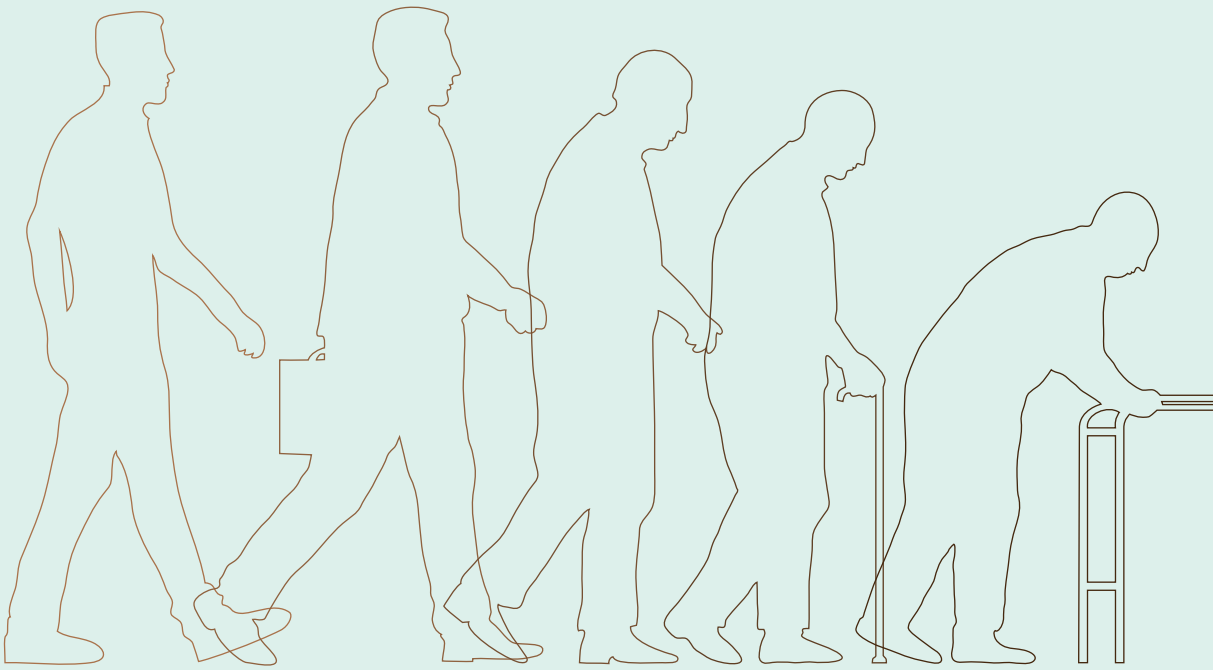
Cardiovascular risk factors over the life course



Gerben Hulsegge

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Cardiovascular risk factors over the life course

Cardiovasculaire risicofactoren gedurende de levensloop
(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. G.J. van der Zwaan, ingevolge het besluit van het college door promoties in het openbaar te verdedigen op donderdag 31 maart 2016 des middags te 12.45 uur

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Gerben Hulsegge
geboren op 7 december 1986 te Bennekom

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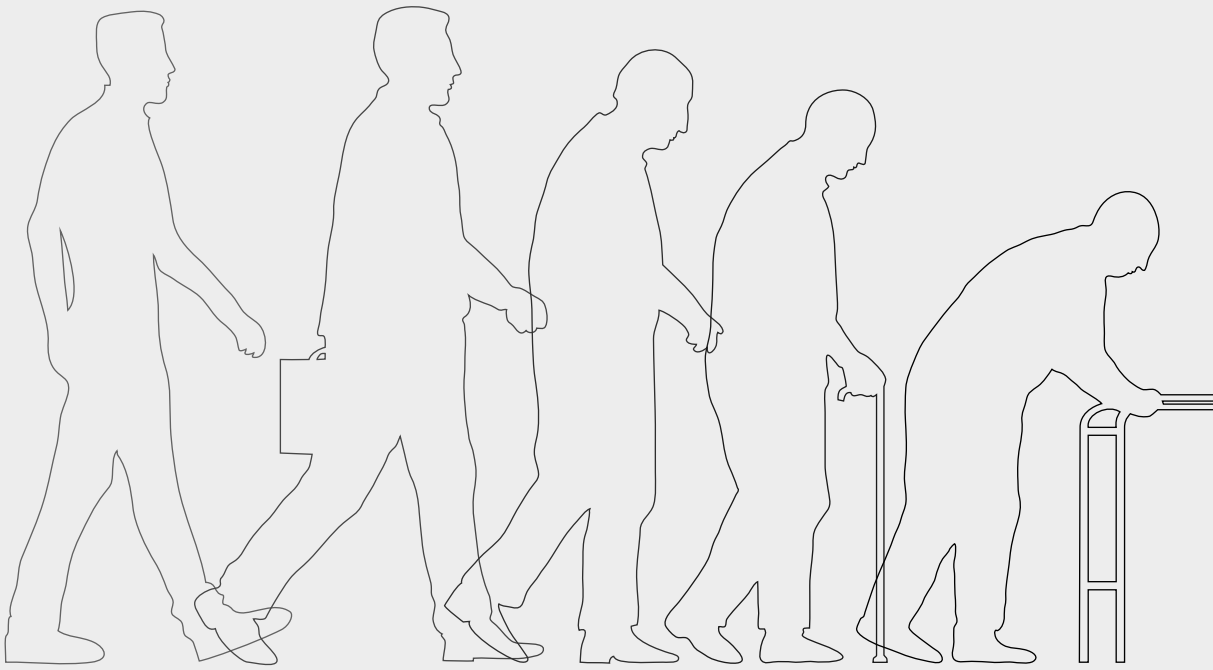
Chapter 1

