genetic variants on the lifetime risk of incident T2D across different CVH categories.

METHODS

Study design and population

This study is embedded within the Rotterdam Study (RS), a prospective cohort study of the community-dwelling population aged 55 years and older in Rotterdam, the Netherlands. The detailed study design has been described elsewhere [157]. Briefly, the baseline examination for the first cohort was completed between 1990 and 1993 (RS-I) with 10,215 participants aged 55 years or over; the response rate was 78%. The Rotterdam Study was extended in 2000 to include all inhabitants who became 55 years of age or moved into the research area after the start of the study (RS-II). There were no eligibility criteria to enter the Rotterdam Study apart from the minimum age and residential area based on postal codes.

We used the third center visit (1997-99, n=4,797) of RS-I and the first visit of RS-II (2000-01, n=3,011) as the baseline for our analyses. An overview of the study population is shown in **Figure S1**.

Cardiovascular health

We included six metrics in calculating the CVH score [27]: body mass index (BMI), smoking behavior, blood pressure, total cholesterol, physical activity, and diet, all measured at baseline. We did not include glucose levels since diabetes was the outcome of interest. BMI was calculated as body weight (in kg) divided by the square of height (in meters). Smoking behavior was assessed using a computerized questionnaire and categorized into three groups: Poor: current smoker (cigarette | cigar | pipe); Intermediate: stopped cigarettes ≤ 12 months or former smoker (cigar | pipe); Ideal: never ((never smoking a cigarette or stopped cigarettes > 12 months) & never-smoking cigar or pipe)) [158]. Blood pressure was measured at the right upper arm with the participant in a sitting position, of which the mean of 2 consecutive measurements was

used. Total cholesterol concentration was obtained from a fasting blood sample using the Hitachi 917 (Roche Diagnostics, Almere, Netherlands). Physical activity levels were assessed using a validated adapted version of the Zutphen Physical Activity Questionnaire [159]. To quantify activity intensity, we assigned metabolic equivalent task (MET) values to all contained activities (cycling, walking, sports, domestic work, gardening) according to the 2011 updated version of the Compendium of Physical Activities. We classified them into moderate (3-5.9 MET) and vigorous (≥6 MET) intensity according to the 2017 Dutch Physical Activity Guideline. A validated food frequency questionnaire was used to assess dietary patterns. Participants indicated which foods they consumed at least twice a month in the preceding year using a self-administered checklist. Subsequently, a semi-quantitative food frequency of 170 food items was collected by trained dieticians [160]. The specific food consumption level was further dichotomized according to the Dutch Dietary Guidelines. We categorized all six metrics into three levels (coded as poor=0, intermediate=1, and ideal=2), following the AHA criteria [27] (**Table S1**).

We used each metric' sum to calculate the CVH score, ranging from 0 to 12 with higher scores corresponding to better cardiovascular health. The cut-points for categorizing total CVH into poor, intermediate, and ideal were defined based on the distribution of the total CVH score in our study (Poor CVH: 0-5 score; Intermediate CVH: 6-7 score; Ideal CVH: 8-12). We calculated biological CVH comprising blood pressure, total cholesterol, and smoking with 0-2 score into poor, 3 score into intermediate, and 4-6 score into ideal CVH categories. Behavioral CVH included BMI, smoking, diet, and physical activity, and subjects with 0-4 score, 5-6 score, and 7-8 score were defined as poor, intermediate, and ideal behavior CVH, respectively [27]. We also derived the number of cardiovascular health metrics at the ideal level, ranging from 0 (none) to 6 (all metrics at the ideal level).

Genotyping and genetic risk score

Genotyping has been performed using the Illumina 550K and 610K quad array (Illumina Inc., San Diego, CA, USA) imputed to the Haplotype Reference Consortium reference panel (version 1.0) with Minimac 3. We included 403 independent genetic variants associated with T2D based on a recent GWAS on European ancestry individuals to calculate a weighted genetic risk score (GRS) [161]. In this study, the definitions of T2D were study-specific. Thirty out of 32 studies identified T2D cases based on laboratory tests, e.g. fasting blood glucose, glycated hemoglobin, use of antidiabetic treatment, and medical data; while in two of them T2D was defined based on validated self-reported T2D history. The GRS was calculated as the sum of the products of single-nucleotide polymorphism allele dosages of the 403 genetic variants and their respective reported effect estimates.

Ascertainment of type 2 diabetes mellitus

Participants were followed up from the date of attending baseline visit onwards. At baseline and during follow-up, cases of T2D were ascertained by the use of general practitioners' records, hospital discharge letters, and serum glucose measurements collected from center visits, which take place roughly every 4 years. T2D was defined as a fasting blood glucose concentration of 7.0 mmol/L (126 mg/dL) or higher, a non-fasting blood glucose concentration of 11.1 mmol/L (200 mg/dL) or higher (when fasting samples were unavailable), or the use of blood glucose-lowering medications. Information about blood glucose-lowering medications was obtained from both structured home interviews and pharmacy dispensing records. At baseline, more than 95% of the Rotterdam Study population was covered by the pharmacies in the study area. Two study physicians independently adjudicated all potential events of T2D. In the case of disagreement, a consensus was sought from an endocrinologist. Participants were followed until

the occurrence of incident T2D, death, or the end of the study period (January 1st, 2015).

Statistical analysis

Baseline characteristics are presented as mean (standard deviation (SD)) for continuous variables and frequency (percentage) for categorical variables. We compared the baseline characteristics of individuals among different CVH categories using Kruskal-Wallis tests for continuous data and Chi-square tests for categorical data.

In the main analyses, the lifetime risk for incident T2D was estimated by a modified version of survival analysis which takes the competing event of death into account, with age as the time scale. We calculated the remaining lifetime risks for incident T2D from index ages 55, 65, and 75 years up to age 107. T2D-related GRS was stratified into tertiles based on the distributions in the total population. All participants were categorized into low (tertile 1), intermediate (tertile 2), and high (tertile 3) genetic risk categories. We then assessed the joint contributions of genetic predisposition and CVH profile by calculating lifetime risks of T2D within cross-tabulated categories of both GRS tertile and CVH categories, resulting in nine risk strata at each index age. After testing the violation of proportionality [162], we compared the overall difference of lifetime risk estimates across GRS tertiles in specific CVH categories by the Fine-Gray method based on sub-hazard distributions [163]. Lifetime risks in intermediate and high GRS groups were also compared with the lifetime risk estimated in the low GRS group by a z ratio test (calculated as the difference in lifetime risk between the two groups divided by its standard error) [164]. We also performed an exploratory analysis using both cause-specific and sub-distribution (Fine-Gray) hazard models, in which we regressed T2D on continuous genetic risk score (normally standardized) and CVH scores in the 55 years of age sample with a multiplicative interaction term included [164, 165].

Additionally, in a series of subgroup analyses, we calculated the lifetime risks of incident T2D related to biological and behavioral CVH, separately. We also conducted sex-specific analyses to explore the difference between men and women, and checked the interaction between sex and CVH scores using both cause-specific and sub-distribution (Fine-Gray) hazard models. To test the robustness of the main findings, we performed the following sensitivity analyses: (1,2) excluding participants who had prediabetes (defined as a fasting blood glucose concentration ≥6.1 mmol/L (110 mg/dL) and <7.0 mmol/L (126 mg/dL)) at baseline or were underweight (BMI ≤18.5 kg/m^2), respectively; (3) using solely BMI-based CVH to test whether the associations are driven solely by BMI; (4) conducting the complete case analyses taking into account the uncertainty of imputed values.

To deal with missing values for CVH metrics, we used single imputation with the expectation-maximization method. Data were handled and analyzed with SPSS Statistics version 25.0.0.1 (IBM Corp., Armonk, NY) and R, CRAN version 3.6.3, with packages survival, etm, and cmprsk. All analyses were performed at the significance level of 0.05 (2-tailed).

RESULTS

A total of 5,993 participants free of T2D at baseline were included in this study. The mean (SD) age of the population was 69.1 (8.5) years and 3,475 (58%) participants were women. At baseline, out of 5,993 participants, 2,020 (17.4%) had ideal CVH, 2,605 (45.6%) had intermediate CVH, and 1,368 (37.0%) had poor CVH. Compared to individuals with poor CVH, those with intermediate or ideal CVH tend to have a more favorable risk profile, e.g. higher education level, and higher high-density lipoprotein (HDL)-cholesterol level. As for the separate CVH metric, the ideal level of blood pressure was the least frequent (9%), whereas ideal physical activity was the most prevalent (79%). Also, the proportion for each ideal metric

showed an increasing trend in the hierarchical CVH categories (**Table 1**).

Table 1. Baseline characteristics of the study population across different cardiovascular health (CVH) categories

	Total participants	Poor CVH	Intermediate CVH	Ideal CVH	p *
Sample size, n	5993	1368	2605	2020	-
Age (years)	69.1 (8.5)	70.5 (9.3)	69.4 (8.4)	67.9 (7.9)	<0.001
Women	3475 (58%)	747 (55%)	1505 (58%)	1223 (61%)	0.003
Height (cm)	167.0 (9.1)	166.7 (9.4)	166.8 (9.1)	167.5 (8.8)	0.009
Weight (kg)	74.8 (12.5)	79.6 (13.0)	75.5 (12.4)	70.5 (11.0)	<0.001
Body mass index (kg/m^2)	26.8 (3.8)	28.6 (4.0)	27.1 (3.8)	25.1 (3.0)	<0.001
Education					
Primary	778 (13%)	223 (17%)	341 (13%)	214 (11%)	
Lower/intermediate or lower vocational	2563 (43%)	587 (43%)	1134 (44%)	842 (42%)	
Intermediate vocational or higher general	1767 (30%)	381 (28%)	772 (30%)	614 (31%)	<0.001
Higher vocational or university	793 (13%)	164 (12%)	313 (12%)	316 (16%)	
Total alcohol intake (g/day)	3.1 (0.1-14.4)	3.3 (0-18.6)	2.9 (0.1-14.4)	3.6 (0.3-13.6)	0.482
Total physical activity (METh/week)	78.4 (53.5-106.9)	60.8 (36.1-91.2)	78.5 (54.9-106.3)	88.6 (65.3-118.4)	<0.001
Total cholesterol (mmol/L)	5.8 (1.0)	6.2 (1.0)	5.9 (0.9)	5.5 (0.9)	<0.001
Glucose (mmol/L)	5.5 (5.2-5.9)	5.6 (5.3-6.0)	5.5 (5.2-5.9)	5.4 (5.1-5.7)	<0.001
Insulin (pmol/L)	66 (47-94)	76 (53-110)	69 (49-95)	57 (41-80)	<0.001
HDL cholesterol (mmol/L)	1.4 (1.1-1.6)	1.3 (1.1-1.6)	1.3 (1.1-1.6)	1.4 (1.2-1.7)	<0.001
Systolic blood pressure (mmHg)	142 (21)	151 (20)	145 (19)	133 (19)	<0.001
Diastolic blood pressure (mmHg)	77 (11)	79 (11)	78 (11)	74 (10)	<0.001
History of cardiovascular disease	674 (11%)	159 (12%)	317 (12%)	198 (10%)	0.036
Use of lipid-lowering agents	982 (16%)	253 (19%)	470 (18%)	259 (13%)	<0.001
Use of blood pressure-lowering drugs	2099 (35%)	566 (41%)	1017 (39%)	516 (26%)	<0.001
Cardiovascular health scores					
Body mass index					
Poor	1030 (17%)	489 (36%)	466 (18%)	75 (4%)	
Intermediate	2979 (50%)	700 (51%)	1450 (56%)	829 (41%)	<0.001
Ideal	1984 (33%)	179 (13%)	689 (26%)	1116 (55%)	
Smoke					
Poor	1201 (20%)	632 (46%)	459 (18%)	110 (5%)	
Intermediate	719 (12%)	184 (13%)	373 (14%)	162 (8%)	<0.001
Ideal	4073 (68%)	552 (40%)	1773 (68%)	1748 (87%)	
Diet					
Poor	1023 (17%)	473 (35%)	436 (17%)	114 (6%)	
Intermediate	3882 (65%)	824 (60%)	1822 (70%)	1236 (61%)	<0.001
Ideal	1088 (18%)	71 (5%)	347 (13%)	670 (33%)	
Physical activity					
Poor	723 (12%)	495 (36%)	206 (8%)	22 (1%)	
Intermediate	548 (9%)	189 (14%)	287 (11%)	72 (4%)	<0.001
Ideal	4722 (79%)	684 (50%)	2112 (81%)	1926 (95%)	
Blood pressure					
Poor	3179 (53%)	1038 (76%)	1587 (61%)	554 (27%)	
Intermediate	2271 (38%)	313 (23%)	904 (35%)	1054 (52%)	<0.001
Ideal	543 (9%)	17 (1%)	114 (4%)	412 (20%)	
Total cholesterol					
Poor	1877 (31%)	697 (51%)	872 (33%)	308 (15%)	
Intermediate	3084 (52%)	595 (43%)	1417 (54%)	1072 (53%)	<0.001
Ideal	1032 (17%)	76 (6%)	316 (12%)	640 (32%)	

Data are n (%), mean (standard deviation), or median (interquartile range) for characteristics with skewed distributions.

* p-value for difference among three CVH categories, Kruskal-Wallis tests for continuous data, and χ^2 tests for categorical data.

During 69,208 person-years of follow-up, at index age 55 years, the median (interquartile range (IQR)) follow-up time was 14 (8-15) years and the number of participants with incident T2D was 869. Trends for estimates of the remaining lifetime risk of incident T2D at index age 55 years are displayed in **Table 2** and **Figure 1**. At index age 55 years, the lifetime risk (95%CI) of T2D was 22.6% (19.4-25.8) for ideal, 28.3% (25.8-30.8) for intermediate, and 32.6% (29.0-36.2) for poor CVH category.

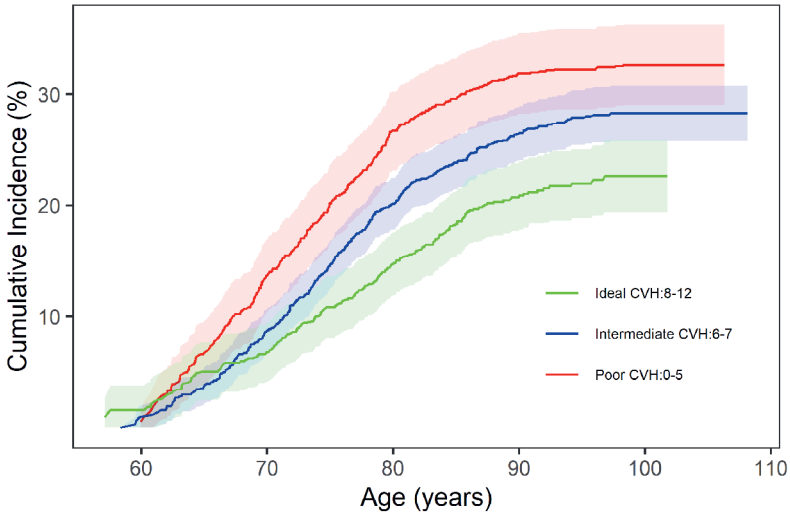

Figure 1. The lifetime risk of incident type 2 diabetes in individuals aged 55 years, across cardiovascular health (CVH) categories.

Among 4,952 (82.6%) participants who had available genotype information, the baseline risk profile showed no significant differences in the distribution of individual CVH metrics between GRS tertiles (**Table S2**). Substantial gradients of lifetime T2D risk were consistently observed within hierarchical CVH categories regardless of the indexed age and genetic predisposition (**Table 3, Figure 2**). For example, at index age 55 years, the remaining lifetime risk for T2D in the high GRS group was 23.5% (18.5-28.6) for ideal CVH, 33.7% (28.9-38.5) for intermediate CVH, and 38.7% (31.4-46.1) for poor CVH. The lifetime risk for T2D was still the lowest for ideal CVH in the lowest GRS tertile (21.5%(13.7-29.3)), in the second GRS tertile (20.8%(15.9-25.8)), and in the highest GRS tertile (23.5%(18.5-28.6)) when compared with the remaining lifetime risk of T2D for poor and intermediate CVH. As shown in **Table S10**, our exploratory analysis to check the interaction between GRS and the CVH scores showed no statistically significant interaction ($p = 0.72$).

Participants were further stratified based on biological or behavioral CVH categories that showed the remaining lifetime risk of T2D decreased gradually from poor to intermediate and to ideal, regardless of the indexed age (**Figure S2, Table S3**). For sex-specific analysis, similar patterns were obtained in both sexes in all attained ages (**Figure S3, Table S4**), with no significant p-value for interaction ($p = 0.98$), as shown in **Table S11**.

In sensitivity analyses, similar patterns for the T2D risk gradients among GRS tertiles in specific CVH categories were observed when we excluded participants who had prediabetes at baseline (**Table S5**), and when underweight participants were excluded (**Table S6**). In further analyses using solely BMI-based CVH (**Table S7**), and analysis based on the complete cases, results remained consistent with our main results.

Table 2. The remaining lifetime risk of incident type 2 diabetes by cardiovascular health (CVH) category

Attained age (years)	Poor CVH		Intermediate CVH		Ideal CVH		p *
	N events / N total	Cumulative incidence, % (95%CI)	N events / N total	Cumulative incidence, % (95%CI)	N events / N total	Cumulative incidence, % (95%CI)	
55	245/1368	32.6 (29.0, 36.2)	405/2605	28.3 (25.8, 30.8)	219/2020	22.6 (19.4, 25.8)	<0.001
65	224/1332	29.2 (25.9, 32.4)	383/2566	26.2 (23.9, 28.5)	195/1984	18.9 (16.3, 21.5)	<0.001
75	125/987	19.9 (16.8, 23.0)	223/1936	18.8 (16.5, 21.1)	120/1481	14.9 (12.2, 17.6)	0.011

* Overall test comparing sub-hazard distributions across CVH categories by Fine-Gray method. p for difference among CVH categories.

Table 3. The remaining lifetime risk of incident type 2 diabetes for each cardiovascular health category, stratified by genetic risk

Attained age (years)	Genetic risk	Poor CVH				Intermediate CVH				Ideal CVH			
		N events / N total	Cumulative incidence, % (95%CI)	p *	p #	N events / N total	Cumulative incidence, % (95%CI)	p *	p #	N events / N total	Cumulative incidence, % (95%CI)	p *	p #
55	Low	51/382	24.5 (18.1, 30.8)	0.003	-	79/713	20.1 (16.0, 24.2)	<0.001	-	50/556	21.5 (13.7, 29.3)	0.214	-
	Intermediate	69/375	33.3 (26.5, 40.0)		0.063	101/711	25.0 (20.6, 29.4)		0.11	62/565	20.8 (15.9, 25.8)		1.118
	High	82/364	38.7 (31.4, 46.1)		0.004	140/719	33.7 (28.9, 38.5)		<0.001	69/567	23.5 (18.5, 28.6)		0.673
65	Low	48/376	22.1 (16.4, 27.7)	0.007	-	78/710	19.8 (15.8, 23.9)	<0.001	-	45/548	17.1 (11.9, 22.4)	0.12	-
	Intermediate	62/364	29.1 (23.0, 35.2)		0.099	97/700	24.5 (20.2, 28.8)		0.119	57/556	18.7 (14.1, 23.3)		0.653
	High	75/351	35.9 (29.3, 42.6)		0.002	134/708	32.1 (27.5, 36.6)		<0.001	64/561	21.2 (16.5, 25.9)		0.254
75	Low	31/289	16.4 (11.1, 21.7)	0.279	-	53/548	16.1 (12.0, 20.2)	0.065	-	27/436	13.7 (8.0, 19.3)	0.051	-
	Intermediate	35/266	20.9 (14.7, 27.1)		0.28	55/546	16.5 (12.4, 20.6)		0.892	35/428	14.4 (9.7, 19.1)		0.852
	High	39/261	22.8 (16.5, 29.1)		0.128	72/526	21.9 (17.3, 26.5)		0.065	46/417	18.4 (13.4, 23.3)		0.22

* Overall test comparing sub-hazard distributions across risk categories by Fine-Gray method. p for difference among genetic risk categories. # Test comparing lifetime risk in intermediate and high-risk groups with the low-risk group by the z ratio test.

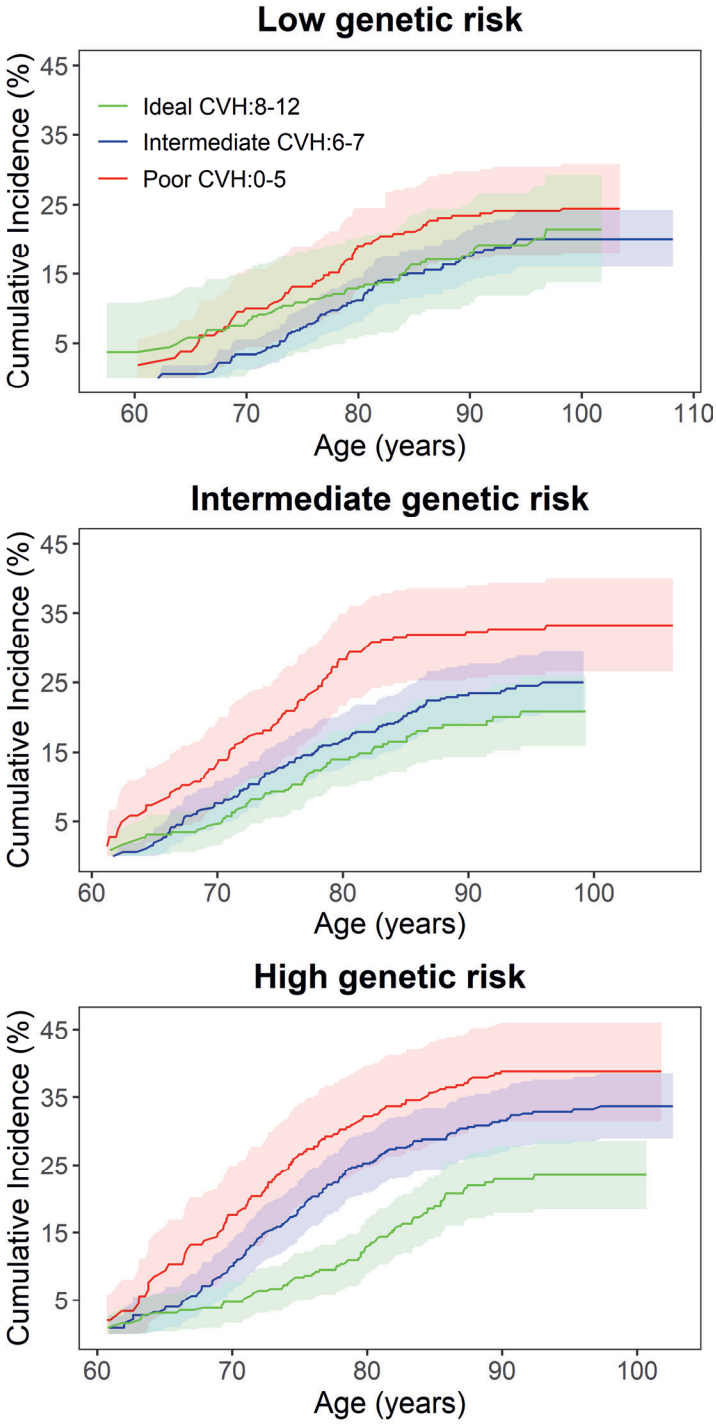

Figure 2. The lifetime risk of incident type 2 diabetes in individuals aged 55 years across cardiovascular health (CVH) categories, stratified by type 2 diabetes genetic risk score.

DISCUSSION

In a large prospective population-based cohort study we showed that at age 55 years, the remaining lifetime risk of incident T2D was the lowest for ideal CVH when compared to intermediate and poor CVH categories. Although more favorable CVH was associated with a lower lifetime risk of T2D, it was not counterbalanced by the genetic susceptibility to T2D.

Our study showed that individuals with more favorable CVH had a lower remaining lifetime risk for incident T2D regardless of the indexed age, highlighting the importance of clustering of both behavioral and biological CVH factors among middle-aged individuals, or even older age for the risk of T2D. For example, the remaining lifetime risk of incident T2D was 14.9% for ideal, 18.8% for intermediate, and 19.9% for poor CVH at the age of 75. Our results complement the results of previous studies. Among the Jackson Heart study, compared with participants with only one or no ideal CVH metric, the risk of T2D was 15% and 37% lower in those with 2 and ≥3 ideal CVH metrics, respectively [155]. Among the REasons for Geographic and Racial Differences in Stroke Study, Joshua et al investigated the association between ideal CVH and long-term risk of incident diabetes. They found that participants with more than 4 vs 0-1 ideal CVH components had an 80% lower risk of T2D [28].

In our study, we did not find statistically significant differences between men and women, our power to detect an interaction was modest as the sample size was further reduced due to sex-specific grouping. A former study indicated that women could have more years of survival free of T2D than men after the age of 45 [151]. Among the Physician's Health Study I and the Women's Health Study, for participants at age 45 years with ≥4 healthy lifestyle factors (never smoking, BMI <25kg/m^2, regular exercise, healthy diets, and moderate drinking), the remaining lifetime risk of diabetes for men was 7.3% (5.7-8.9), and 6.4% (4.2-8.6) for women [152].

When we stratified participants based on biological and behavior CVH scores, we found that the pattern of lifetime risk of T2D remained similar. Despite the dose-dependent relationship between the number of ideal CVH components and the risk of incident T2D, results from individual CVH components suggested that behavioral factors, especially BMI, have the most influence [28, 152, 155]. Here we also noticed that compared with the poor level, participants benefit more from decreasing lifetime T2D risk from adhering to an ideal behavioral CVH than adhering to an ideal biological CVH. The AHA Simple 7 considers BMI a behavior. This is controversial because it may suggest that obesity is a choice. However, others suggest obesity should be considered a health metric in the same way as blood pressure, dyslipidemia or T2D would [166].

In the present analyses, the unfavorable impact of a particular behavioral factor i.e., physical inactivity might be diluted because 79% of the included participants achieved an ideal physical activity level. As physical inactivity is a major determinant of T2D risk, our results are likely to underestimate the detrimental effect of unfavorable behavioral CVH, following by unfavorable CVH on the lifetime risk of T2D. This type of asymmetric distribution of individual CVH components has also been reported in several studies [28, 154, 167]. Although these studies consistently suggest that favorable CVH is associated with a lower risk of cardiometabolic disease, the different skew-distributed components indicate that the region-specific adjustment for cardiovascular health score might be needed to enhance its preventive effect.

We showed that the beneficial impact of adhering to a favorable CVH on the lifetime risk for incident T2D is not counterbalanced by genetic susceptibility to T2D. It has been widely known that T2D is concordantly determined by environmental and genetic factors [168].

Despite abundant evidence in this field, studies have mostly evaluated potential interplays between individual lifestyle factors (e.g. obesity, physical activity, and particular food consumption) prone to confounding by each other and individual genetic variants [169, 170]. Our study adds to the findings of previous studies on lifetime risk associated with CVH by also considering T2D-related genetic susceptibility. Meanwhile, the trends of lifetime risk of T2D in different genetic risk groups lose their significance in the ideal CVH category, which indicates that individuals with higher genetic predisposition might benefit more from adhering to a favorable CVH. Recently, the Finnish study reported that polygenic risk score could improve reclassification of incident T2D over traditional clinical risk factors [171]. Although the interaction effect between genetic risk and clinical risk on the lifetime risk of T2D was also tested in their study, the factors they used to estimate the clinical risk were derived from the ADA's diabetes risk test [172]. Unlike the metrics of CVH, most of these factors were unmodifiable such as age, sex, history of gestational diabetes et al. Therefore, their findings may have more prognostic than interventional utility. Our results were comparable with the previous two studies that have reported the long-term effects of modifiable lifestyle factors across genetic risk groups for incident T2D. In line with our findings, two Asian cohorts (the China Kadoorie Biobank Study and the Singapore Chinese Health Study) reported that a healthy lifestyle was associated with a significantly lower risk of T2D within any genetic risk category (49 related genetic variants) among the Chinese population [173]. However, only five risk factors - BMI, alcohol intake, smoking, physical activities, and diets - were considered. The UK Biobank Study also found that the relative effects of the ideal lifestyle were comparable between genetic risk groups (38 related genetic variants) for developing T2D [153]. By combining biological and health factors, we investigated an extended risk factors profile and more comprehensive genetic information.

Given the increasing clinical application of genomic data, epidemiologically derived estimates are necessary for providing accurate personalized risk assessments. We did not observe a significant interaction between T2D-GRS and the CVH scores for incident T2D. These findings in total indicate the substantial potential benefits of adherence to an ideal level of modifiable risk factors regardless of GRS. Therefore, preventive strategies should promote stricter adherence to favorable cardiovascular health for all.

The key strengths of this study include the prospective cohort design, relatively long follow-up time, and meticulous assessment of T2D diagnosis. We also had detailed information regarding the components of cardiovascular health as well as access to genotype information. However, our study has some limitations that need to be addressed. Firstly, the measurements of individual CVH metrics were collected at baseline, which could have led to participants' misclassification across the different categories. Both biological and behavioral CVH factors could have changed with aging [174]. Secondly, in genetic-stratified analyses, estimations in several groups were based on relatively small samples eventually resulted in wide confidence intervals. Nevertheless, we have shown the robustness of our findings in several sensitivity analyses. Finally, our study mainly included individuals of European ancestry, limiting our findings' generalizability to other populations.

CONCLUSIONS

In conclusion, our findings suggest that adhering to favorable cardiovascular health in midlife could lower the remaining lifetime risk for incident T2D, regardless of T2D genetic predisposition. Multilevel intervention measures to improve the prevention of T2D are warranted.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://academic.oup.com/eurjpc/article/28/16/1850/6357208?login=false

Part III

Implication of blood pressure management in type 2 diabetes

3.1

Chapter 3.1

Comparison of blood pressure guidelines among diabetes

3.2

Chapter 3.2

Multimorbidity, blood pressure, and mortality in diabetes

Part IV

Burden of complications across the glycemic spectrum

4.1

Chapter 4.1

Glycemic spectrum and lifetime risk of cardiovascular disease

ABSTRACT

Background: Data on sex-specific lifetime risk of cardiovascular disease (CVD) across the glycaemic spectrum, in particular in impaired fasting glucose (IFG) state, are scarce. Whether overweight/obesity modifies the CVD burden also remains unclear.

Methods: Using a prospective population-based Rotterdam study, normoglycemia, IFG, and type 2 diabetes mellitus (T2D) were defined. First incident cases of coronary heart disease, heart failure, and stroke during a follow-up time until January 1st, 2015 were identified and formed the composite CVD endpoint. The remaining lifetime risks of CVD were estimated in each glucose category at 55, 65, 75, and 85 years of age, using a modified version of survival analysis adjusted for the competing risk of death.

Results: Among 5698 women and 3803 men free of CVD at baseline, the mean age was 64.5 years (SD 9.6) and 60.0% of participants were women. At age 55 years, the remaining lifetime risk of any CVD event among women was 55.1% (48.3-61.9) for IFG, compared to 52.7% (95% CI 49.5-55.9) for normoglycemia and 61.5% (54.7-68.3) for T2D. For men, the remaining lifetime risk of any CVD event was 62.1% (55.2-69.1) for IFG, compared to 59.1% (55.5-62.7) for normoglycemia and 60.3% (53.1-67.5) for T2D. At age 55 years, the lifetime risk for incident CVD was higher, albeit not statistically significant, among overweight/obese women and men with IFG compared to normal-weight women and men.

Conclusions: Impaired fasting glucose carried a large lifetime risk for incident CVD among both women and men compared with normoglycemia. In particular among men, the risk was comparable to that of T2D. Overweight/obesity modifies the risk and conferred a larger burden of lifetime CVD risk among women and men with IFG.

INTRODUCTION

As diabetes develops and progresses towards micro-and macro-vascular complications, treatment becomes more challenging, and the costs dramatically rise [43]. Impaired fasting glucose (IFG), known as prediabetes, is a state of elevated blood glucose level, yet below the threshold of type 2 diabetes mellitus (T2D) [213]. IFG is a high-risk state of T2D with a conversion rate of 5–10% annually [214]. The prevalence rate of IFG is increasing and the worldwide prevalence is estimated to reach 548.4 million in 2045 [43].

Cardiovascular disease (CVD) is the leading cause of morbidity and mortality among individuals with hyperglycemia [215]. Independently from other conventional risk factors, diabetes alone confers about the twofold excess risk for CVD [15]. However, metabolic and physiological features are dysregulated in individuals with IFG, and traditional CVD risk factors such as obesity, and dyslipidemia become more prevalent among this population [216]. Therefore, the IFG state also carries a considerable risk for CVD [217]. However, large-scale population-based studies addressing the long-term CVD burden across the entire glycaemic spectrum are limited [44, 218].

While men are at a larger risk for clinical vascular damages earlier in life, women are more susceptible to age-related vascular changes at midlife [219]. Sex differences are a major contributor to CVD heterogeneity at older ages. However, sex differences in the long-term CVD burden across the entire glycemic spectrum, in particular IFG state, remain unclear. Several studies have shown hyperglycemia associated with long-term CVD risk among men only [218], while others indicated that the risk for CVD is higher in women [44]. Besides, whether obesity modifies the CVD burden across the glycaemic spectrum in women and men remains unclear.

Using data from the large population-based Rotterdam Study, we evaluated the 10-year and lifetime risk for incident CVD across the glycaemic spectrum among women and men. In particular, we focused on the CVD risk among women and men with IFG. We have previously shown that among the general population and at age 55, though men and women have similar lifetime risks of CVD, there are considerable differences in the first manifestation. Men were more likely to develop coronary heart disease (CHD) as a first event, while women were more likely to have a heart failure (HF) or stroke as their first event [220]. Therefore, we evaluated the differences in first manifestations of CVD across different glycemic spectrums. We further studied whether the lifetime CVD burden differed by overweight/obesity status.

METHODS

Study design and population

This study is embedded within the framework of the Rotterdam Study, a prospective population-based cohort among participants of European ancestry aged ≥40 years living in the well-defined Ommoord district of Rotterdam, the Netherlands. Initially, in 1990 all inhabitants (n=10,215) aged 55 years or over were invited to participate; 7,983 of invitees agreed to participate. In 2000, 3011 participants who had reached the age of 55 years (out of 4,472 invitees) were invited to participate in the second cohort. In 2006, a third cohort included inhabitants aged 45 years and older (n=3,932), bringing the total study population to 14,926 individuals by the end of 2008. There were no eligibility criteria to enter the Rotterdam Study apart from the minimum age and residential area based on postal codes. The complete design and rationale behind the Rotterdam Study have been described in a separate publication [54].

This study included participants from the third examination of the first cohort (1997-1999), the first examinations of the second (2000-2001) and the third (2006-2008) cohorts. We included participants if they had information on prevalent diabetes status with at least one

baseline interview or clinical examination (n=10,962). We excluded prevalent CVD cases at baseline (n=1,300), and participants with missing values on prevalent CVD (n=161), eventually including 9,501 eligible people for the present analyses. **Figure 1** shows the flowchart of the study population.

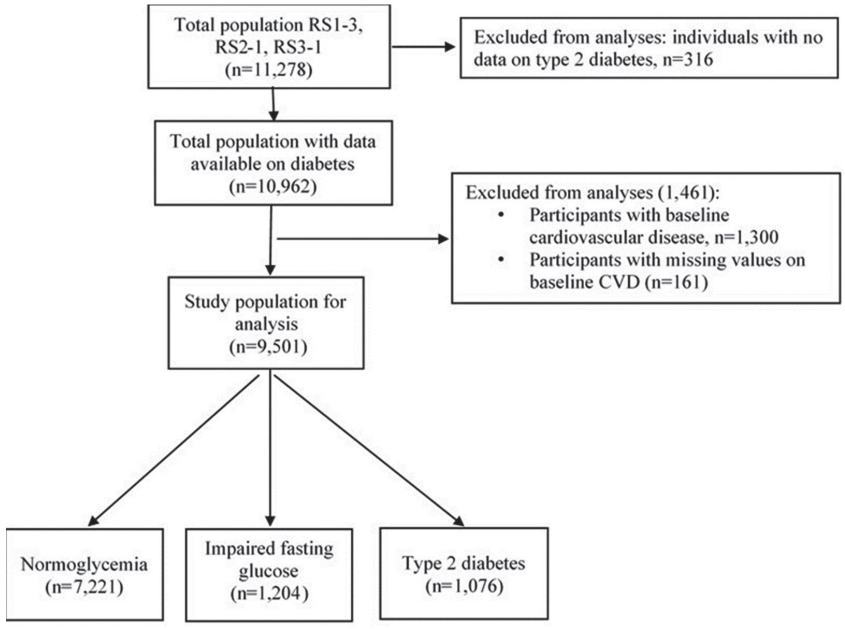

Figure 1. Flowchart for the study population.

Baseline measurements

At baseline, information was obtained on individuals' characteristics, health status, medical and medication history, and lifestyle factors. Normoglycemia, IFG, and T2D were defined based on World Health Organization (WHO) guideline [221]: normoglycemia defined as a fasting blood glucose concentration of 6.0 mmol/l or lower; IFG defined as a fasting blood glucose concentration between 6.1-6.9 mmol/l; T2D defined as a fasting blood glucose concentration of 7.0 mmol/l or higher or the use of blood glucose-lowering medications. WHO and American Diabetes Association (ADA) use different thresholds for defining normoglycemia and IFG. Therefore, due to considerable debate regarding the definition of IFG, as a sensitivity analysis, we also repeated our analyses according to the ADA guideline [222]: normoglycemia defined as a fasting blood glucose concentration below 5.6 mmol/l, IFG defined as a fasting blood glucose concentration between 5.6-6.9 mmol/l, and T2D as a fasting blood glucose concentration of 7.0 mmol/l or higher or the use of blood glucose-lowering medications. BMI was calculated as body weight (in kg) divided by the square of length (in meters). Overweight/obesity were defined as BMI ≥25 kg/m^2 *vs* normal BMI (18.5<BMI < 25 kg/m^2). All biochemical variables were assessed in serum samples taken after overnight fasting. Serum glucose (mmol/l) concentration was measured using the glucose hexokinase method and insulin concentration by metric assay (Biosource Diagnostics, Camarillo, CA). Hypertension was defined as systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg or a prescription for an antihypertensive agent. Serum total cholesterol (TC) (mmol/l), and high-density lipoprotein-cholesterol (HDL-c) (mmol/l) were both measured on the COBAS 8000 Modular Analyzer (Roche Diagnostics GmbH, Germany).

Follow-up measurements

Follow-up data on vital status and CVD events for all individuals included in the study were available. Outpatient clinic reports, hospital discharge letters, electrocardiograms, and imaging data were collected from general practitioner records and hospital records. Information on vital status was obtained from the central registry of the municipality of the city of Rotterdam. Follow-up started at baseline and individuals were followed until the occurrence of the first incident CVD event; including incident CHD, HF, and stroke, or death or the end of follow-up, January 1st, 2015.

Assessment of cardiovascular diseases

Incident CVD was a composite endpoint comprised of first incident CHD, HF, or stroke. Definitions and procedures on the adjudication of cardiovascular outcomes have been described in detail previously [223, 224]. Incident CHD was defined as fatal or non-fatal myocardial infarction, surgical or percutaneous coronary revascularization procedure, or death from CHD. Incident HF was defined following the guidelines of the European Society of Cardiology as the combination of typical symptoms and signs, confirmed by objective evidence of cardiac dysfunction or a positive response to initiated treatment. Incident Stroke was defined according to WHO criteria as a syndrome of rapidly developing clinical signs of focal (or global) disturbance of cerebral function, with symptoms lasting 24 hours or longer or leading to death, with no apparent origin other than vascular.

Statistical analysis

Data were firstly assessed visually for normality. We performed descriptive statistics by reporting mean (standard deviation (SD)) or median (interquartile range (IQR)) for continuous variables and numbers (percentage) for categorical variables. Baseline characteristics in individuals with different levels of baseline serum glucose were compared using the ANOVA test.

All analyses were performed across different levels of baseline serum glucose including normoglycemia, IFG, and T2D, separately for women and men. Among women and men with normoglycemia, IFG and T2D, we calculated the remaining lifetime risks for any CVD (first incident of CHD, HF, or stroke) at age 55, 65, 75, and 85 years taking into account competing risks in all calculations of observed risks. The lifetime risk estimates reflect the remaining risk at the indexed age to the age of last observation; in our study maximum age was 105.8 years.

To compare the lifetime risks with the absolute risks in a shorter period, we further calculated a 10-year risk for all outcomes of interest at all index ages. We used a modified version of survival analysis for the calculation of the absolute short and lifetime risks. In this type of analysis, at each age category, the incidence of each CVD outcome is calculated during follow-up [225]. When there is a competing event, the Cumulative Incidence Function (CIF) uses the overall survival function S(t) that counts failures from competing events in addition to the event of interest. By using the overall survival function, CIF bypasses the need to make unverifiable assumptions of independence of censoring on competing events. To assess the impact of overweight/obesity, the analyses were additionally stratified by BMI (BMI$\geq$25 kg/m^2 compared to 18.5<BMI<25 kg/m^2).

As a sensitivity analysis, we repeated the analyses based on ADA-defined thresholds to test whether the different thresholds for normoglycemia and IFG according to the ADA guideline influence the results. In another set of sensitivity analyses, individuals on lipid-lowering medications were excluded from the analyses. We also calculated the lifetime risk of any CVD event only among diabetic individuals on glucose-lowering medication at baseline,

as they are a group of patients with a more severe diabetes profile. We also compared the overall difference of lifetime risk estimates in women and men across glycemic categories and subgroup analyses by the Fine-Gray method based on sub-hazard distributions [163]. Fine-Gray proposes a proportional hazards model by treating the CIF curve as a sub-distribution function.

Missing values on CVD risk factors were imputed using 10-fold multiple imputations. Covariates included in our imputation models were baseline age, sex, prevalent IFG, T2D/glycemic status, first incident outcomes, vital status, hypertension, total cholesterol, HDL-cholesterol, and lipid-lowering medications. We used $P<0.05$ as the significance level. All measures of association are presented with 95% confidence intervals. All analyses were conducted in SPSS software version 26 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) and R statistical software, version 3.6.3.