General introduction



Background

Cardiovascular diseases (CVD) remain the main contributor to morbidity and mortality, in Europe and worldwide.[1-3] Nearly half of all deaths in Europe are caused by CVD,[3] and CVD was the leading cause of Disability Adjusted Life Years lost in 2010.[2] CVD places an enormous burden not only on patients and their families but also on the health care system.[4]

CVD usually manifests itself at middle age or beyond, but it is the result of a disease process, which often starts in childhood and progresses with age.[5-7] It is a multifactorial process, where unhealthy lifestyles and metabolic risk factors interact and accumulate with ageing, eventually leading to CVD.[5, 7] This emphasises the importance of insight into the combined effects of lifestyle factors and metabolic risk factors on CVD. It also stresses the need for insight into the changes in lifestyles and metabolic risk factors that occur throughout the life course, and their impact on CVD. In this thesis, several aspects of medium-term and long-term changes in lifestyle and metabolic risk factors in relation with CVD are addressed.

Metabolic risk factors across generations

A generation refers to a group of people (e.g. 10-year age group) born and living at the same time which may each have unique demographics and life experiences.[8] As each generation ages in distinct circumstances, age-specific levels of metabolic risk factors may change across successive generations (referred to as 'generation shift'). An increasing or decreasing trend of the overall prevalence of risk factors in the general population may be the result of a higher or lower, respectively, age-specific prevalence of risk factors in each younger generation than in older generations.

Although mortality rates for CVD continue to decline in the Netherlands, the number of CVD cases is likely to increase due to ageing of the population.[9-14] The present prevalence of risk factors in current generations, will also influence the absolute number of CVD cases in the future. Knowledge about the current and future prevalence of metabolic risk factors in each generation is needed for indications about future public health problems in the currently young generations when they reach old age. From a public health perspective, it is therefore important to understand changes that are taking place in the population.

Prominent changes in the prevalence of CVD risk factors in high-income countries over the last decades are the decline in smoking prevalence,[15-19] the increase in obesity prevalence[19-22] and improvements in for example the treatment and control of risk factors.[23] These changes indicate that the risk profile of more recently born generations will not be the same at old age as the risk profile of the preceding generations. The more recently born generations in high-income countries smoked less often than earlier born adult generations, particularly men.[15, 16, 24] However, less is known whether similar generation shifts in age-specific prevalence also occurred for overweight, obesity and for other cardiovascular risk factors.

Cardiovascular risk factor profiles and risk of cardiovascular disease

To study the combined effect of multiple risk factors on CVD broadly two kinds of, partially overlapping, cardiovascular risk profiles are distinguished in the field of cardiovascular epidemiology. One risk factor profile emphasises lifestyle, and is labelled the 'lifestyle profile' (Figure 1.1).[25-31] The second risk factor profile focusses on the 'classic' major CVD risk factors. In line with other studies,[32-35] this profile should be labelled 'risk profile'. In the context of this thesis, the term 'metabolic risk profile' will be used for a clearer distinction with the 'lifestyle profile'. It is noted, however, that the lifestyle profile also contains one metabolic risk factor (body mass index) and that the metabolic risk profile also contains one lifestyle factor (smoking).