RESULTS

We used data from 9,501 participants of the Rotterdam Study. In total, the mean age of the population was 64.1 years (SD 9.7) and 5,698 (60.0%) participants were women. On average, women were older than men. At baseline, the majority had normoglycemia (76.0%), whereas 12.7% had IFG and 11.3% had T2D. Prevalence rates of IFG and T2D were significantly higher among men (15.0% and 12.6% respectively) compared to women (11.1% and 10.5% respectively) (**Table 1**). In both women and men, compared with normoglycemia, individuals with IFG or T2D had a more unfavorable CVD risk profile including higher BMI, and a larger proportion of individuals used blood pressure-lowering or lipid-lowering medications.

Table 1. Baseline characteristics of women and men in the study population across the glycaemic spectrum

	Women (n= 5,698)			Men (n= 3,803)		
	Normoglycemia (n=4,468)	**IFG (n=634)**	**Type 2 diabetes (n=596)**	**Normoglycemia (n=2,753)**	**IFG (n=570)**	**Type 2 diabetes (n=480)**
Age, years	63.8 (9.8)	67.0 (9.6)	68.3 (10.3)	62.7 (9.2)	64.1 (8.3)	65.5 (9.0)
BMI, kg/m^2	26.8 (4.3)	29.0 (5.0)	30.2 (5.2)	26.5 (3.3)	28.0 (3.6)	28.7 (4.4)
Overweight, n (%)	2777 (62.2)	509 (80.3)	508 (85.2)	1830 (66.5)	468 (82.1)	382 (79.6)
Hypertension, n (%)	2392 (53.5)	472 (74.4)	479 (80.4)	1499 (54.4)	410 (71.9)	387 (80.1)
Antihypertensive medication, n (%)	1184 (26.5)	260 (41.0)	315 (52.9)	607 (22.0)	184 (32.3)	215 (44.8)
Total cholesterol, mmol/l	5.9 (1.0)	5.9 (0.9)	5.7 (1.0)	5.6 (0.9)	5.7 (1.0)	5.3 (1.1)
HDL cholesterol, mmol/l	1.6 (0.4)	1.5 (0.4)	1.3 (0.4)	1.3 (0.3)	1.2 (0.3)	1.1 (0.3)
Lipid-lowering medication, n (%)	628 (14.1)	124 (19.6)	139 (23.3)	366 (13.3)	82 (14.4)	128 (26.7)

P-values for all cardiovascular risk factors for both women and men were significant at <0.001.

During a median follow-up of 8.4 years, 1,071 CVD events (18.9 per 1000 person-years (PY)) occurred among women and 910 CVD events (26.0 per 1000 PY) among men. The corresponding numbers of events were 352 for CHD (6.1 per 1000 PY), 489 for HF (8.2 per 1000 PY), and 474 for stroke (8.0 per 1000 PY) among women and 463 CHD (13.0 per 1000 PY), 323 HF (8.5 per 1000 PY), 330 stroke (8.7 per 1000 PY) among men. CVD mortality rates were almost 8% in both women and men.

Table 2. Remaining lifetime risks versus 10-year risks of first incident cardiovascular disease by glycaemic status

		Normoglycemia		Impaired fasting glucose		Type 2 diabetes	
		Cumulative incidence, % (95%CI)		Cumulative incidence, % (95%CI)		Cumulative incidence, % (95%CI)	
		Women (n=4,468)	Men (n=2,753)	Women (n=634)	Men (n=570)	Women (n=596)	Men (n=480)
Cardiovascular disease							
N events		740	608	149	147	182	155
Age 55	10-year	47.7 (44.3-51.1)	53.9 (50.1-57.7)	47.2 (39.9-54.4)	56.5 (49.0-64.0)	65.3 (58.6-72.1)	63.8 (56.9-70.7)
	Lifetime	52.7 (49.5-55.9)	59.1 (55.5-62.7)	55.1 (48.3-61.9)	62.1 (55.2-69.1)	61.5 (54.7-68.3)	60.3 (53.1-67.5)
Age 65	10-year	46.6 (43.1-50.1)	47.5 (43.3-51.8)	45.9 (38.5-53.3)	52.5 (44.6-60.4)	63.7 (57.3-70.1)	57.5 (50.1-64.9)
	Lifetime	52.4 (49.2-55.7)	55.5 (51.7-59.4)	49.9 (48.1-61.8)	59.4 (52.1-66.7)	61.4 (54.8-67.9)	56.4 (48.9-63.9)
Age 75	10-year	43.2 (39.2-47.2)	39.7 (34.4-45.0)	43.0 (34.3-51.7)	46.9 (37.0-56.7)	58.8 (51.7-66.0)	50.2 (41.0-59.3)
	Lifetime	50.8 (47.1-54.5)	51.3 (46.5-56.0)	53.6 (45.5-61.7)	56.0 (47.0-65.1)	58.1 (50.9-65.4)	51.1 (42.0-60.3)
Age 85	10-year	37.4 (32.1-42.7)	32.8 (23.7-41.8)	34.2 (22.9-45.4)	36.6 (20.0-53.2)	45.9 (35.3-56.5)	44.9 (29.0-60.8)
	Lifetime	43.9 (38.8-49.0)	48.1 (39.9-56.3)	49.7 (39.3-60.2)	46.0 (29.7-62.4)	47.9 (37.5-58.4)	45.6 (29.0-62.2)
Coronary heart disease							
N events		238	328	50	65	64	70
Age 55	10-year	16.8 (14.4-19.2)	29.3 (26.2-32.5)	15.1 (10.4-19.7)	25.9 (19.4-32.3)	28.7 (22.2-35.1)	34.7 (27.8-41.6)
	Lifetime	18.8 (16.4-21.2)	33.3 (29.9-36.6)	18.0 (12.8-23.1)	30.2 (23.7-36.6)	24.0 (18.0-30.2)	30.9 (24.2-37.6)
Age 65	10-year	15.5 (13.1-17.9)	21.1 (18.2-24.1)	13.4 (9.1-17.8)	18.9 (13.3-24.6)	25.0 (19.3-30.8)	27.9 (21.6-34.3)
	Lifetime	18.0 (15.6-20.4)	27.5 (24.2-30.8)	17.6 (12.5-22.7)	24.6 (18.7-30.6)	21.0 (15.6-26.4)	27.1 (20.7-33.4)
Age 75	10-year	12.5 (10.0-15.0)	13.1 (10.1-16.0)	10.4 (6.2-14.7)	11.3 (5.8-16.9)	19.2 (13.8-24.6)	20.8 (14.3-27.4)
	Lifetime	15.7 (13.2-18.3)	21.2 (17.5-24.8)	14.3 (9.0-19.6)	18.9 (12.6-25.1)	18.0 (12.7-23.3)	22.2 (15.5-29.0)
Age 85	10-year	9.9 (7.0-12.9)	8.0 (4.1-12.0)	3.2 (0.1-6.3)	6.9 (0.3-13.5)	12.6 (6.6-18.6)	10.4 (2.4-18.3)
	Lifetime	11.8 (8.9-14.8)	16.1 (10.8-21.5)	11.1 (5.1-17.0)	7.5 (1.1-13.9)	12.7 (6.6-18.8)	14.8 (5.2-24.4)
Heart failure							

N events		329	212	76	51	84	60
Age 55	10-year	23.2 (20.3-26.1)	24.2 (20.9-27.5)	23.8 (18.0-29.6)	29.5 (22.9-36.1)	35.0 (28.4-41.6)	32.9 (26.8-39.0)
	Lifetime	26.9 (24.0-29.9)	30.3 (26.7-33.9)	32.6 (26.4-38.9)	31.8 (25.2-38.5)	35.1 (28.6-41.6)	31.2 (25.2-37.2)
Age 65	10-year	22.9 (20.0-25.9)	23.2 (19.8-26.6)	23.1 (17.3-28.9)	28.5 (21.9-35.2)	32.9 (26.8-39.0)	31.3 (25.4-37.3)
	Lifetime	27.2 (24.2-30.2)	29.8 (26.1-33.6)	32.3 (26.0-38.7)	31.0 (24.3-37.7)	33.5 (27.4-39.6)	30.3 (24.4-36.2)
Age 75	10-year	22.0 (18.8-25.2)	20.6 (16.8-24.4)	22.0 (15.6-28.4)	26.8 (19.3-34.2)	27.3 (21.2-33.4)	30.0 (23.3-36.8)
	Lifetime	27.3 (24.0-30.6)	28.3 (24.1-32.6)	32.5 (25.3-39.7)	31.1 (23.6-38.7)	29.5 (23.4-35.6)	30.5 (23.8-37.2)
Age 85	10-year	18.8 (14.8-22.8)	18.8 (12.9-24.7)	14.8 (7.5-22.1)	22.5 (11.1-33.8)	18.3 (11.0-25.6)	19.9 (10.4-29.5)
	Lifetime	23.4 (19.3-27.6)	28.9 (22.1-35.8)	29.0 (20.1-37.8)	28.2 (16.6-39.8)	24.2 (16.5-31.9)	21.7 (12.0-31.4)
Stroke							
N events		322	213	64	52	88	65
Age 55	10-year	22.7 (20.0-25.5)	22.2 (18.8-25.5)	23.9 (18.2-29.7)	24.8 (18.3-31.2)	37.6 (30.1-45.2)	30.7 (24.4-37.1)
	Lifetime	25.5 (22.8-28.2)	24.9 (21.5-28.3)	27.5 (21.5-33.5)	28.2 (21.6-34.8)	33.1 (26.4-39.8)	28.7 (22.5-34.9)
Age 65	10-year	22.1 (19.4-24.5)	20.6 (17.1-24.1)	24.5 (18.6-30.4)	22.4 (16.5-28.3)	33.2 (27.1-39.4)	24.0 (18.6-29.5)
	Lifetime	25.3 (22.6-28.1)	24.2 (20.7-27.6)	28.1 (22.0-34.2)	26.1 (20.0-32.3)	31.7 (25.6-37.8)	23.0 (17.6-28.4)
Age 75	10-year	20.7 (17.7-23.6)	16.7 (12.7-20.6)	20.7 (14.4-27.0)	19.7 (13.2-26.2)	30.8 (24.6-37.1)	20.9 (14.9-26.8)
	Lifetime	24.0 (21.1-27.0)	21.1 (17.1-25.0)	24.8 (18.1-31.5)	22.4 (15.6-29.3)	29.0 (22.8-35.2)	21.6 (15.6-27.7)
Age 85	10-year	17.3 (13.7-20.8)	14.7 (8.5-20.9)	17.2 (9.-24.5)	12.3 (3.6-21.1)	24.8 (16.9-32.7)	20.2 (10.6-29.8)
	Lifetime	18.5 (15.0-22.0)	19.9 (13.8-26.1)	22.9 (14.9-30.9)	16.7 (7.1-26.4)	22.8 (15.1-30.6)	21.2 (11.2-31.1)

As shown in **Table 2** and **Figure 2**, at age 55 years, the remaining lifetime risk of incident CVD event was 55.1% (95% CI 48.3-61.9) for IFG, compared to 52.7% (49.5-55.9) for normoglycemia, and 61.5% (54.7-68.3) for T2D in women. Among women, the lifetime risk for CVD was larger among T2D individuals compared to non-diabetic and the difference was statistically significant.

In men, the remaining lifetime risk for incident CVD event was 62.1% (55.2-69.1) for IFG, compared to 59.1% (55.5-62.7) for normoglycemia, and 60.3% (53.1-67.5) for T2D. Among men at 65 years of age, corresponding estimates were 59.4% (52.1-66.7) for IFG, compared to 55.5% (51.7-59.4) for normoglycemia, and 56.4% (48.9-63.9) for T2D. In men, the magnitude of differences in lifetime CVD risk between the three glucose categories was smaller and the risk in men with IFG was as high as men with T2D.

The gradient in cumulative incidence risk of any CVD across the glycaemic spectrum event differed by sex. Compared with women, the overall remaining lifetime risk for incident CVD events was higher in men with baseline glucose levels below the threshold of T2D (both normoglycemia and IFG). In both women and men, the cumulative incidence of CVD increased steadily with age (**Table 2** and **Figure 2**).

Compared with the lifetime risks, the 10-year risk of any CVD events was lower at all glucose spectrums and all the index ages (**Table 2**).

At age 55 years, the lifetime risks of CHD were 18.0% (12.8-23.1) for IFG, compared to 18.8% (16.4-21.2) for normoglycemia, and 24.0% (18.0-30.2) for T2D among women and 30.2% (23.7-36.6) for IFG, 33.3% (29.9-36.6) for normoglycemia, and 30.9% (24.2-37.6) for T2D among men. Compared to women, men were more likely to develop CHD as the first manifestation of CVD across all glucose spectrum (p-value<0.001).

At age 55 years, the lifetime risks of HF were 32.6% (26.4-38.9) for IFG, 26.9% (24.0-29.9) for normoglycemia, and 35.1% (28.6-41.6) for T2D among women and 31.8% (25.2-38.5) for IFG, 30.3% (26.7-33.9) for normoglycemia, and 31.2% (25.2-37.2) for T2D among men. Among women, there was a clear trend for increasing the remaining lifetime risk of HF from normoglycemia to T2D (p-value=0.02). This trend did not exist among men.

At age 55 years, the lifetime risks of stroke were 27.5% (21.5-33.5) for IFG, 25.5% (22.8-28.2) for normoglycemia, and 33.1% (26.4-39.8) for T2D among women and 28.2% (21.6-34.8) for IFG, 24.9% (21.5-28.3) for normoglycemia, and 28.7% (22.5-34.9) for T2D among men. Compared to men with T2D, diabetic women were more likely to develop stroke as the first manifestation of CVD (p-value<0.001).

Stratification by BMI showed that overweight/obese individuals had an increased risk of CVD throughout the glucose spectrum. At age 55 years, among IFG category, compared to women [48.5% (34.6-62.5)] and men [56.9% (40.6-73.1)] with normal BMI, the lifetime risk of CVD was higher among both women [56.4% (48.6-64.2) and men [66.1% (58.3-74.0)] who were overweight/obese. The lifetime risk of CVD was statistically significantly the highest among overweight/obese women with T2D [64.7% (57.5-71.6)], compared to diabetic women with normal BMI [42.8% (25.5-60.1)]. In men, the patterns were different in which diabetic men with normal BMI [75.1% (61.5-88.8)] compared to diabetic men with overweight/obesity [55.8% (47.2-64.4)] were more likely to develop CVD events (**Table S1, Figure 3&4**).

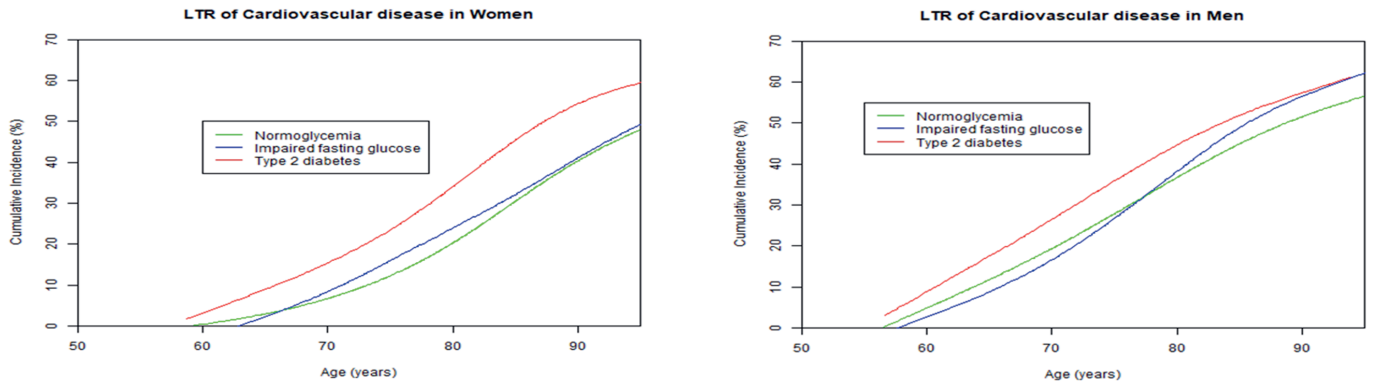

Figure 2. Cumulative incidence of cardiovascular disease defined as the composite endpoint of coronary heart disease, heart failure, and stroke in individuals aged 55 years, adjusted for the competing risk of death.

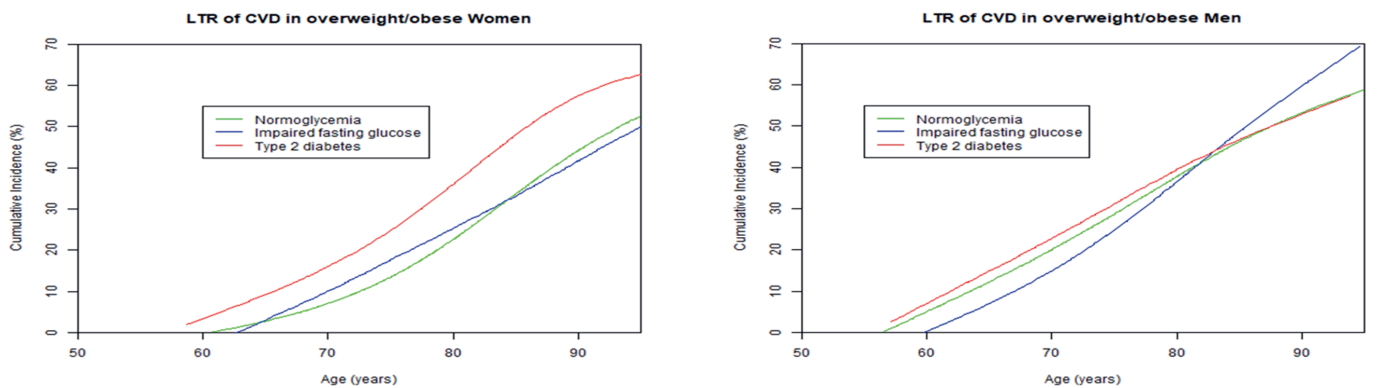

Figure 3. Cumulative incidence of cardiovascular disease defined as the composite endpoint of coronary heart disease, heart failure, and stroke in individuals aged 55 years, adjusted for the competing risk of death, for overweight/obese women and men.

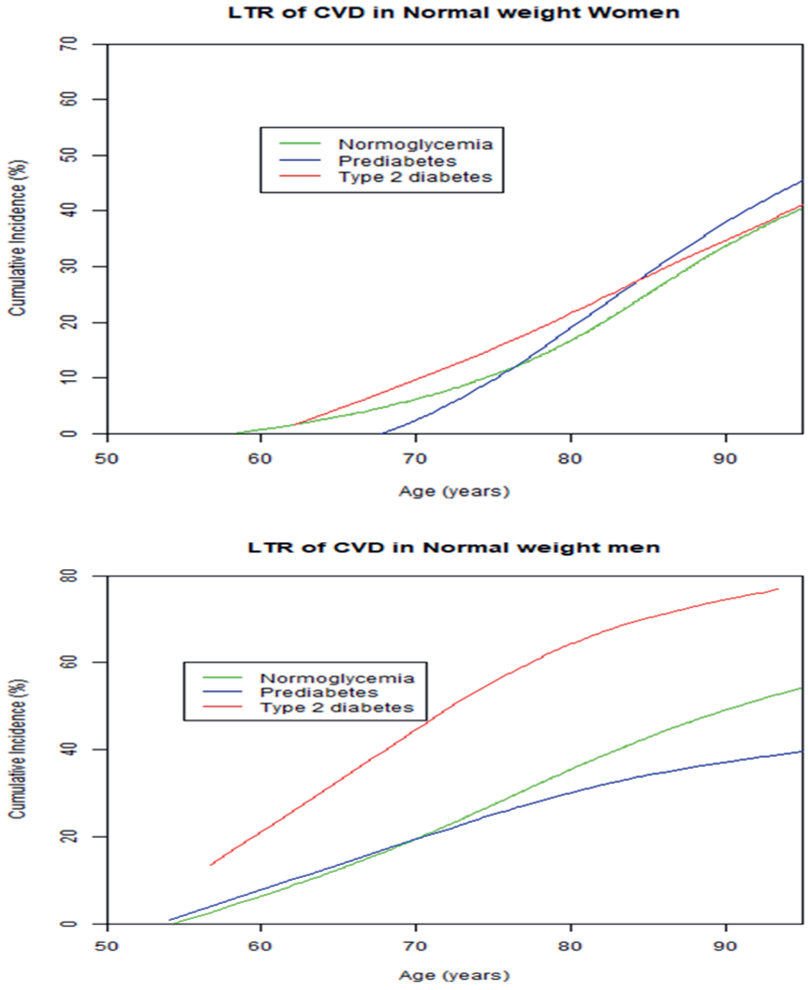

Figure 4. Cumulative incidence of cardiovascular disease defined as the composite endpoint of coronary heart disease, heart failure, and stroke in individuals aged 55 years, adjusted for the competing risk of death, for women and men with normal weight.

When we defined IFG according to the ADA guideline, men with IFG at age 55 years had greater attenuation in remaining lifetime risk for incident CVD event [59.6% (55.1-64.0)] compared to women with IFG [54.5% (50.3-58.8)] (**Figure S1**). Excluding individuals on lipid-lowering medications did not change the overall picture (data not shown). When the analyses were limited to only T2D individuals who took glucose-lowering medications at baseline, the remaining lifetime risk of any CVD event at age 55 remained the same (data not shown).

DISCUSSION

In a large well-designed prospective population-based study, impaired fasting glucose at age 55 years carried a large lifetime risk for CVD among both women and men. In particular among men, the remaining lifetime risk of CVD in those with IFG was comparable to that of T2D. At age 55 years, the lifetime risk of any CVD event was higher among overweight/obese individuals with IFG compared with normal weight individuals, albeit not statistically significant.

Impaired fasting glucose is a prevalent condition in the general population. In our study, the prevalence rate of IFG was 11% in women and 15% in men. Although several studies have suggested that individuals with IFG are not necessarily at increased risk of CVD [44, 226], it is well established that the initiation and progression of vascular dysfunction occur during the prediabetes stage [227]. Similar to diabetes, IFG coincides with the presence of other common cardiovascular risk factors such as obesity, hypertension, and dyslipidemia. Therefore, individuals with IFG are at high risk of developing CVD outcomes. Several common pathophysiological features underlie the effect of long-term hyperglycemia on vascular damage. Among them, the excess glucose level in the bloodstream may contribute to endothelial dysfunction through an imbalance between endothelium-derived relaxing and contracting factors. NADPH oxidases (NOX) are membrane-bound proteins that catalyze the conversion of oxygen to superoxide particularly under conditions of hyperglycemia. It has been well established that endothelial dysfunction contributes to the onset of CVD [228].

In our study, at age 55 years, IFG carried a large lifetime risk for CVD particularly among men that were comparable to that of T2D. In line with our findings, in the Koran Heart Study including 408,022 individuals, a greater CVD risk has been associated with IFG only in men [229]. While a previous meta-analysis of 29 prospective studies (n= 194,658) in 2004 and the Framingham Heart Study (n=4,058) in 2008 have reported a greater CVD risk only in women [230, 231]. Similar CVD risks for women and men (n= 237,468) have been reported in the Asia Pacific region [232]. Levels of cardiovascular risk factors have been shown to differ between normoglycemic women and men before the conversion to prediabetes and, eventually, diabetes [233, 234]. Sex differences might, at least partly, be explained by sex-specific patterns in the management of hypertension and dyslipidemia. In our study, men compared to women with IFG, had lower proportions of antihypertensive (34% vs. 44%) and lipid-lowering (14% vs. 20%) medication use. Controlling blood pressure and lipid levels is widely recommended to prevent vascular risk in individuals with hyperglycaemia. Our findings, together with previous results, raise a question of whether sex differences in CVD burden associated with IFG are due to biological differences (e.g., sex hormones), or are driven by a coincidence of several metabolic and behavioral risk factors (e.g., BMI and physical activity) than sex-differences per se.

The lifetime risk at age 55 for developing CVD among individuals with T2D was 62% for women and 60% for men through 106 years of age (in our study maximum age was almost 106 years). In line with our study, the Framingham Heart Study showed a greater risk of developing CVD among diabetic individuals: 57% for women and 67% for men through 95 years of age [235]. However, in a recent study performed in seven observational cohorts of U.S. black and white men and women, the reported risk was almost 20% lower compared with our results [218]. Different CVD risk estimates might be due to several factors including demographic differences in participants included in these studies. In particular different cardiovascular risk profiles at baseline, different practices to treat cardiovascular risk factors or more effective prevention of CVD outcomes, varying definitions of CVD events, and CVD burden in different populations, as well as the different durations of follow-up. Additionally,

different methods of assessing glucose levels such as fasting glucose or glycosylated hemoglobin A_{1c} level (HbA_{1c}) may have contributed to the heterogeneous results so far [236]. Of note, most of the studies, including the previous study by Michael and colleagues [218], have not included HF as an outcome. Several previous studies have reported that the risk of HF in patients with T2D is more than twice compared with individuals free of diabetes. In our study, the lifetime risk of HF was significantly higher among individuals with IFG and T2D compared to normoglycemic individuals. The increased risk for HF in patients with hyperglycemia could partly be explained by shared risk factors such as age, obesity, dyslipidemia, and hypertension [237].

The 10-year risk of any CVD events was lower at all glucose spectrums and all the index ages when compared with the lifetime risks. 10-year risk corresponds to the risk for an individual to develop CVD in the coming 10 years while lifetime risk for an individual is the risk to develop CVD for the remaining of his/her lifespan and thus over a longer course than 10-year.

Overweight/obesity modifies the CVD risk in which overweight/obese women and men with IFG had a greater lifetime risk of CVD compared with their counterparts with normal BMI. Our study also revealed that overweight/obese women with T2D had a higher lifetime risk of CVD compared with diabetic women with normal BMI. This implies that the risk of cardiovascular complications associated with hyperglycemia could partly be driven by overweight/obesity. Moreover, despite that the lifetime risk of CVD among overweight/obese men with IFG was higher than women with IFG (66% vs. 56%), this risk was higher in women with T2D than their men counterparts (65% vs. 56%). Different risk profiles in women and men could be due to physiological differences between women and men including the levels of subcutaneous fat storage, hormonal, or genetic differences [234]. A higher risk of CVD that we observed in T2D women compared to men with T2D may be the result of greater deterioration in cardiovascular risk profile in diabetic women [238]. Women need to attain a larger average BMI to be diagnosed with T2D [234]. Therefore, compared to men, diabetic women might require a greater metabolic deterioration to develop CVD. Better management of hyperglycemia through sex- or gender-tailored lifestyle or pharmacological interventions helps to modify BMI, which can eventually be used as an effective tool to prevent both IFG and T2D and their complications. Previous studies suggest that lifestyle interventions result in significant improvements in reducing CVD risk [239], particularly in women [240].

This study has some strengths and limitations. The population-based nature, the large sample size, long-term follow-up, and the availability of detailed data on various forms of cardiovascular events, as well as cardiovascular risk factors, are among the strengths of our study. However, the limitations of our study also merit attention. To categorize the status of IFG and T2D, HbA_{1c} measurement was not available, and we used fasting serum glucose measurements which may have led to some misclassification of T2D. However, our findings indicated that fasting glucose level, even in the non-diabetic range, could be a marker of CVD risk. Moreover, the measurements of fasting blood glucose were at baseline, which could have led to participants' misclassification during follow-up. Our results regarding the magnitude of the differences between women and men in several categories did not reach statistical significance. This might be due to the relatively small sample size of individuals with IFG and T2D and therefore limited statistical power to detect the potential sex differences. Furthermore, to estimate the impact of BMI on the lifetime risk of CVD, we used anthropometric data at baseline. This may lead to some misclassifications as BMI tends to change with age. Finally, as nearly all the Rotterdam Study participants are from European ancestry, our results may not be generalizable to other ethnicities.

CONCLUSIONS

Our results underscore the importance of cardiovascular risk assessment across the glycaemic spectrum particularly among individuals with impaired fasting glucose. Our study suggests that guideline recommendations to prevent CVD need to go beyond the diabetes status and also consider the high risk of CVD in the prediabetes stage, in particular among middle-aged men. Future studies are warranted to investigate the sex-specific impact of modifiable cardiovascular risk factors over time and their preventive implications for women and men.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://drc.bmj.com/content/9/1/e002406.long

4.2

Chapter 4.2

Prediabetes and risk of cardiovascular disease and mortality

4.3

Chapter 4.3

Glycemic status and cognitive and physical functioning decline

ABSTRACT

Background: Evidence about the decline of cognition and physical function across glycemic status (normoglycemia, prediabetes, and diabetes) are inconsistent. We evaluated longitudinal changes in cognition and physical function according to glycemic status and also different glycemic transitions.

Methods: 9307 participants (mean age: 59.7 years, 53.7% women) were included from the China Health and Retirement Longitudinal Study (2011-2018). Global cognition (assessed by orientation, memory, and executive function) and physical function (calculated as the sum of impaired basic and instrumental activities of daily living) were assessed in each wave. The glycemic status was assessed in waves 2011 and 2015 (Diabetes, defined as fasting blood glucose ≥7.0 mmol/L, HbA1c ≥6.5%, self-reported diabetes, or glucose-lowering medication use; Prediabetes, defined as fasting blood glucose between 5.6 to 6.9 mmol/L or an HbA1c of 5.7-6.4%).

Results: Compared to normoglycemia, baseline diabetes was associated with a faster decline in orientation (-0.018 SD/year, 95%CI -0.032, -0.004) and a faster increase in physical function score (0.082 /year, 95%CI 0.038, 0.126). We did not observe any effect of prediabetes on the changing rate of cognition and physical function. Progression from normoglycemia to diabetes between waves 2011 and 2015 was associated with a significantly faster decline in global cognition, memory, executive function, and physical function compared to stable normoglycemia.

Conclusions: Baseline diabetes was associated with accelerate decline of cognition and physical function. Associations with prediabetes were not observed, suggesting an important short diagnostic window when diabetes de novo presents.

INTRODUCTION

Type 2 diabetes (hereinafter referred to as diabetes), characterized by hyperglycemia, constitutes a serious health burden affecting approximately 463 million adults worldwide [260]. Compared to well-known diabetes-related complications such as cardiovascular disease, the effects of hyperglycemia on cognition and physical functioning have so far been less investigated. Diabetes has been associated with a slight cognitive decline, probably starting during the prediabetic stage [261]. Nevertheless, the relationship between prediabetes and cognitive function is less clear, with results of both harmful [262-265], and null effects reported [266]; and most of these studies were conducted in Western populations. In addition, prediabetes is also related to various vascular disorders, potentiating cognitive and functional decline early before the onset of diabetes [267]. By now, only one longitudinal study, conducted among the Swedish elderly (≥60 years old), has specifically reported the accelerated physical function decline among participants with prediabetes and diabetes [268]. As such, significant knowledge gaps remain.

In China, the prevalence rate of diabetes has increased dramatically, rising from 0.67% in 1980 to 11.2% in 2017. Currently, Chinese patients account for 24% of the global patients with diabetes [179]. Even more Chinese adults live with prediabetes: a shocking prevalence rate of 35.2% has been estimated [269]. Ethnic differences substantially contribute to differences in the prevalence and health effects of prediabetes [270, 271] and might influence the age of onset of diabetes-associated cognitive decline [272]. The latter has not been analyzed in the Asian population and in middle age (45~60 years old). Moreover, prediabetes is not a robust diagnostic entity, especially at older age (≥60 years old) [242, 273]. The effects of transitions between normoglycemia, prediabetes, and diabetes during follow-up in changes of cognition and physical function have never been studied.

Therefore, we investigated the longitudinal changes in cognition and physical function across the glycemic spectrum (including normoglycemia, prediabetes, and diabetes) in the middle-aged and older Chinese population of the population-based China Health and Retirement Longitudinal Study (CHARLS). In addition, we determined the impact of the transition of glycemic statuses during follow-up.

METHODS

Study design and population

The CHARLS is a community-based longitudinal survey of the Chinese population of middle-aged and elderly (≥45 years old). The detailed study design has been described elsewhere [56, 186]. Briefly, the baseline survey was conducted between June 2011 and March 2012 with individuals selected through multistage probability sampling. Follow-up is performed every two years with physical measurements and blood samples. Three follow-up waves (2013, 2015, 2018) were completed after the baseline survey. The CHARLS has been approved by the Peking University Institutional Review Board. Written informed consent was obtained for all study participants.

A detailed flow chart for participants' selection is shown in **Figure S1**. Of the 9882 participants, who attended physical and clinical visits at baseline, 575 were excluded for the

following reasons: self-reported doctor-diagnosed mental disease (n=390) or unavailable information to define baseline glycemic statuses (n=185). Then, from the 6553 participants who had complete cognition measurement at baseline, 1271 were excluded because of loss to follow-up (n=1064) or missing covariates (n=207). Hence, 5282 participants were included in the present cognition analyses. Similar to the disability analysis, from 9162 participants who had complete baseline physical function measurements, 721 were excluded because of loss to follow-up (n=334) or missing covariates (n=387), and 8441 participants were included in the final analysis.

Measurements

Structured questionnaires were administered by trained field workers using a face-to-face computer-assisted personal-interview system to collect demographic, lifestyle, and medical history data. Body mass index (BMI) was calculated as body weight divided by the square of height (kg/m^2). Education level was classified as no formal education, elementary school, middle school, and high school or higher level of education. Smoking status was assessed using the questions: "Have you ever chewed tobacco, smoked a pipe, smoked self-rolled cigarettes, or smoked cigarettes/cigars?" and "Do you still have the habit or have you quit?", and was further defined as never, former, and current. Alcohol consumption was assessed using the question, "Did you drink any alcoholic beverages, such as beer, wine, or liquor in the past year? If yes, how often?". Blood pressure was measured three times at a sitting resting position, and the mean was used for the analyses. Information about the use of blood pressure- and blood glucose-lowering drugs were collected using the question, "Are you now taking any of the following treatments to treat hypertension/diabetes? Taking Chinese traditional medicine, Western modern medicine, or other treatments?". We defined prevalent chronic diseases as the number of self-reported doctor-diagnosed chronic diseases (heart disease, stroke, chronic lung disease, asthma, and cancer) [274].

Blood samples were available from the baseline and the wave 2015 [56, 186]. Participants were asked to fast overnight before collection. Blood glucose was tested using the Hexokinase method, and glycated hemoglobin (HbA1c) was measured using the high-performance liquid chromatography method. Triacylglycerol, total cholesterol, and HDL-cholesterol (HDL) were assessed with routine clinical chemistry methods.

Definitions of prediabetes and diabetes

We defined fasting status as the participant reported fasting over 8 hours before blood sample collection. According to the 2020 American Diabetes Association criteria [275], diabetes was defined as fasting blood glucose ≥7.0 mmol/L (126 mg/dL), non-fasting blood glucose ≥11.1 mmol/L (200 mg/dL), HbA1c level ≥47.5 mmol/mol (6.5%), self-reported doctor-diagnosed diabetes, or current use of blood glucose-lowering medication. Prediabetes was defined as fasting blood glucose in the range of 5.6-6.9 mmol/L (100-125 mg/dL) or an HbA1c level of 38.8-46.4 mmol/mol (5.7-6.4%). In addition, we also studied the glycemic transition statuses (normoglycemia - normoglycemia; normoglycemia - prediabetes; normoglycemia - diabetes; prediabetes - normoglycemia; prediabetes - prediabetes; and prediabetes - diabetes) between baseline and wave 2015 among baseline nondiabetic participants.

Cognitive and disability assessments

Both cognitive and disability assessments have been performed in all waves. Participants underwent a battery of three cognitive tests, for the cognitive assessment, including orientation, memory, and executive function, with higher scores indicating better cognitive function. Orientation was assessed by asking questions regarding the date (year, month, day of month, and day of week) and season, and then allocated 1 point to each correct answer with the sum score ranging from 0 to 5. Memory was determined by testing immediate and delayed recall of ten unrelated words. The sum of words, which were successfully recalled in these two recall tests, was used as the composite memory score, ranging from 0 to 20. The executive function was assessed by the figure drawing test. The participant was asked to observe and draw a picture of two overlapping pentagons and the serial seven test. The participant was asked to subtract 7 from 100 (up to five times). The executive function score was the sum of these two tests, ranging from 0 to 6. The reliability and validity of these cognitive tests have been well-documented [264, 276].

The *z* scores were calculated to allow direct comparisons across different cognitive tests. Specifically, we standardized the follow-up score by subtracting the mean of the baseline score and then dividing it by the baseline standard deviation (SD). The global cognitive *z* score was estimated by averaging the *z* scores from the three tests and then standardizing it to baseline using the mean and SD of the global cognitive *z* score. Therefore, a unit of *z* score would mean the one SD above the mean baseline score.

Physical function was assessed by the activities of daily living (ADL: dressing, bathing, eating, getting in or out of bed, toileting, continence) and instrumental activities of daily living (IADL: doing household chores, preparing hot meals, shopping for groceries, managing money, taking medications). Each item was scored from 0 to 3 (0: no difficulty, 1: some difficulty but can still do it, 2: much difficulty and need help, 3: unable). The ADL and IADL scores were the sums of their components, and the level of physical dysfunction was assessed as the sum of these two, ranging from 0 to 33, with higher scores indicating worse ability.

Statistical analysis

Baseline characteristics are presented as mean (SD) or median (interquartile range, IQR) for continuous variables and frequency (percentage) for categorical variables. Linear mix-effect models were used to investigate the difference in annual changes in cognition and functioning between baseline glycemic statuses, using normoglycemia as the reference. We used the follow-up time (years since baseline) as a time scale. We fitted the models with the intercept and the time term as random effects accounting for inter-individual differences at baseline and changing rates in outcome variables during follow-up. For the fixed-effects part, we first included baseline glycemic status, time, glycemic status × time interaction, baseline age, and sex. The "glycemic status × time" interaction term indicated a differential changing rate. We also adjusted for possible confounders, including education level, BMI, smoking status, alcohol consumption, triacylglycerol, total cholesterol, HDL, systolic blood pressure, blood pressure-lowering medication, and prevalent chronic diseases.

To check for any possible effect modification caused by age or sex, we separately added a

three-way product interaction term (glycemic status × time × baseline age; glycemic status × time × sex) into the model and further explored these by stratification. A p-value <0.10 indicated a significant interaction as a relaxation of type I error. Age stratification was based on the median age (60 years) to ensure the sample size for subgroup analysis and the comparability to the former study [268].

In addition, we used the Sankey plot to explore the impact of glycemic transition statuses between baseline and wave 2015 among baseline nondiabetic participants. Then we investigated the difference in annual changes in cognition and functioning between different glycemic transitions with the stable normoglycemia (normoglycemia - normoglycemia) used as the reference.

To deal with the missing values, we used a multiple imputation, chained-equations method to replace missing data for cognitive and functioning reassessments, respectively. Variables used in the predictive mean matching model to impute the missing values of outcomes included the baseline information (baseline age, sex, education level, body mass index, smoking status, alcohol consumption, systolic blood pressure, use of blood pressure-lowering medications, triacylglycerol, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and prevalent chronic diseases [heart disease, stroke, chronic lung disease, asthma, and cancer]) and baseline outcome measurements. For each longitudinal analysis, we created 20 imputed data sets and obtained estimates by pooling results using Rubin's rules. In addition, we included age as a spline term in a sensitivity analysis to check for any possible nonlinear effect. Data were handled and analyzed with SPSS Statistics version 25.0.0.1 (IBM Corp., Armonk, NY) and R, CRAN version 4.0.5, with packages “lme4”, “lmerTest”, and “mice”. All analyses were performed at the significance level of 0.05 (2-tailed) unless specified otherwise.

Table 1. Baseline characteristics of the study population

	Cognition analysis (n=5282)			Physical function analysis (n=8441)		
	Normoglycemia (n=2363)	Prediabetes (n=2111)	Diabetes (n=808)	Normoglycemia (n=3764)	Prediabetes (n=3402)	Diabetes (n=1275)
Age, years	58.1 ± 8.9	58.7 ± 8.5	59.7 ± 8.4	58.8 ± 9.3	59.7 ± 9.1	60.4 ± 8.9
Women, n (%)	1091 (46.2)	983 (46.6)	392 (48.5)	2000 (53.1)	1818 (53.4)	704 (55.2)
Education						
No formal education	375 (15.9)	345 (16.3)	147 (18.2)	1069 (28.4)	988 (29.0)	370 (29.0)
Elementary school	1057 (44.7)	917 (43.4)	338 (41.8)	1575 (41.8)	1383 (40.7)	523 (41.0)
Middle school	606 (25.6)	566 (26.8)	209 (25.9)	731 (19.4)	706 (20.8)	250 (19.6)
High / vocational / university	325 (13.8)	283 (13.4)	114 (14.1)	389 (10.3)	325 (9.6)	132 (10.4)
Body mass index, kg/m^2	23.1 ± 3.8	24.0 ± 3.7	25.1 ± 4.7	22.9 ± 3.7	23.8 ± 3.9	24.8 ± 4.5
Smoking status, n (%)						
Never	1303 (55.1)	1206 (57.1)	468 (57.9)	2260 (60.0)	2102 (61.8)	785 (61.6)
Former	197 (8.3)	224 (10.6)	101 (12.5)	283 (7.5)	320 (9.4)	137 (10.7)
Current	863 (36.5)	681 (32.3)	239 (29.6)	1221 (32.4)	980 (28.8)	353 (27.7)
Alcohol consumption, n (%)						
No alcohol consumption	1474 (62.4)	1336 (63.3)	532 (65.8)	2500 (66.4)	2286 (67.2)	870 (68.2)
Less than once a month	219 (9.3)	167 (7.9)	69 (8.5)	315 (8.4)	249 (7.3)	104 (8.2)
More than once a month	670 (28.4)	608 (28.8)	207 (25.6)	949 (25.2)	867 (25.5)	301 (23.6)
Systolic blood pressure, mmHg	127.7 ± 20.6	131.1 ± 20.8	134.5 ± 20.7	128.0 ± 21.3	131.4 ± 21.2	135.2 ± 21.4
Use of blood pressure-lowering medication, n (%)	352 (14.9)	427 (20.2)	255 (31.6)	546 (14.5)	670 (19.7)	400 (31.4)
Triacylglycerol, mg/dL	97.4 (71.7, 138.1)	110.6 (77.9, 163.7)	140.7 (91.2, 220.4)	96.5 (70.8, 136.3)	109.3 (77.0, 160.2)	137.2 (92.0, 221.3)
Total cholesterol, mg/dL	187.1 ± 35.4	197.6 ± 37.8	202.2 ± 44.1	187.3 ± 35.6	198.0 ± 38.8	202.6 ± 45.0
High-density lipid cholesterol, mg/dL	52.1 ± 14.5	50.5 ± 15.0	45.6 ± 15.6	52.5 ± 14.6	51.4 ± 15.4	46.4 ± 16.0
Use of lipid-lowering medication, n (%)	81 (3.4)	106 (5.0)	103 (12.7)	116 (3.1)	154 (4.5)	151 (11.8)
Prevalent chronic disease, n (%)						
Heart disease	230 (9.7)	254 (12.0)	141 (17.5)	357 (9.5)	401 (11.8)	208 (16.3)
Stroke	39 (1.7)	32 (1.5)	22 (2.7)	67 (1.8)	51 (1.5)	36 (2.8)
Chronic lung disease	237 (10.0)	206 (9.8)	95 (11.8)	378 (10.0)	338 (9.9)	153 (12.0)
Asthma	93 (3.9)	75 (3.6)	28 (3.5)	135 (3.6)	122 (3.6)	53 (4.2)
Cancer	15 (0.6)	18 (0.9)	8 (1.0)	27 (0.7)	29 (0.9)	15 (1.2)

Values are mean (standard deviation) or median (interquartile range) for continuous variables and number (percentage) for categorical variables.

RESULTS

Baseline characteristics

Among the 5282 participants included in the cognition analysis, the mean (SD) age was 58.6 (8.7) years, the median (IQR) follow-up duration was 6.9 (4.0-7.0) years, 2111 (40.0%) were classified as having prediabetes, and 808 (15.3%) as diabetes. For the physical function analysis, 8441 participants were included, 3402 (40.3%) were classified as having prediabetes, and 1275 (15.1%) were classified as having diabetes. The mean (SD) age was 59.4 (9.2) years, and the median (IQR) follow-up duration was 7.0 (6.9-7.0) years. The distributions of baseline characteristics by glycemic statuses were virtually the same for these two analyses. As shown in **Table 1**, the participants with prediabetes or diabetes tended to be older at baseline; a larger proportion was women and had a poorer cardiovascular risk profile.

Cognition and physical function changes among various baseline glycemic statuses

Table 2 and **Figure 1** demonstrate the baseline difference and annual changes in cognition *z* scores among different glycemic statuses. Compared with normoglycemia, people with diabetes had significantly worse performance in global cognition, memory, and executive function at baseline, but no difference was observed for individuals with prediabetes. Over the follow-up period, only diabetes was associated with a faster decline in orientation (-0.018 SD/year, 95%CI -0.032, -0.004). A significant interaction was detected between age and glycemic statuses associated with orientation decline (p for interaction = 0.002). After stratification, the association between diabetes and orientation decline was only significant among older participants (≥60 years old) (**Table S1**). No significant interaction between glycemic statuses and sex was found concerning changes in cognition function (**Table S2**).

Table 2 and **Figure 2** provide the baseline difference and annual changes in physical function among glycemic statuses. There was no difference in physical function among different glycemic groups at baseline. Whereas during the follow-up, diabetes was associated with a faster increase in physical function score (0.082 /year, 95%CI 0.038, 0.126), ADL (0.036 /year, 95%CI 0.015, 0.056), and IADL (0.043 /year, 95%CI 0.015, 0.071), no difference was observed for prediabetes. No significant interaction was found between glycemic status and age, and sex on physical function (**Table S3**, **Table S4**).

Table 2. The associations between glycemic status and baseline performance (intercept), and annual changes in cognition *z* scores (SD/year) and functioning scores, using linear mixed models

		Glycemic status (intercept)		Time, years [#]	Glycemic status × time (years) [*]	
		Prediabetes	Diabetes		Prediabetes × time	Diabetes × time
Global cognition	**Model 1**	0.026 (-0.027, 0.079)	-0.030 (-0.102, 0.042)	-0.039 (-0.045, -0.032)	0.002 (-0.008, 0.012)	-0.009 (-0.022, 0.005)
	Model 2	-0.011 (-0.059, 0.037)	-0.109 (-0.176, -0.041)	-0.038 (-0.045, -0.032)	0.003 (-0.007, 0.012)	-0.008 (-0.021, 0.006)
Orientation	**Model 1**	0.044 (-0.010, 0.097)	0.051 (-0.022, 0.123)	-0.039 (-0.046, -0.032)	-0.001 (-0.011, 0.010)	-0.019 (-0.033, -0.005)
	Model 2	0.011 (-0.039, 0.062)	-0.021 (-0.091, 0.050)	-0.039 (-0.046, -0.032)	-0.000 (-0.010, 0.010)	-0.018 (-0.032, -0.004)
Memory	**Model 1**	-0.005 (-0.055, 0.045)	-0.050 (-0.118, 0.019)	-0.020 (-0.027, -0.012)	0.001 (-0.010, 0.012)	0.002 (-0.013, 0.016)
	Model 2	-0.036 (-0.084, 0.013)	-0.114 (-0.182, -0.047)	-0.020 (-0.027, -0.013)	0.002 (-0.009, 0.012)	0.002 (-0.012, 0.017)
Executive function	**Model 1**	0.021 (-0.030, 0.073)	-0.056 (-0.126, 0.015)	-0.034 (-0.041, -0.027)	-0.003 (-0.013, 0.007)	-0.004 (-0.017, 0.010)
	Model 2	-0.001 (-0.050, 0.048)	-0.101 (-0.169, -0.032)	-0.035 (-0.041, -0.028)	-0.003 (-0.013, 0.007)	-0.003 (-0.017, 0.011)
Physical function	**Model 1**	-0.018 (-0.133, 0.097)	0.150 (-0.008, 0.308)	0.176 (0.154, 0.198)	0.003 (-0.029, 0.035)	0.082 (0.038, 0.127)
	Model 2	-0.024 (-0.140, 0.091)	0.042 (-0.122, 0.205)	0.176 (0.154, 0.198)	0.003 (-0.029, 0.035)	0.082 (0.038, 0.126)
ADL	**Model 1**	-0.027 (-0.078, 0.023)	0.075 (0.006, 0.144)	0.062 (0.052, 0.072)	0.007 (-0.007, 0.022)	0.036 (0.016, 0.056)
	Model 2	-0.034 (-0.084, 0.017)	0.026 (-0.046, 0.098)	0.062 (0.052, 0.072)	0.007 (-0.007, 0.022)	0.036 (0.015, 0.056)
IADL	**Model 1**	0.004 (-0.076, 0.083)	0.070 (-0.038, 0.179)	0.111 (0.097, 0.125)	-0.004 (-0.024, 0.017)	0.043 (0.015, 0.072)
	Model 2	0.001 (-0.078, 0.081)	0.004 (-0.108, 0.116)	0.111 (0.097, 0.125)	-0.004 (-0.024, 0.017)	0.043 (0.015, 0.071)

Results were reported using normoglycemia as the reference. Model 1 was adjusted for baseline age and sex. Model 2 was further adjusted for education level, body mass index, smoking status, alcohol consumption, systolic blood pressure, use of blood pressure-lowering medications, triacylglycerol, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and prevalent chronic diseases (heart disease, stroke, chronic lung disease, asthma, and cancer) at baseline.

[#] Time (years) represents the annual change in cognition and functioning scores (slope) for the reference group.

[*] Glycemic status × time (years) represents the additional annual change in cognition and functioning scores (slope) for the prediabetes or diabetes group.

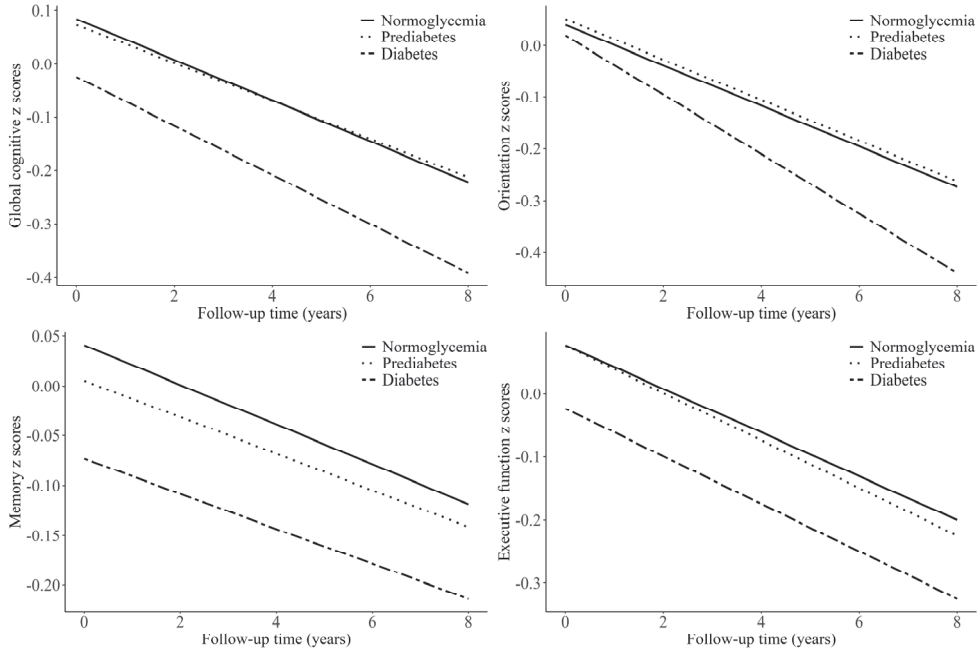

Figure 1. Predicted trajectories of cognitive *z* scores according to baseline glycemic status.
Note: Analyses adjusted for baseline age, sex, education level, body mass index, smoking status, alcohol consumption, systolic blood pressure, use of blood pressure-lowering medications, triacylglycerol, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and prevalent chronic diseases (heart disease, stroke, chronic lung disease, asthma, and cancer).

Cognition and physical function changes across various glycemic transitions during follow-up

Figure S2 shows the transitions of glycemic statuses between baseline and wave 2015 among baseline nondiabetic participants, using the data from the physical function analysis. Specifically, among the 7166 nondiabetic participants (normoglycemia or prediabetes) at baseline, 5055 had available data on glycemic definition at wave 2015, which was used here. Of the 2414 baseline prediabetic participants, 358 (14.8%) progressed to diabetes, 555 (23.0%) regressed to normoglycemia, and 1501 (62.2%) remained to be prediabetes. Of the 2641 baseline normoglycemic participants, 1410 (53.4%) progressed to prediabetes, 214 (8.1%) progressed to diabetes, 1017 (38.5%) remained normoglycemic. Similar Sankey plot from the cognition data was shown in **Figure S3**.

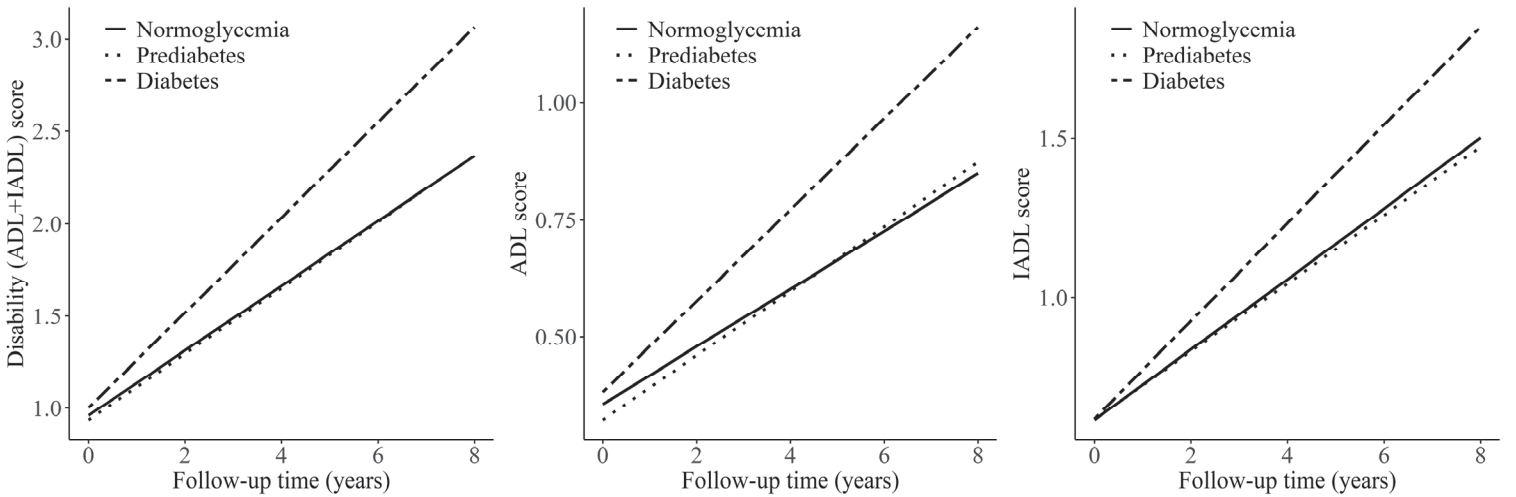

Figure 2. Predicted trajectories of functioning scores according to baseline glycemic status.

Note: Analyses adjusted for baseline age, sex, education level, body mass index, smoking status, alcohol consumption, systolic blood pressure, use of blood pressure-lowering medications, triacylglycerol, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and prevalent chronic diseases (heart disease, stroke, chronic lung disease, asthma, and cancer).

Table 3. Longitudinal analysis of the mean difference in the rate of concomitant change in cognition *z* scores (SD/year) and functioning scores comparing different glycemic transitions, using linear mixed models

Glycemic transition (2011-2015)	Normoglycemia - Normoglycemia	Normoglycemia - Prediabetes	Normoglycemia - Diabetes	Prediabetes - Normoglycemia	Prediabetes - Prediabetes	Prediabetes - Diabetes	Diabetes-
Cognition analysis							
N	659	916	119	376	947	227	808
Global cognition	Reference	-0.006 (-0.023, 0.010)	-0.041 (-0.074, -0.009)	-0.007 (-0.027, 0.014)	-0.004 (-0.020, 0.013)	-0.015 (-0.040, 0.009)	-0.017 (-0.034, 0.001)
Orientation	Reference	0.004 (-0.013, 0.021)	0.005 (-0.028, 0.039)	-0.002 (-0.023, 0.020)	-0.003 (-0.020, 0.014)	-0.009 (-0.035, 0.016)	-0.018 (-0.036, -0.001)
Memory	Reference	-0.013 (-0.030, 0.005)	-0.049 (-0.084, -0.014)	-0.005 (-0.028, 0.017)	-0.007 (-0.025, 0.010)	-0.013 (-0.040, 0.014)	-0.010 (-0.028, 0.009)
Executive function	Reference	0.001 (-0.015, 0.018)	-0.033 (-0.066, -0.000)	0.002 (-0.020, 0.022)	-0.009 (-0.025, 0.008)	-0.015 (-0.041, 0.010)	-0.005 (-0.023, 0.012)
Disability analysis							
N	1017	1410	214	555	1501	358	1275
Physical function	Reference	0.013 (-0.037, 0.064)	0.157 (0.064, 0.250)	-0.009 (-0.074, 0.056)	-0.001 (-0.051, 0.049)	0.040 (-0.036, 0.116)	0.111 (0.059, 0.164)
ADL	Reference	0.000 (-0.023, 0.023)	0.038 (-0.004, 0.080)	-0.008 (-0.037, 0.021)	0.004 (-0.018, 0.027)	0.025 (-0.009, 0.059)	0.044 (0.020, 0.068)
IADL	Reference	0.014 (-0.019, 0.048)	0.117 (0.055, 0.179)	0.001 (-0.042, 0.044)	-0.005 (-0.038, 0.028)	0.012 (-0.038, 0.063)	0.062 (0.028, 0.097)

Adjusted for baseline age, sex, education level, body mass index, smoking status, alcohol consumption, systolic blood pressure, use of blood pressure-lowering medications, triacylglycerol, total cholesterol, high-density lipoprotein, use of lipid-lowering medications, and prevalent chronic diseases (heart disease, stroke, chronic lung disease, asthma, and cancer) at baseline.

Table 3 provides the mean difference in the rate of concomitant change in cognition and physical function comparing different glycemic transition statuses. By using the stable normoglycemia as the reference, we observed that those who progressed from normoglycemia to diabetes had a significantly faster decline in global cognition (-0.041 SD/year, 95%CI -0.074, -0.009), memory (-0.049 SD/year, 95%CI -0.084, -0.014), and executive function (-0.033 SD/year, 95%CI -0.066, -0.000). Similar trends were also found for the disability analysis; we observed that those who progressed from normoglycemia to diabetes had an accelerated increase in concomitant physical function score (0.157 /year, 95%CI 0.064, 0.250) and IADL (0.117 /year, 95%CI 0.055, 0.179); these effect sizes were even larger than prevalent diabetes.

Sensitivity / Nonresponse analyses

Sensitivity analysis results using imputed data were similar to those from the main analyses (**Tables S5**). Results remain robust after we included age as a spline term (**Tables S6**). Of the 9307 participants, who attended physical and clinical measurements at baseline, 4025 (43.2%) were excluded from the cognition analysis because of missing information or loss of follow-up. Compared to the included participants, those excluded participants were older, more often women, and had lower education and lower cognition scores at baseline (**Table S7**). Similarly, 866 (9.3%) individuals were excluded from the physical function analysis. Those excluded participants were older, had a higher proportion of blood-pressure-lowering medication use, and had a higher prevalence of chronic diseases (**Table S8**).

DISCUSSION

In this population-based middle-aged and older Chinese cohort, we found that prevalent diabetes was associated with an accelerated decline in cognition and physical function over time. Baseline prediabetes was not associated with the changing rate of cognition and physical function over time. The transition of normoglycemia to diabetes during follow-up was also related to an accelerated concomitant decline in cognition and physical function.

Although former studies have shown an increased risk of dementia in diabetic patients, cognition changes in people with prediabetes have been less studied [261-266]. As a prodromal feature of dementia, the accelerated cognitive decline becomes evident years before diagnosis [277]. However, the results are inconsistent among the few available longitudinal studies investigating the relationships between prediabetes/diabetes and cognition decline. Compared to normoglycemia, those with prediabetes or diabetes had faster cognitive decline [262, 265], while others found that these problems were restricted to diabetes [264, 266]. As for the specific cognitive domain, two studies reported that diabetes was associated with a faster decline in perceptual speed and executive function tasks but not with episodic memory [262, 263]. In contrast, others found a relationship with a faster memory decline but not with the executive function [266]. Associations between prediabetes and a specific cognitive domain have not been reported. Methodological discrepancies, such as the cohort characteristics (age range, ethnicity), follow-up duration, and cognitive assessment tools, may explain the differences between the findings. In particular, we did not find a significant association with prediabetes despite a relatively large sample size (2111 individuals with prediabetes). Our findings and that of Zheng et al. do not support that the sample size is responsible for the heterogeneous findings on prediabetes and cognitive decline [264].

The present study is the first prospective investigation of the trajectory of physical function among different glycemic statuses in the Chinese population. Our results are compatible with prior studies and found that diabetes is associated with a substantially increased risk of physical dysfunction. In complement to a prior meta-analysis mainly based on cross-sectional studies [267], our findings showed that despite a similar level of physical function at baseline, the rate of functioning decline during follow-up is faster for diabetic patients. Regarding the relationship between prediabetes and physical function, the literature is inconsistent and mainly based on cross-sectional studies. For example, according to a study in the UK among people aged 60–70 years, prediabetes was associated with weaker muscle strength and impaired physical function [278]. Among the Japanese elderly (mean age 71 years), however, prediabetes was not associated with walking speed and chair stand time tests [279]. In addition, the Helsinki Birth Cohort Study (mean age 70 years) indicated that only impaired glucose tolerance and not impaired fasting glucose are related to poor physical performance [280]. Only one longitudinal study, conducted among Swedish older adults over 60, has specifically investigated the physical function decline among different glycemic statuses and concluded that prediabetes (defined by HbA1c) is associated with faster functional decline and disability compared to normoglycemia [268]. Although our study had a much larger sample size, we could not confirm this relationship between prediabetes and the accelerated decline of physical function. Differences in age range, duration of follow-up, ascertainment of prediabetes, and measurements of physical function may explain the discrepancy between the two studies.

Notably, when using baseline glycemic status as a non-chronicity with possible transitions, we found that compared to stable normoglycemia, those who progressed from normoglycemia to diabetes have an accelerated decline in global cognition, memory, executive function, and physical function. The transition to prediabetes did not return such complications. Supported by former evidence [242, 263], our findings indicate that prediabetes might not be a reliable high-risk entity, at least not to prevent cognitive decline or physical dysfunction.

Although the pathophysiological pathways through which baseline diabetes may cause cognitive and physical dysfunction remain to be elucidated, several mechanisms have been proposed. For example, diabetes directly causes atherosclerotic diseases, such as stroke, which contributes to cognitive decline and physical dysfunction. Prior studies based on the Swedish National Study on Aging and Care in Kungsholmen (SNAC-K) reported that diabetes, but not prediabetes, is associated with an increased risk of ischemic stroke and post-stroke dementia [281], and the association between diabetes and functional decline is partly mediated by cardiovascular disease [268]. Another possible mechanism involves shared risk factors. Many diabetes-related risk factors, such as obesity and depression, are also related to a faster decline in cognition and physical functioning [282-285].

A major strength of our study is the large, well-designed population-based cohort with repeated outcome measurements which enabled us to generate the trajectories of cognition and physical function among different glycemic statuses. Additionally, with repeated data on glycemic status during follow-up, we explored any possible effect caused by the glycemic transition. Taken together, our study filled in a specific knowledge gap about the cognition and functioning changes across glycemic status in China. Our study has some limitations that should be acknowledged. First, although ethnicity information was not collected in the CHARLS, we

still can reasonably presume that most of the participants were Han Chinese, limiting our findings' generalizability to other ethnicities and countries. Secondly, only those with complete baseline information and at least one repeated measurement were eligible for the current study, possibly leading to selection bias. Results from the nonresponse analysis show that the responding participants were relatively healthier than those excluded, which may limit the internal validity and generalization. Moreover, our analysis of responders' data may have underestimated complications by excluding nonresponders' potentially faster functioning decline, especially cognition function [286]. Also, limited by the available waves in the CHARLS, the follow-up interval was relatively short, and we could not investigate the association between glycemic transition and subsequent decline of cognition and physical function. Other studies with longer follow-up times are required to deal with the possible reverse causation. Finally, unlike performance-based measures such as walk time, ADL and IADL generally display weak validity and reproducibility, and are susceptible to ceiling effect [287]. As the emphasis has changed toward early detection in community-dwelling older adults, the measurements of physical functioning we used here may not be sensitive enough to detect the minor deficits present during its onset. A former study reported that combining self-reported and performance-based measurements can refine prognostic information, particularly among older persons with high self-reported functioning [288]. Therefore, future studies using more comprehensive physical function measurements are needed.

CONCLUSIONS

In conclusion, our results indicate that diabetes, but not prediabetes, is associated with an accelerated decline in cognition and physical function in middle-aged and older Chinese. Additionally, a transition from normoglycemia towards the development of diabetes was also related to a faster concomitant decline in cognition and physical function, suggesting a critical short diagnostic window when diabetes de novo presents.

SUPPLEMENTARY MATERIAL

Supplementary material are available on:

https://www.maturitas.org/article/S0378-5122(23)00054-3/fulltext

4.4

Chapter 4.4

Cardiovascular health and lifetime risk of multimorbidity

Part V

General Discussion and Summary

5.1

Chapter 5.1

General discussion

The main aims of this thesis were to investigate the role of cardiometabolic health in the development of type 2 diabetes (T2D), to investigate the implication of hypertension management in T2D, and to investigate the burden of complications across the glycemic spectrum. Regarding the role of cardiometabolic health in T2D risk, I was interested in several novel cardiometabolic-related risk factors, including heart rate variability (HRV), relative fat mass (RFM), and arterial stiffness/remodeling because the related evidence was limited and inconsistent. To enable future better prevention of incident T2D, I also investigated the role of composite cardiovascular health (CVH) metric and genetic variants on the lifetime risk of incident T2D. In addition, since hypertension is a major preventable risk factor for cardiovascular disease (CVD) and mortality among T2D, but the health benefit of intensive blood pressure control for T2D patients remains unclear [34, 35], I estimated the concordance and discordance in the hypertension management for Chinese adults with diabetes using definitions from different guidelines. I also investigated the associations between blood pressure and mortality within different multimorbidity patterns among T2D patients. Another area of interest in my research was to investigate the burden of diabetes complications. I firstly explored the differences in the first manifestations of CVD across different glycemic spectrums. Then, considering the potential of prediabetes in T2D management, I also investigated the role of incident T2D and healthy lifestyle in the associations between prediabetes and risk of CVD and mortality. Regarding other emerging diabetes complications, I assessed longitudinal changes in cognition and disability according to glycemic status and different glycemic transitions. I also explored the effect of CVH on the lifetime risk for multimorbidity of non-communicable diseases. Detailed findings and discussion points from each of these studies are reported in previous chapters.

In this chapter, I summarize the main findings from this thesis and discuss the most important methodological issues. Finally, I report the public health and clinical implications of my findings and also provide directions for future research.

Main Findings

Part 2: Maintaining cardiometabolic health to prevent incident T2D

In **Part 2**, I investigated the role of cardiometabolic health in T2D development. In **Chapter 2.1**, I observed that longitudinal evolutions of both heart rate and different HRV metrics were significantly associated with new-onset T2D. High heart rate and HRV were related to an increased T2D risk, especially among younger individuals. However, Mendelian randomization (MR) analyses suggested no causal association between HRV and incident T2D; thus, more studies are needed to validate our findings and to further elucidate the underlying mechanisms. Since weight control is the most important factor reducing the risk of incident T2D [313], I examined associations of RFM, body mass index (BMI), waist circumference (WC) and waist-hip ratio (WHR) with incident T2D in **Chapter 2.2**. By enrolling individuals from multiple Dutch cohorts, I found that RFM was more strongly associated with incident T2D than commonly used measures of obesity. These associations were present across all age categories and were most pronounced in younger individuals. These findings highlight that adequate obesity control, particularly in young individuals, would substantially reduce the risk of developing T2D in the community. In **Chapter 2.3**, I studied if large artery stiffness is involved

in T2D pathogenesis and showed that arterial stiffness and remodeling markers were associated with incident T2D, with stronger associations observed among individuals with a higher T2D genetic risk score (GRS). Although the MR approach indicated that the relationship between arterial stiffness and T2D is not causal, GRS for arterial stiffness index showed significant associations with fasting insulin and insulin resistance. Finally, to better prevent incident T2D, in **Chapter 2.4,** I investigated the role of composite CVH metric on the lifetime risk of incident T2D. I observed that at age 55 years, the remaining lifetime risk of incident T2D was the lowest for ideal CVH compared to intermediate and poor CVH categories. Although more favorable CVH was associated with a lower lifetime risk of T2D, it was not counterbalanced by the genetic susceptibility to T2D.

Part 3: Rethinking blood pressure management in T2D

In **Part 3**, I studied the implication of blood pressure management in T2D. Considering the notable difference that exists regarding hypertension management across various diabetes guidelines [29], in **Chapter 3.1**, I estimated the concordance and discordance between the 2020 Chinese Diabetes Society (CDS), the 2022 American Diabetes Association (ADA), and the 2017 American College of Cardiology/American Heart Association (ACC/AHA) guidelines using data from the nationally representative China Health and Retirement Longitudinal Study (CHARLS). I found that among middle-aged and older Chinese with diabetes, compared with the 2020 CDS guideline, the 2017 ACC/AHA guideline would additionally recommend to initiate antihypertensive medication for 19.3% (15.5%, 23.1%) and to intensify antihypertensive treatment for 16.6% (12.5%, 20.7%). The overall discordance for the recommendation of intensified antihypertensive treatment between the 2020 CDS and 2022 ADA guidelines was 24.0% (17.8%, 30.2%). In a nutshell, only a modest degree of discordance between the 2020 CDS, the 2022 ADA, and the 2017 ACC/AHA guidelines were found among middle-aged and older Chinese with diabetes, and adopting the 2017 ACC/AHA guideline among Chinese adults with diabetes would double the number of people qualified to initiate antihypertensive treatment. In addition, achieving blood pressure targets is a key component of T2D management and is important in reducing mortality risk; however, little is known about whether different multimorbidity patterns would modify the associations between blood pressure and mortality among patients with T2D. In **Chapter 3.2**, using data from the UK Biobank, I observed that a U-shaped association was found between systolic blood pressure (SBP) and mortality risk among T2D patients across total, concordant, and discordant multimorbidity patterns, and the related effect estimations increased with accumulating multimorbidity counts. A reverse J-shaped association was indicated for diastolic blood pressure (DBP) in specific multimorbidity patterns. Notably, the lowest risk of death was consistently observed around 130~140 mmHg for SBP and 80~90 mmHg for DBP within different multimorbidity patterns. These findings imply that different multimorbidity counts or types should not affect optimal blood pressure targets in diabetes management.

Part 4: Emerging complications across the glycemic spectrum

In **Part 4**, I investigated the burden of complications across the glycemic spectrum. The complications included not only CVD, but also cognitive decline, physical disability, and multimorbidity of non-communicable diseases. In **Chapter 4.1**, I found that hyperglycemia

status, either prediabetes or T2D, carried a large lifetime risk for incident CVD among women and men compared with normoglycemia. In particular among men, the risk was comparable to that of T2D. Overweight/obesity modified the risk and conferred a larger burden of lifetime CVD risk among women and men with hyperglycemia. Furthermore, in **Chapter 4.2**, I observed that prediabetes is independently associated with an increased risk of atherosclerotic cardiovascular disease and mortality, and favorable lifestyle habits could lower the risk of transitioning to cardiovascular disease and mortality among people with prediabetes. These findings highlight the benefits of promoting a healthy lifestyle to prevent cardiometabolic disease among people with prediabetes. Compared to these traditional cardiovascular complications, the effects of hyperglycemia on emerging complications such as cancer, cognition, and physical function have been less investigated [47]. Therefore, in **Chapter 4.3**, I also evaluated longitudinal changes in cognition and physical function according to glycemic status and different glycemic transitions and found that compared to normoglycemia, baseline T2D was associated with a faster decline in orientation and physical function, and progression from normoglycemia to diabetes during follow-up was also associated with a significantly faster decline in global cognition, memory, executive function, and physical function compared to stable normoglycemia. In **Chapter 4.4**, I also observed that adherence to favorable CVH would infer a lower lifetime risk of cardiovascular and non-cardiovascular diseases, and that women had a lower lifetime risk of NCD and multimorbidity in all CVH categories, compared to men. Moreover, the trend towards lower lifetime risk of individual NCDs and comorbidities with more favorable CVH was more distinct among women.

Methodological Considerations

Novel epidemiological methods

In this thesis, I conducted several novel statistical methods for epidemiological research. Over the past decades, the repertoire of analytical techniques in epidemiology has been growing fast, following the need to answer complex research questions of population health. The new techniques are necessary and generally convey new information from the data. However, the correct interpretation of the results of such techniques is essential for the clinical research and epidemiology community. Not all researchers and clinicians are familiar with various sophisticated methodologies, so careful and precise explanations of the methodology, analysis, and results are critical.

Joint model: Prospective cohorts, such as the Rotterdam Study, usually have multiple waves of data collection during follow-up visits, which will provide both repeated measurement of various covariates and also survival outcomes. Since repeated measurement and survival data require different statistical methods, common practice has been to analyze these data separately. However, given the typical properties of these data: 1) repeated measurement sequences are intermittently collected and subject to measurement error; 2) occurrence of the survival event terminates the underlying measurement process, potentially in an informative manner; and 3) the underlying measurement process affects the hazard for survival. A separate analysis of repeated measurement and survival outcomes is potentially inefficient because it does not fully exploit the dependence between the repeated measurement process and the hazard for survival, thus, leading to a biased estimation of the association between the exposure and outcome since

it ignores measurement error [314]. Therefore, to produce valid inferences, a model for the joint distribution of the longitudinal and survival outcomes is required instead. In **Chapter 2.1**, to investigate the prospective association of the evolution of HRV with incident T2D during follow-up, I performed joint models for longitudinal and time-to-event data using the R package "**JMbayes2"**. This Joint model was implemented by combining the joint distribution of HRV metrics in the linear mixed effects model with the Cox model. It can estimate continuous profiles of each HRV metric based on the repeated measured data, collected during the whole follow-up period for each individual. Therefore, it would consider individual variations and reduce the bias associated with missing data. In addition, a joint model is more appropriate to estimate the hazard of T2D incident for the HRV metrics as time-varying covariates because it accounts for their endogenous nature. The principal advantage of this approach over separate analyses of each outcome is the correct treatment of noisy and incompletely observed time-varying covariate information, which enables unbiased estimation of the relationship between exposure and outcome.

Mendelian randomization: Causal inference is central to almost all epidemiologic studies. However, limited by various types of bias, we can never interpret an estimate of the association from an observational study as a causal effect. MR is an analytical method that uses genetic variants as instrumental variables for modifiable risk factors that affect population health [315]. Valid instrumental variables are the key to performing MR analysis and they are defined by three key assumptions: that they associate with the risk factor of interest (the relevance assumption); that they share no common cause with the outcome (the independence assumption); and that they do not affect the outcome except through the risk factor (the exclusion restriction assumption). MR is less likely to be affected by confounding or reverse causation than conventional observational studies and can provide evidence about putative causal relations. Despite all these advantages, MR is also subject to certain biases. For example, weak instrument bias can occur when using one or more genetic variants that only explain a small proportion of the variation in the risk factor, coupled with a small sample size. Another relevant topic is pleiotropic effects, meaning the effects of a genetic variant on multiple biological pathways. These can either affect the outcome through another trait or pathway to the one under investigation, known as horizontal pleiotropy, or affect other traits through the risk factor of interest, known as vertical pleiotropy [315, 316]. In addition, as genetic effects on phenotypes are typically small, MR estimates of association usually have much wider confidence intervals than conventional epidemiological estimates [317]. For example, in **Chapter 2.3**, I adopted traditional Cox regression and MR analysis to evaluate the causal associations between arterial stiffness and incident T2D. Unlike results from Cox regression analysis which suggested elevated arterial stiffness was associated with incident T2D, MR analysis found positive but insignificant associations. This was also the case in **Chapter 2.1** when I used MR analysis to validate the observational findings that suggested HRV would increase T2D risk. Despite these, the rationale for using MR is that an unbiased, imprecise estimate is preferable to a precise, biased estimate of causal association [316, 318]. As such, MR analysis fills an important missing piece in the causal inference puzzle.

Multistate model: The multistate model is a model for time-to-event data in which all individuals start in one or possibly more starting states (e.g., baseline healthy status) and

eventually may end up in one (or more) absorbing or final state(s) (e.g., incident CVD or death). In between, intermediate states can be visited, possibly more than once. Some individuals may be censored before they reach any absorbing state. Thus, multistate model is a valuable extension of traditional survival analysis, as it can help to give more biological insight into the disease/recovery process of a patient and also enables clinicians to obtain more accurate predictions of survival probabilities and to calculate dynamic predictions [252]. Prediabetes is not a robust diagnostic entity, but few data were available that evaluate whether the increased risk resulted from prediabetes per se or the transition from prediabetes to diabetes during follow-up. Therefore, in **Chapter 4.2**, I used the multistate model to examine whether incident T2D (intermediate states) affect the association between prediabetes and CVD/mortality. I also investigated how baseline lifestyle factors influence the temporal transition process of cardiometabolic diseases. Although multistate model is a very useful tool to answer a wide range of questions in survival analysis, it is not frequently applied. The two main reasons are the need for more handy software and difficulty interpreting the results. Over the past decade, with the efforts of more researchers, the multistate model has gradually gained popularity in epidemiologic research.

Methodological considerations related to the studies included in this thesis

Selection bias: Selection bias in epidemiological studies occurs when there is a systematic difference between the characteristics of those selected for the study and those who are not. Most studies in this thesis were based on the Rotterdam Study and the UK Biobank. Both studies are population-based cohorts. The Rotterdam Study consists of the community-dwelling population aged 55 years and older in the Ommoord district of Rotterdam city. The UK Biobank contains general population aged 40-69 years across the U.K. The probability of selection bias at baseline of the Rotterdam Study was relatively low, with quite high baseline participation rates for the initial three study waves (ranging from 64.9% to 78.1% of all eligible individuals). However, the response rate of the most recent wave in the Rotterdam Study is lower (45%), which may be partly due to the changing social landscape or the willingness of citizens to contribute to science. As for the UK Biobank, a former study reported that UK Biobank participants were more likely to be older, to be female, and to live in less socioeconomically deprived areas than non-participants [258]. Compared with the general U.K. population, participants within the UK Biobank were less likely to be obese, to smoke, and to drink alcohol on a daily basis and had fewer self-reported health conditions. Thus, UK Biobank has been considered to be not representative of the sampling population [258]. Nonetheless, valid assessment of exposure-disease relationships based on these two cohorts may still be widely generalizable and do not require participants to be representative of the larger sampling population. Also, selective non-participation at baseline is not likely to be related to future disease risk [319].

Another possible cause of selection bias during the longitudinal analyses is loss to follow-up [320]. Loss to follow-up is considered differential when dropout rate during follow-up differs according to specific characteristics, i.e. when those with adverse levels of the determinant under the study are more likely to drop out than those with optimal levels. In my studies, the possible bias caused by loss to follow-up has been minimized through continuous linkage of the study database with medical records from general practitioners and pharmacies,

as well as hospitals, in the Rotterdam Study and nationwide hospital inpatient records and death certificates in the UK Biobank. Therefore, the follow-up status of the outcomes of interest was virtually complete.

Confounding: Usually, a confounder is defined as a variable that influences both the dependent and independent variables, causing a spurious association. Selecting an appropriate set of confounders to control is critical for reliable causal inference. The exposure and selected confounding covariates, in most of the included studies, are all assessed at the same time. This could be a concern as it can be challenging to determine whether a covariate assessed at the same time as the exposure may, in fact, be affected by it [321]. For example, in **Chapter 4.2**, when I investigated the role of individual lifestyle metrics, including physical activity, in the transition of cardiometabolic disease among individuals with prediabetes, BMI was considered essential and then controlled for as a confounder. However, given that BMI was measured at baseline when the physical activity level was assessed, it is also conceivable that BMI is on the pathway from baseline physical activity to incident cardiometabolic disease and that controlling for it may block some of the impacts of physical activity. Conversely, it may also be the case that BMI itself affects both physical activity and incident cardiometabolic disease. Thus, BMI may be both a confounder and a mediator on the pathway from physical activity to cardiometabolic disease. It is thus difficult to know whether or not to adjust for BMI, if both BMI and physical activity are measured at the same time. It is also the case, in **Chapter 4.3**, when I adjusted for prevalent CVD while investigating the association between baseline glycemic status and cognitive decline. It is possible that prevalent CVD is on the pathway from baseline hyperglycemia to accelerate cognitive decline. To adequately address this situation, from the perspective of study design when multiple waves of data are available, it may be desirable to control for the covariates in the wave prior to the exposure of interest which could better rule out the possibility that the covariate used in the analysis is a mediator [321]. However, this is not always an option when only two waves of data are available (one for the exposure and covariates and one for the outcome), the same situation I encountered when using the UK Biobank or the CHARLS data. Nonetheless, adjusting for any possible mediators can only underestimate the true underlying association between exposure and outcome. Thus the observed significant results will be more robust if this bias can be properly account for.

Another important concern here is residual confounding. Residual confounding is the distortion that remains after controlling for confounding in the analysis of a study. There are two main sources of residual confounding. The first source of residual confounding concerns the situations when there are additional confounders that are not considered, or there is no attempt to adjust for them, because data on these factors is not available. For example, in **Chapter 4.3**, when I investigated the association between baseline glycemic status and cognitive and physical functioning decline, I thoroughly adjusted for a large set of potential confounders. However, I cannot exclude any residual confounding in parts of these analyses that were of etiological origins, such as physical activity and diet. The second source of residual confounding stems from the situations when control of confounding is not tight enough. For example, obesity is an important confounder for many disease-related observational studies. However, obesity itself is a heterogeneous entity and simply adjusting for BMI during the analysis is not precise enough [20]. Therefore, in **Chapter 4.2**, when I investigated the role of

incident T2D and healthy lifestyle in the associations between prediabetes and risk of CVD and mortality, I adjusted for waist circumstance in addition to BMI to further address the confounding bias induced by obesity [251]. However, this is not always an option when the required data is unavailable.

Single exposure measurement: Typically, exposure variables are only assessed at baseline without considering any possible changes during follow-up, as in most of our studies. This may be reasonable when conducting research to devise disease prediction models but can induce a bias when investigating the etiological associations and may also reduce the validity of our research findings in real life. For example, in **Chapter 2.4**, using the exposure variable CVH only measured at baseline, I found that adhering to favorable CVH could lower the remaining lifetime risk for incident T2D. This suggests that multilevel intervention can improve the prevention of T2D. Similar study designs and conclusions have also been reported by many former studies investigating the association between CVH and CVD [296]. Nevertheless, CVH was only measured at baseline. Thus, whether participants remained in the same status during follow-up was not evaluated. As lifestyle behaviors may change throughout life, the association of incident CVD with baseline risk profile does not account for within-person variation over the long term, potentially diluting the protective effects or even providing false positive results [322]. For example, a prior study based on the Norway HUNT Study found that compared to inactive maintainers and relapsers, only participants who maintained their physical activity from adolescence to young adulthood had a significantly lower CVD risk and better mental health. Adopting physical activity (i.e., being inactive as adolescents and physically active as young adults) was not associated with lower CVD risk [323]. Similarly, results from the Whitehall II cohort found that there was no consistent relationship between the direction of change in category of CVH (between 1985/1988 and 1997/1999) and risk of CVD [324]. Another study, also based on the Whitehall II cohort, observed that participants who deteriorated from moderate or high to low CVH (between 1991/93 and 2002/04) were still at lower risk of T2D compared with those remaining at low CVH [174]. Although speculative, these aberrant findings may suggest that there may be no relationship between change in healthy lifestyle and incident cardiometabolic disease, or that initial attainment of favorable lifestyle pattern buffers the deleterious consequences of future worsening in these. Either way, this means that a single baseline measurement cannot fully capture the underlying health effects caused by the exposure variables.

This kind of bias caused by single exposure measurement is especially the case when using multistate model to investigate how certain covariates influence different phases of the disease transition. For example, in **Chapter 4.2**, I investigated the role of lifestyle factors in the transition from prediabetes to incident T2D and the subsequent transition to CVD or death. The measurements of lifestyle factors were only performed at baseline and therefore possible changes in modifiable behaviors during follow-up could not be accounted for here. Potential improvements in health behaviors after the diagnosis of first event (i.e. disease) would most likely lead to an underestimation of the risks of subsequent disease(s). Nevertheless, previous study shown that in the absence of interventions, most individuals do not make major lifestyle changes following diagnosis of a serious chronic disease [259]. One recent multi-cohort study also reported that a substantial proportion of participants continued or initiated physical

inactivity or continued smoking after the diagnosis of non-communicable diseases (diabetes, CVD, chronic lung diseases, and cancer) [325]. In addition, low socioeconomic status was associated with a more than fourfold increase in the odds of initiating physical inactivity, more than twofold increase in the odds of continuing physical inactivity and continuing smoking [325]. This also suggests that we need to be cautious about generalizability when interpreting the results of multistate models regarding the associations between lifestyle factors and disease outcomes.

External validity / generalizability: Most participants within the Rotterdam Study and the UK Biobank are of Caucasian descent (over 90%) and live in high-income western European countries with adequate medical resources. Since regional heterogeneity has been widely reported in the prevention and management of diabetes [15, 326, 327], our findings based on these two cohorts may have limited generalizability to other countries. For example, obesity management is a primary treatment goal for T2D [328]. In **Chapter 2.2**, I assessed the association of RFM, a new adiposity index, with incident T2D in three Dutch cohorts (the PREVEND, the LifeLines, and the Rotterdam Study). According to former studies, the association between obesity and diabetes risk is subject to substantial regional variability, with greater diabetes risk at lower BMI thresholds than reflected in currently used BMI cutoffs for assessing diabetes risk found in low- and middle-income countries [329, 330]. Such ethnic variations could be attributed to different fat distributions and percentages of body fat and may also be attributable to the variations in both genetic background and phenotype. For example, compared with white individuals, a much earlier role of beta cell dysfunction is observed in South Asians, leading to rapidly increasing blood glucose trajectories and an increased risk of diabetes [331]. Also, South Asians appear to store more ectopic fat in the liver compared with their white European counterparts with similar BMI levels [332]. Therefore, whether our findings are valid for other ethnicities warrants further investigation. In addition to the regional-specific association, the public effectiveness of available prevention strategies also requires validation. For example, in **Chapter 2.4** I investigated the role of CVH on the lifetime risk of incident T2D and found that the prevalence of ideal physical activity was relatively high (nearly 80%) in the Rotterdam Study. This suggests that interventions targeting physical activity may need more room for improvement among the sampling population. Of note, diet is also a critical component of lifestyle intervention but dietary habit is subject to tremendous regional variability. Therefore, the healthy diet pattern suggested in **Chapters 2.4**, **4.2**, and **4.4** warrants further investigation in other countries and ethnicities to check for its usefulness.

Healthcare system should also be considered when examining the generalizability of our findings. In both the Dutch and U.K. healthcare systems, the entire population is entitled to primary care covered by their obligatory health insurance. In this primary care setting, a general practitioner provides primary prevention for any disease conditions. Therefore, generalizing the results from studies embedded in the Rotterdam Study and the UK Biobank to healthcare systems that are organized differently, or to those with limited availability of primary preventive healthcare, should be done with caution, especially for diabetes management. A former study identified important variations in health system performance in managing diabetes by region, World Bank income group, and individual-level sociodemographic factors [333]. Specifically, individuals with diabetes who live in upper-middle-income countries are more likely to be

tested, diagnosed, and treated than those in low-income and lower-middle-income countries. This suggests that countries with greater wealth and, in turn, more health systems resources are effectively reaching and engaging more people with diabetes. Nevertheless, given the expected increase in both economic and medical resources in coming years in low- and middle-income countries, our findings based on the Rotterdam Study and the UK Biobank may still be informative to guide these countries preparing future resource allocation.

Public Health and Clinical Implications & Future Research

Multilevel lifestyle intervention in T2D management

Lifestyle interventions are considered the first-line treatment in T2D management, supported by multiple studies showing that adherence to a healthy lifestyle is associated with substantial risk reduction in T2D and its complications [26]. Nonetheless, significant knowledge gaps remain, especially as the effectiveness of lifestyle intervention in the real world can be influenced by various factors. Meanwhile, even though low- and middle-income countries bear the greatest diabetes burden, the majority of translational diabetes prevention interventions have been implemented in high-income countries [25]. Understanding the real-world impacts of lifestyle intervention for preventing T2D and associated comorbidities is imperative to inform resource allocation. However, how best to help people achieve and maintain lifestyle changes remains uncertain and warrants further research.

Timing of lifestyle intervention is important to exert and maintain its protective effects. The development of T2D follows a gradual and continuous process, which provides a time window for early identification of high-risk subgroups, such as individuals with prediabetes or overweight. Advances in medical technology, such as rapid blood glucose tests, also make routine screening feasible and reasonable in cost, even at the home level. Thus, substantial time exists before the development of overt diabetes during which one can intervene. Several studies from Diabetes Prevention Programs (DPPs) showed that lifestyle interventions could be cost-effective and effective in preventing or delaying progression to T2D among high-risk individuals, especially those with impaired glucose tolerance [108, 256, 257, 334]. Therefore, prediabetes represents a window of opportunity to initiate lifestyle intervention to help prevent incident T2D. Regarding the appropriate timing of lifestyle intervention among T2D patients, multiple cohort studies found that among patients with newly diagnosed diabetes, worse glycemic control for the first year after diagnosis was independently associated with worse outcomes [335, 336]. This phenomenon, usually referred to as legacy effect or metabolic memory, suggests that immediate, intensive treatment for newly diagnosed patients is necessary to avoid irremediable long-term risk for diabetes complications and mortality. Diagnosing T2D is also a teachable moment for some patients, which can stimulate and increase their motivation to respond positively to educational information and adopt new behaviors [337]. Indeed, healthy behavior changes in the year after diagnosis of diabetes are also associated with significant risk reductions in CVD, independent of cardioprotective medication use [338]. In addition, health behavioral change programs conducted in primary care settings have demonstrated effectiveness in reducing body weight and blood pressure and increasing healthy components of both diet and physical activity with little additional burden on the primary care professionals [339]. Taken together, this evidence indicates that the period shortly after T2D

diagnosis is an important window for lifestyle intervention to help prevent future T2D complications.

How to choose the most effective intervention strategy also needs to be considered. Currently, the most influential strategies of lifestyle intervention are from the DPPs, including physical activity and dietary intervention. Health-care policies recommend that individuals with prediabetes undertake these lifestyle interventions, irrespective of whether the prediabetes phenotype is defined by hyperglycemia in the postprandial state (impaired glucose tolerance; IGT), fasting state (impaired fasting glucose; IFG), or by intermediate HbA1c levels. However, the evidence to date indicates that these lifestyle interventions in their current format have not succeeded in preventing the relentless progression to T2D in individuals with isolated IFG [243]. In addition, a prior review study reported a higher prevalence of isolated IFG in Caucasian cohorts, whereas a higher prevalence of isolated IGT in Asian cohorts [270]. Thus, future intervention approaches should target the distinct pathophysiological and clinical course of T2D progression, emphasizing isolated IFG and possible ethnic differences. These DPPs strategies generally focus on weight reduction by modifying nutrition and increasing physical activity. However, other lifestyle factors, including environmental stressors (i.e., air pollution and noise), sleep quality, and psychosocial stress (i.e., loneliness and depression), are also increasingly recognized as modifiable risk factors for cardiometabolic disease [340, 341]. Future research about these novel lifestyle factors may further improve current intervention strategies. Another area of concern for lifestyle intervention is the duration of their effectiveness. Most related trials have follow-up durations of shorter than five years, leaving the effectiveness over longer time spans less studied [342]. Prior meta-analysis investigating the long-term sustainability of DPPs strategies reported that in adults at risk for diabetes, lifestyle intervention and medications (weight loss and insulin-sensitizing agents) reduced diabetes incidence. Medication effects were short lived. The lifestyle interventions were sustained for several years; however, their effects declined with time, suggesting that interventions to preserve effects are needed [343]. Hence, whether there are other intervention strategies with more prolonged effects also deserve further investigation.

Another topic about real-world lifestyle intervention is whether we have targeted the right population. I believe that population-level lifestyle intervention strategies should be prioritized over individualized strategies that focus on a high-risk segment of the population. First, it has been estimated that the prevention yield of targeting individuals at high risk of developing T2D is limited. As shown by the Da Qing Diabetes Prevention Outcome Study, reductions in T2D progression have been sustained for up to 30 years after the active intervention phase among individuals with impaired glucose tolerance. However, more than 80% of participants still developed T2D eventually [344]. The Look AHEAD trials also reported that during a median follow-up of 9.6 years, an intensive lifestyle intervention focusing on weight loss did not lead to reduction in the rate of cardiovascular events in overweight or obese adults with T2D [345]. Second, the binary thinking about high or low risk not only pertains to citizens or clinicians, but may also mislead policymakers into thinking that they have solved the problem of primary prevention by a strict focus on “high-risk” individuals [346]. T2D is usually considered a “geriatric disease” since the related disease burden occurs mainly in the older population. This leaves younger individuals with adverse risk factors falsely reassured about their low risk of

disease. Over the past decade, T2D has become an increasing burden in children and young adults [347]. Youth-onset T2D is different not only from type 1 but also from type 2 diabetes in adults, and appropriate lifestyle interventions targeting this population is also strongly recommended. Finally, those lifestyle factors identified for hyperglycemia, are also strongly related to other prevailing diseases, such as cancer and dementia. These factors are well established and should also be widely recommended for prevention strategies among the general population.

Harnessing heterogeneity in obesity

Obesity is recognized as a significant public health hazard associated with increased morbidity and mortality. Weight loss is the most important factor reducing the risk of incident T2D and can exert benefits that extend beyond glycemic control to improve the prevention of diabetes complications [313]. Weight loss also reverses the underlying metabolic abnormalities among T2D patients. Sustained loss of at least 15% bodyweight can have a disease-modifying effect in T2D, an outcome that is not attainable by any other glucose-lowering intervention [348]. Despite all these advantages, obesity has remained a puzzling condition for clinicians due to its remarkable heterogeneity.

The comprehensive measurement of excess adiposity includes: assessing total body adiposity (i.e., body weight, BMI), assessing the distribution of body fat (i.e., waist and hip circumference), assessing body composition (i.e., skinfold thickness), and assessing ectopic fat (i.e., triglycerides in non-adipose tissues) [349]. Among those metrics, BMI is currently the main diagnostic indicator of overweight/obesity in clinical practice. Significant heterogeneity exists for its optimal cutoff point, especially among different ethnicities or countries. Lower BMI cutoffs for detecting metabolic risk have been recommended for Asian populations compared with all other populations globally [350]. One recent study using data from 57 low- and middle-income countries showed substantial regional variability in the association between BMI and diabetes risk and further provided suggested sex-stratified and region-stratified BMI cutoffs for diabetes risk when used as a sole anthropometric measurement to identify which individuals should be screened for diabetes [330]. Moreover, since BMI alone is not sufficient to properly assess or manage the cardiometabolic risk associated with increased adiposity in adults, the International Atherosclerosis Society and International Chair on Cardiometabolic Risk Working Group on Visceral Obesity recommended including waist circumference in the evaluation and management of patients with overweight or obesity [251]. They also reinforced the notion that sex- and ethnicity-specific waist circumference thresholds increase across BMI categories and that the combination of waist circumference and BMI provide improved predictions of health risk than either anthropometric measure alone [251]. All these findings underscore the heterogeneity of obesity measurements that should be considered during clinical practice and patient-center decision-making.

Another manifestation of the heterogeneity of obesity is “healthy obesity”. Not all people who are categorized as having obesity (BMI $\geq$30 kg/m^2) have excessive adiposity. Even among people who do have excess adiposity, not all people will have metabolic complications. Some obese people may nevertheless show trivial or even no metabolic complications, the so-called metabolically healthy obesity (MHO). However, whether or not there is a form of healthy

obesity remains controversial [351]. The 30-year Nurses' Health Study examined the association between metabolic health and its change over time and cardiovascular disease risk across BMI categories. It showed that even when metabolic health is maintained during long periods of time, obesity remains a risk factor for cardiovascular disease. A large proportion of metabolically healthy women converted to an unhealthy phenotype over time across all BMI categories, which is associated with an increased cardiovascular disease risk [352]. Similarly, the China Kadoorie Biobank also demonstrated that metabolic health is a transient state for a large proportion of Chinese adults and obesity remains a risk factor for cardiometabolic diseases independent of major metabolic factors. In their studies, individuals converted from metabolically healthy status to unhealthy phenotypes across all BMI categories still raise future risk of cardiometabolic diseases [353, 354]. Notably, most epidemiological studies defined MHO using BMI, waist circumference, and metabolic components such as blood pressure, lipid values, and fasting blood glucose, which may not identify the true healthy obesity entity [351]. Moreover, at any given BMI level, individuals with subcutaneous obesity are at much lower risk of future cardiometabolic outcomes than those with visceral obesity [21]. Even more striking was the finding that a selective gluteal-femoral accumulation of body adipose tissue was protective against the development of cardiometabolic disease [355]. Thus, one recent commentary argued that contemporary imaging techniques might help answer this question. The investigators proposed that low levels of visceral adipose tissue and ectopic adipose tissue combined with a preferential accumulation of gluteal and femoral adipose tissue might define a "super healthy obesity" phenotype [356].

In conclusion, I believe that the singular term obesity cannot properly describe different forms of obesity in terms of adipose tissue mass, body fat distribution, adipose tissue function, and patient lifestyle habits. On this basis, some researchers proposed that we should move away from the term "obesity" and instead refer to "obesities" that would better reflect the clinical reality and distinct treatment challenges associated with the heterogeneity of obesity [20].

Harnessing heterogeneity in T2D

Despite being simply diagnosed based on hyperglycemia, T2D is actually a complex, heterogeneous disease entity. Failure in proper classification followed by the lack of tailored strategies might be responsible for the poor control of diabetes complications. According to McCarthy's "palette model of diabetes", development of T2D is a result of defects in multiple etiological pathways including: beta cell function, insulin action (liver and muscle), glucagon secretion/action, incretin secretion/action and fat distribution; and each T2D case is the result of a combination of defects in these pathways [357]. As shown in **Figure 1**, by given a color to each pathway, a single T2D patient can be represented by different shades in the palette model reflecting the relative contribution of each pathophysiological process to their diabetes. This theory can help us understand and predict the diabetes phenotype with respect to progression, treatment response and outcome. Suggested by another review study [358], the palette model of diabetes also provides us several routes to deconvolute the underlying etiological mechanisms of T2D. To this end, one can (1) directly measure the physiological processes, along with clinical variables, to determine the relative contribution of each process to an individual's phenotype; (2) measure the underlying genetic contribution, where the genetic variants are partitioned into groups reflecting the underlying etiological process (partitioned

polygenic scores); (3) measure an intermediate phenotype, such as those captured by the metabolome and proteome, that integrates both genetic and lifestyle factors; and (4) measure and combine all of the above in an integrative multi-omics approach.

The heterogeneity may exist before the clinical diagnostic threshold for T2D is reached. In **Chapter 4.2**, using multistate models, I observed that among participants with incident T2D during follow-up, those with baseline prediabetes tended to have a lower risk for post-T2D cardiovascular disease and death. These aberrant associations suggest that despite all patients being clinically diagnosed as having T2D, glycemic status preceding diagnosis still affects the future risk of diabetes complications. The Whitehall II study, investigating 18-year trajectories of traditional cardiometabolic risk factors preceding the diagnosis of T2D based on fasting glucose or 2 h glucose, also concluded that individuals with prediabetes differed in their natural history and underlying pathogenesis dependent on whether they have increased fasting glucose concentrations or increased 2 h glucose concentrations, or both, and such differences were also present in individuals with incident T2D [359]. Moreover, the European Group for the Study of Insulin Resistance: Relationship between Insulin Sensitivity and Cardiovascular Disease study, examining the association of pathophysiological characteristics and biomarkers of metabolic functions with different glucose curve patterns during oral glucose tolerance test among individuals without diabetes, identified four glucose curve patterns that differed from each other concerning insulin sensitivity, insulin secretion, obesity, plasma lipids, and low-grade inflammatory markers. They also found that the identified glucose curve patterns were robust over time and transitions between classes were associated with changes in cardiometabolic risk factors [360]. These findings suggest that T2D is not a single disease entity, but rather a heterogeneous disease with different underlying mechanisms preceding its diagnosis in different groups of individuals.

The heterogeneity of T2D has also been suggested during the diagnosis stage. Several key studies have explored the different T2D phenotypes using some readily measured variables involved in the underlying pathogenesis. For example, Ahlqvist and colleagues challenged the current paradigm of adult-onset diabetes using data-driven cluster analysis [361]. Based on six variables: glutamate decarboxylase antibodies, age at diagnosis, BMI, HbA1c, and homeostasis model estimates of b-cell function and insulin resistance, they stratified patients with T2D into five phenotypes with different clinical features and risks for developing diabetes complications [361]. This is an exciting study reporting, for the first time, the mapping of individuals with T2D on a scale according to these feasible clinical and physiological variables at diagnosis. Different T2D phenotypes with various degrees of whole-body and adipose-tissue insulin resistance resulted in a different prevalence of diabetes complications such as early stages of non-alcoholic fatty liver disease and diabetic neuropathy [362]. This T2D sub-phenotyping could eventually could help improve targeted prevention and treatment.

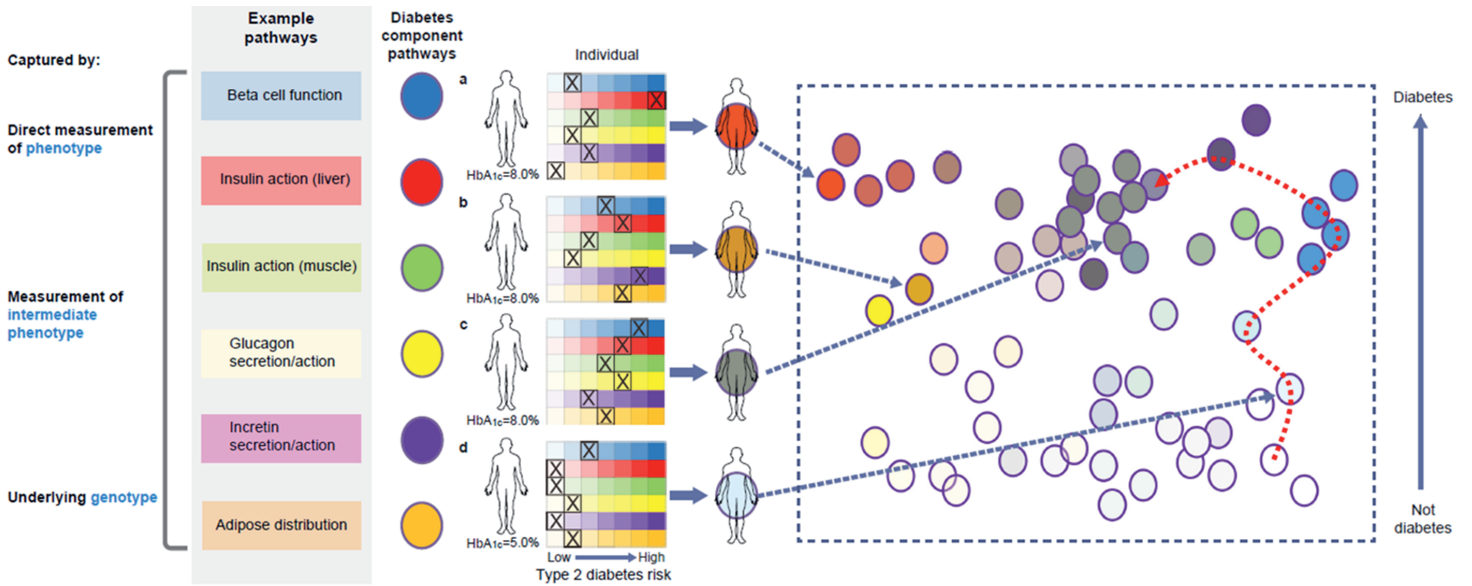

Figure 1. Deconvoluting the diabetes component pathways.

Note: The concept of "McCarthy palette model of diabetes" are illustrated using a model of six diabetes component pathways ("base colors") and four individuals, three of whom have diabetes. The grids display the range of trait variation for each of these component pathways and the position of each individual on each of those spectra. Each person as a color, resulting from different contributions of the colors representing the various pathophysiological processes that can contribute to diabetes. To convert values for HbA1c in % into mmol/mol, subtract 2.15 and multiply by 10.929. Adapted from McCarthy [357] under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium.

The genetic basis of T2D also plays an important role here. More than 400 genetic variants have been found related to T2D risk [363], and further investigation into the underlying etiological heterogeneity is warranted. For example, Udler et al. applied the Bayesian nonnegative matrix factorization to cluster variant-trait associations for 94 known T2D variants and 47 diabetes-related traits. They identified five novel clusters of T2D loci [364]. These different clusters represent five broad pathophysiological processes: classic beta cell deficiency with high proinsulin, beta cell deficiency with low proinsulin, obesity, lipodystrophy, and a process characterized by fatty liver and abnormal lipids. More recently, they also found that these genetically driven pathways leading to T2D predispose differentially to clinical outcomes, including blood pressure, renal function, and coronary artery disease [365]. Unlike other serum biomarkers, these genetic variants associated with T2D are more likely to point to T2D causal mechanisms and remain constant regardless of developmental stage, disease state, or treatment. Therefore, such genetic approaches offer insight into biological pathways causing T2D and associated comorbidities.

Recent advances in omics and wearable monitoring enable deep molecular and physiological profiling and may provide essential tools for harnessing T2D heterogeneity. For example, Sophia et al. conducted a small-scale (n=109) but in-depth study to explore the ability of deep longitudinal profiling to make health-related discoveries, identify clinically-relevant molecular pathways and affect behavior [366]. The cohort was enriched for individuals with prediabetes but also included some T2D patients and underwent integrative personalized omics profiling from samples collected quarterly for up to 8 years using clinical measures and emerging technologies including genome, immunome, transcriptome, proteome, metabolome, microbiome and wearable monitoring. They discovered more than 67 clinically actionable health discoveries and identified multiple molecular pathways associated with metabolic, cardiovascular and oncologic pathophysiology. This omics profiling led to prediction models of insulin resistance. The early return of results to participants also contributed to increased acceptance of diet and exercise changes in research participants [366]. Although time-consuming and requiring elaborate protocol, this study shows that deep longitudinal profiling can lead to actionable health discoveries and provide relevant information for precision health.

Sex differences reflects another manifestation of the heterogeneity of T2D [45]. CVD, the most common diabetes complication, has traditionally been seen as a “man’s problem”. Despite that CVD mortality rates declined considerably over the past decades in both sexes and with stronger age-specific reductions in CHD observed in men than women, CVD mortality remains higher among men than women until old age [367]. CVD is also the leading cause of death in women, and women are disadvantaged in managing CVD. For example, diabetes confers a greater proportional excess cardiovascular risk to women than to men [368-370], whilst adverse pregnancy-related outcomes and factors concerned with the female reproductive cycle (i.e., gestational diabetes) give women added vulnerability to future cardiometabolic diseases [371, 372]. Sex differences exist in the prescription of cardiovascular medication among patients at high risk or with established cardiometabolic disease in primary care, with a lower prevalence of aspirin, statins, and angiotensin-converting enzyme inhibitors prescription in women [373]. Sex differences can also affect the effectiveness of prevention strategies. Results from the Da Qing Diabetes Prevention Study found that lifestyle interventions may improve cardiovascular

mortality more in women with prediabetes than their male counterparts [374]. Hence, there is an urgent need to explore T2D heterogeneity by encompassing the sex differences. Sex-specific approach should also be the norm, whenever feasible, in diabetes research.

Emerging complications of T2D

The classical diabetes complications such as cardiovascular disease are well-known and continue to pose a tremendous burden on people living with T2D. However, with advances in diabetes management and increased life expectancy, the face of diabetes complications is changing [47]. By using data from the England Clinical Practice Research Datalink, researchers found that traditional complications accounted for more than 50% of hospitalization in patients with diabetes in 2003, but only 30% in 2018 in England [375]. The large decline in vascular disease death rates led to a transition from vascular disease causes to cancer as the leading contributor to the gap in death rates between individuals with and without diabetes. Overall, a general diversification of causes of death away from vascular causes towards dementia, cancer, and liver disease was reported [49]. These reductions in cardiovascular morbidity and mortality may be partly attributable to the improvement in primary and secondary prevention strategies, such as statin therapy during the past decades [376]. In addition, T2D can accelerate biological ageing and lead to early-onset frailty, and consequently to impaired physical function. The prevalence of disability is high among patients with T2D. Disability-related conditions, such as frailty and fracture, can also complicate diabetes management through hypoglycemia, polypharmacy and treatment burden [53]. Changes in the composition of diabetes-related complications profile mean that preventative and clinical measures should evolve to reflect the diverse set of causes and to further improve its management.

Although substantial studies support the links between diabetes and these emerging complications such as cancer, cognitive decline, and physical dysfunction, our understanding of their underlying mechanisms is incomplete. Several mechanisms including hyperinsulinemia, hyperglycemia, inflammation and cellular signaling mechanisms, have been proposed to link diabetes to cancer [377]. In terms of dementia or cognitive decline, the enhanced blood-brain barrier permeability (caused by hyperglycemia) and impaired insulin signaling may play a key role in diabetes-related cognitive dysfunction [378]. Regarding functional disability, the rapid loss of skeletal muscle strength and quality among patients with diabetes is a crucial cause of functional disability [379].Moreover, diabetes complications such as CVD can also obviously cause physical disability [268].

To complement our findings from **Chapter 4.3** that diabetes was associated with an accelerated decline in cognition and physical function, the long-term trajectories of cognition and daily functioning before and after diabetes onset are modelled in **Figure 2**. Taken together, these findings suggest careful monitoring for cognitive and physical dysfunction after a diabetes diagnosis. However, in current clinical practice, the required assessments in diabetes management are rarely undertaken. This calls for increasing the awareness regarding these emerging complications, among primary care physicians at the frontline of diabetes care, and developing reliable point-of-care tools for assessing these conditions. These situations should especially be considered for older people with T2D.

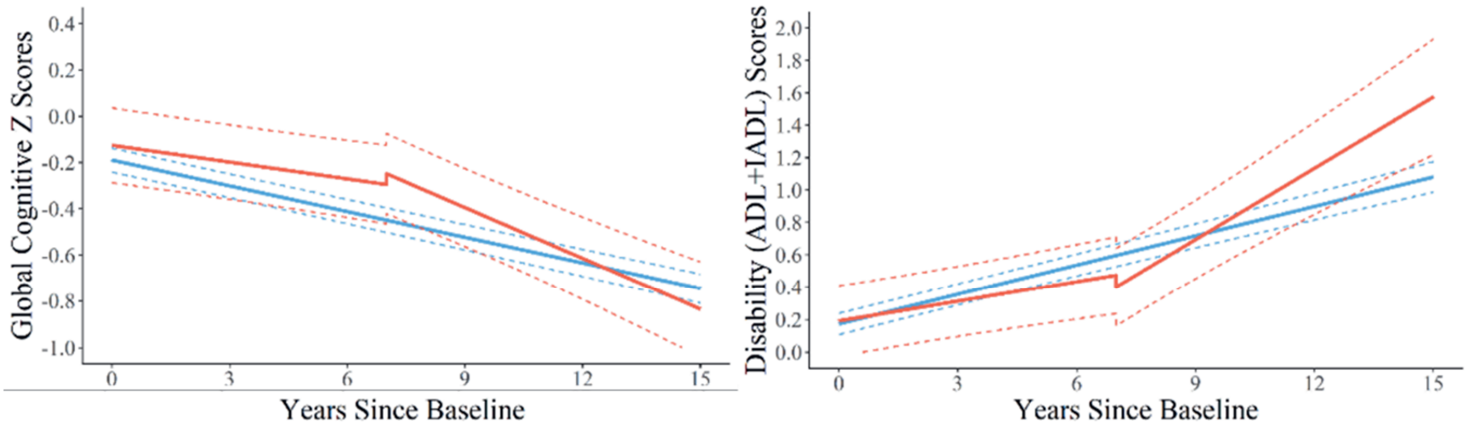

Figure 2. Predicted mean change in cognitive z scores (SD) and daily functioning (point) before and after incident diabetes at year 7.
Note: Global cognition was assessed by orientation, memory, and executive function and daily functioning was calculated as the sum of impaired basic (ADL) and instrumental activities (IADL) of daily living. Blue lines represent the trajectory for diabetes-free participants. Red lines represent the trajectory for participants with incident diabetes. The dashed lines represent the 95% CIs.

5.2

Chapter 5.2

Summary

Since the discovery of insulin in 1921, great progress has been made in preventing and treating type 2 diabetes (T2D). Yet, T2D is still one of the fastest-growing global health emergencies of the 21st century and substantial barriers to changing the course of the epidemic remain. Disease management also moved toward more personalised medicine, emphasising primordial prevention, etiological heterogeneity, and multimorbidity conditions. In this thesis, I studied the role of cardiometabolic health in the development and management of T2D.

Part 2: The role of cardiometabolic health in T2D risk

In **Chapter 2.1**, I demonstrated that longitudinal evolutions of both heart rate and different heart rate variability (HRV) metrics were significantly associated with new-onset T2D, with high heart rate and HRV related to an increased T2D risk. In **Chapter 2.2**, I reported that relative fat mass was more strongly associated with incident T2D than commonly used measures of obesity, especially among younger individuals. Subsequently, in **Chapter 2.3**, I showed that arterial stiffness and remodelling markers were associated with incident T2D, with stronger associations observed among individuals with a higher T2D genetic risk score. However, Mendelian randomisation analyses from **Chapters 2.1** and **2.3** suggested no causal association exist. More studies are needed to validate our findings and further elucidate the underlying mechanism. Finally, in **Chapter 2.4**, I demonstrated that adhering to favourable cardiovascular health (CVH) in midlife could lower the remaining lifetime risk for incident T2D, regardless of T2D genetic predisposition.

Part 3: Implication of blood pressure management in T2D

In this part, I zoomed in on blood pressure management in T2D. By comparing recommendations of hypertension management among different diabetes guidelines in **Chapter 3.1**, I demonstrated that only a modest degree of discordance between the 2020 Chinese Diabetes Society, the 2022 American Diabetes Association, and the 2017 American College of Cardiology/American Heart Association (ACC/AHA) guidelines were found among middle-aged and older Chinese with diabetes, and adopting the 2017 ACC/AHA guideline among Chinese adults with diabetes would double the number of people qualified to initiate antihypertensive treatment. Subsequently, in **Chapter 3.2**, I demonstrated that the lowest risk of death was consistently observed around 130~140 mmHg for systolic blood pressure and 80~90 mmHg for diastolic blood pressure within different multimorbidity patterns, implying that multimorbidity counts or types cannot assist in determining the optimal blood pressure targets in diabetes management.

Part 4: Burden of complications across the glycemic spectrum

In this part, I started with the burden of traditional diabetes complications and moved toward emerging complications. In **Chapter 4.1**, I demonstrated that hyperglycemia status, either prediabetes or T2D, carried a large lifetime risk for incident cardiovascular disease (CVD) among both women and men compared with normoglycemia, and obesity conferred a large burden of lifetime CVD risk. In **Chapter 4.2**, I showed that prediabetes is independently associated with an increased risk of atherosclerotic cardiovascular disease and mortality, and favourable lifestyle habits could lower the risk of transitioning to CVD and mortality among people with prediabetes. Subsequently, in **Chapter 4.3**, I demonstrated that compared to

normoglycemia, baseline T2D was associated with a faster decline in orientation and physical function, and progression from normoglycemia to diabetes during follow-up was also associated with a significantly faster decline in global cognition, memory, executive function, and physical function compared to stable normoglycemia. In **Chapter 4.4**, I showed that adherence to favourable CVH would infer a lower lifetime risk of cardiovascular and non-cardiovascular diseases, and that women had a lower lifetime risk of noncommunicable diseases and multimorbidity in all CVH categories than men.

Part 5: General discussion

In this part, I looked back to my findings, discussed the limitations of my studies and shed light on future diabetes research. In particular, I focused on how we can further improve the effectiveness of lifestyle intervention in the management of T2D. In addition, I discussed the potential of harnessing the heterogeneity of obesity and T2D and highlighted the need to increase awareness of emerging complications in current diabetes care.

5.3

Chapter 5.3

Samenvatting

Sinds de ontdekking van insuline in 1921 is er grote vooruitgang geboekt in het voorkomen en behandelen van type 2 diabetes (T2D). Desondanks blijft T2D wereldwijd een van de snelst groeiende gezondheidscrises van de 21e eeuw. Er blijven aanzienlijke obstakels bestaan voor het veranderen van het verloop van de epidemie. De zorg voor de ziekte is verschuift naar meer gepersonaliseerde geneeskunde, waarbij de vroege primaire preventie, etiologische heterogeniteit en multimorbiditeit de nadruk krijgen. In deze thesis heb ik de rol van cardiometabole gezondheid bij de ontwikkeling en behandeling van T2D bestudeerd.

Deel 2: De rol van cardiometabole gezondheid bij het risico op T2D.

In **Hoofdstuk 2.1** heb ik aangetoond in longitudinale onderzoek dat zowel de hartslag als de hartslagvariabiliteit (HRV) metingen significant geassocieerd waren met nieuw gediagnosticeerde T2D, waarbij een hoge hartslag en HRV gerelateerd waren aan een verhoogd T2D-risico. In **Hoofdstuk 2.2** heb ik gerapporteerd, dat de relatieve vetmassa vooral bij jonge personen sterker geassocieerd was met het ontstaan van T2D dan de gangbare parameters voor obesitas. Vervolgens heb ik in **Hoofdstuk 2.3** aangetoond dat arteriële stijfheid en remodeleringsmarkers geassocieerd waren met nieuw gediagnosticeerde T2D, waarbij sterkere associaties werden waargenomen bij personen met een hoge T2D-genetische risicoscore. De Mendeliaanse randomisatie-analyses in **Hoofdstuk 2.1** en **2.3** suggereren echter dat er geen causaal verband bestaat. Meer onderzoeken zijn nodig om mijn bevindingen te valideren en het onderliggende mechanisme dieper te begrijpen. Ten slotte heb ik in **Hoofdstuk 2.4** aangetoond, dat het trouw volhouden van gezonde cardiometabole leefstijl in het midden van het leven het resterende levenslange risico op nieuw gediagnosticeerde T2D kan verlagen, ongeacht de genetische aanleg voor T2D.

Deel 3: Implicaties van bloeddrukbehandeling bij T2D.

In dit deel heb ik de bloeddrukregulatie bestudeerd bij patiënten met T2D door de aanbevelingen van verschillende diabetesrichtlijnen te vergelijken. In **Hoofdstuk 3.1**, heb ik aangetoond dat er slechts geringe discordantie was tussen de richtlijnen van de Chinese Diabetes Society 2020, de American Diabetes Association 2022 en de American College of Cardiology/American Heart Association (ACC/AHA) 2017 bij middelbare en oudere Chinezen met diabetes, maar dat de ACC/AHA-richtlijn 2017 toepassen op de Chinese volwassenen met diabetes een verdubbeling van het aantal mensen met antihypertensieve behandeling zou opleveren. Vervolgens heb ik in **Hoofdstuk 3.2** aangetoond dat het laagste risico op overlijden consequent werd waargenomen rond een systolische bloeddruk van 130-140 mmHg en een diastolische bloeddruk van 80-90 mmHg dwars door de verschillende multimorbiditeitspatronen, hetgeen impliceert dat multimorbiditeitsscores en -typen niet kunnen helpen bij het vaststellen van de optimale bloeddrukwaarden in het kader van diabetesbehandeling.

Deel 4: De complicatielast over het gehele glykemische spectrum.

In dit deel beschreef ik eerst de traditionele diabetescomplicaties en vervolgens recent ontdekte complicaties. In **hoofdstuk 4.1** liet ik zien dat hyperglykemie, tijdens prediabetes of T2D, een groot levenslang risico op het ontwikkelen van hart- en vaatziekten ziekten (HVZ) oplevert bij zowel vrouwen als mannen in vergelijking met normoglykemie, en obesitas eveneens een grote

last van levenslang HVZ-risico met zich meebrengt. In **hoofdstuk 4.2** toonde ik aan dat prediabetes onafhankelijk geassocieerd is met een hoog risico op atherosclerotische HVZ ziekte en sterfte, en gunstige levensstijlgewoonten de kans op HVZ en sterfte bij mensen met prediabetes kunnen verlagen. Vervolgens toonde ik in **hoofdstuk 4.3** aan dat, in vergelijking met normoglykemie, prevalente T2D geassocieerd was met een snelle afname van de oriëntatie en fysieke functies. Tijdens de follow-up was de progressie van normoglykemie naar T2D (incidente T2D) ook geassocieerd met een significant afname van globale cognitie, geheugen, uitvoeren van testen en fysieke functies. In **hoofdstuk 4.4** liet ik zien dat het naleven van gunstige cardiovasculaire gezondheid een lager levenslang risico op HVZ en niet-cardiovasculaire ziekten met zich meebrengt, en dat vrouwen in alle cardiovasculaire gezondheid categorieën een lager levenslang risico op niet-overdraagbare ziekten en multimorbiditeit hebben dan mannen.

Deel 5: Algemene discussie

In dit deel keek ik terug op mijn bevindingen, besprak ik de beperkingen van mijn onderzoeken en wierp ik licht op toekomstig diabetesonderzoek. In het bijzonder richtte ik me op hoe we de effectiviteit van leefstijlinterventies voor de behandeling van T2D verder kunnen verbeteren. Daarnaast besprak ik de potentie van het benutten van de heterogeniteit van obesitas en T2D en benadrukte ik de noodzaak om meer bewustzijn te creëren voor opkomende complicaties in de huidige diabeteszorg.

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Appendices

PhD Portfolio

List of publications

About the author

Acknowledgements

PhD portfolio

Name PhD student	Kan Wang
Departments	Epidemiology
Research school	Netherlands Institute for Health Sciences (NIHES)
PhD period	September 2019 - June 2023
Promotor	Prof. dr. Eric Sijbrands
Co-promotor	Dr. Maryam Kavousi
	Dr. Fariba Ahmadizar

Training	Year	ECTS
1. MSc in Clinical Epidemiology (*cum laude*)	**2019-2021**	
Study design		4.3
Biostatistical Methods I: Basic Principles		5.7
Biostatistical Methods II: Classical Regression Models		4.3
Principles of Research in Medicine and Epidemiology		0.7
M Research		33.0
Introduction to Medical Writing		2.0
LLS Scientific Integrity		0.3
LLS SteLA Workshop		0.3
LLS Intervision		0.4
Clinical Translation of Epidemiology		2.0
Clinical Epidemiology		3.7
Repeated Measurements in Clinical Studies		1.7
Principles in Causal Inference		1.4
Methods of Public Health Research		0.7
Health Economics		0.7
Introduction to Global Public Health		0.7
The Practice of Epidemiology Analysis		0.7
Fundamentals of Medical Decision Making		0.7
Advances in Clinical Epidemiology		0.7
Using R for Statistics in Medical Research		1.4
Cardiovascular Epidemiology		0.9
Advanced Clinical Trials		1.9
Missing Values in Clinical Research		1.7
Causal Mediation Analysis		1.4
2. Attended Seminars		
Cardiometabolic Group Meeting	2019-2023	2.0
2020 Meetings	2019-2023	2.0
3. Inter(national) Conference and other activities		
Oral presentation at virtual EASD Annual Meeting 2020	2020	1.0
ePoster presentation at ESC Congress 2021 - The Digital Experience	2021	1.0
Oral presentation at ESC Preventive Cardiology 2023, Malaga, Spain	2023	1.0
Peer-review of articles for scientific journals	2019-2021	1.0

List of publications

1. **Wang K**, Kavousi M, Voortman T, Ikram MA, Ghanbari M, Ahmadizar F: Cardiovascular health, genetic predisposition, and lifetime risk of type 2 diabetes. Eur J Prev Cardiol 2022, 28(16):1850-1857.

2. Suthahar N *, **Wang K** *, Zwartkruis VW *, Bakker SJL, Inzucchi SE, Meems LMG, Eijgenraam TR, Ahmadizar F, Sijbrands EG, Gansevoort RT et al: Associations of relative fat mass, a new index of adiposity, with type-2 diabetes in the general population. Eur J Intern Med 2023, 109:73-78.

3. **Wang K**, Gao H, Sijbrands EJG, Kavousi M, Ahmadizar F: Associations of baseline glycemic status and its transitions with cognitive and physical functioning decline. Maturitas 2023, 171:25-32.

4. **Wang K**, Ahmadizar F, Geurts S, Arshi B, Kors JA, Rizopoulos D, Sijbrands EJG, Ikram MA, Kavousi M: Heart rate variability and incident type 2 diabetes in general population. J Clin Endocrinol Metab 2023.

5. Ahmadizar F, **Wang K**, Aribas E, Fani L, Heshmatollah A, Ikram MK, Kavousi M: Impaired fasting glucose, type 2 diabetes mellitus, and lifetime risk of cardiovascular disease among women and men: the Rotterdam Study. BMJ Open Diabetes Res Care 2021, 9(1).

6. Ahmadizar F, **Wang K**, Roos M, Bos M, Mattace-Raso F, Kavousi M: Association between arterial stiffness/remodeling and new-onset type 2 diabetes mellitus in general population. Diabetes Res Clin Pract 2023, 196:110237.

* denotes equal contribution

About the author

Kan Wang was born on the 14th of December, 1992 in Tongcheng, Anhui Province, China. In 2011, he moved to Shanghai to start a bachelor's program in Preventive Medicine at Shanghai Jiao Tong University School of Public Health. After obtaining his medical degree in 2016, he started a master's program in Environmental and Occupational Medicine in Shanghai Medical College of Fudan University and obtained his master degree in 2019. In September of that same year, he started his PhD study at the Department of Epidemiology of the Erasmus Medical Center Rotterdam in the Netherlands. He worked in the Cardiometabolic Epidemiology group under supervision of Prof. Eric Sijbrands and Dr. Maryam Kavousi. His PhD research focused on the role of maintaining cardiometabolic health in the management of type 2 diabetes. During his PhD, he also received a master's degree in Clinical Epidemiology at the Netherlands Institute of Health Sciences in 2021. After finalizing his PhD study, Kan will return to Shanghai to continue his diabetes research as a postdoctoral fellow.

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不同血糖状态下的心血管健康研究

——王侃——