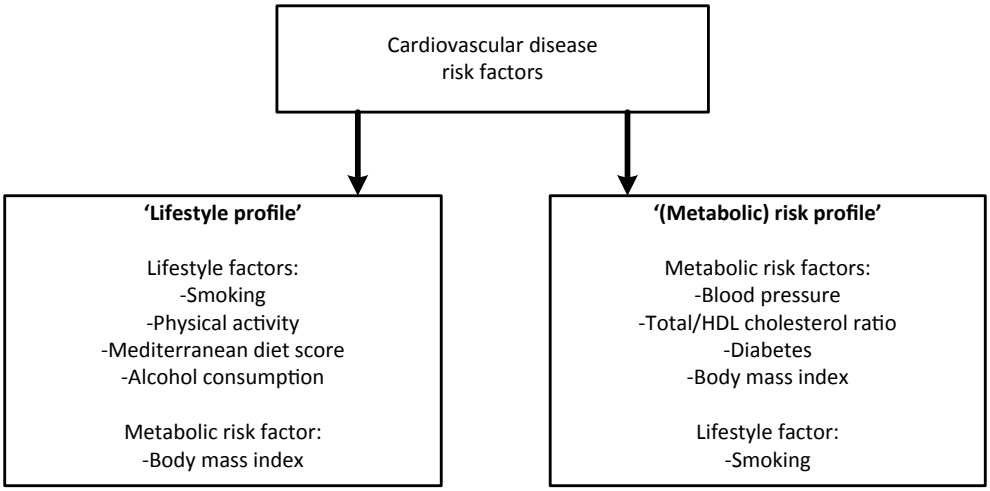


Figure 1.1. The definitions of lifestyle profile and metabolic risk profile used throughout this thesis.

Most research on CVD risk factors has compared individuals with unhealthy lifestyle factors to those with healthy lifestyle factors, and those with high metabolic risk factor levels to those with low metabolic risk factor levels. However, the risk of CVD increases continuously with increasing levels of metabolic risk factors and with increasing number of unfavourable lifestyle factors[27-29, 31, 36] and metabolic risk factors.[37-39] Only a relatively small proportion of the total population is in the high-risk group and the larger part of the total population has low or moderately elevated risk factor levels. The CVD risk in the groups with low or moderately elevated risk factor levels is smaller than in the group with high risk factor levels. Nevertheless, a higher absolute number of CVD cases arise from the groups with a low or moderate risk than from the high-risk group, due to the large number of people in those groups.[27, 29, 31-34, 40, 41]

Several studies have shown the benefits of a healthy lifestyle profile and a low metabolic risk profile. Adherence to four or five healthy lifestyle factors (i.e. healthy diet, being physically active, no smoking, drinking alcohol in moderation and having a healthy weight) was associated with a 46-68% lower risk of CVD compared to having none of these healthy lifestyle factors.[28, 30, 31] The risk of CVD in young and middle-aged adults with favourable levels of all 'major' CVD risk factors (i.e. low metabolic risk profile) at baseline was half that of adults with a higher level in one 'major' CVD risk factor, and each additional risk factor further increased the risk of CVD.[32-35, 40, 42-44]

These studies on lifestyle profiles and metabolic risk profiles were based on single (baseline) assessment of risk factors and did not take into account changes in lifestyle and metabolic risk profiles over the life course. Yet, it seems obvious that the longer a healthy lifestyle and low metabolic risk profile are maintained, the greater the benefits in lowering CVD risk over the life course. To investigate the full importance of living a healthy life throughout the life course, the magnitude of the benefits of maintenance of a healthy lifestyle profile and a low metabolic risk profile is quantified in this thesis. Such quantitative estimates help to provide insight into the potential effects of primary prevention targeted at attaining and maintaining a healthy lifestyle profile and a low metabolic risk profile.

Trajectories of metabolic risk factors and biochemical markers preceding cardiovascular disease and type 2 diabetes

The term 'trajectory' indicates the development of a risk factor level over an individual's life course. Comparison of long-term trajectories of metabolic risk factors and biochemical markers between people who do and people who do not develop disease may help to identify at which time point these factors start deteriorating before occurrence of symptomatic disease. Such insight may provide indications about the optimal timing of preventive actions. It is not fully understood whether symptomatic CVD or type 2 diabetes is preceded by a gradual accumulation of the adverse effects of risk factors starting at a young age, by a relatively sudden deterioration in risk factors before disease onset, or by a combination of both.

CVD and type 2 diabetes share a number of lifestyle factors such as unhealthy diets and physical inactivity, as well as biological risk factors such as obesity and chronic inflammation.[45-53] Apart from being a disease in itself, type 2 diabetes is a major risk factor of CVD,[54, 55] and changes that occur before the development of type 2 diabetes are also relevant for CVD.

A few studies have thrown light on trajectories of body mass index[56] and C-reactive protein before symptomatic CVD,[57] but information about the long-term course of most other metabolic risk factors and biochemical markers before the onset of CVD is lacking. With regard to type 2 diabetes, only one large prospective cohort study described long-

term trajectories of metabolic risk factors before the onset of type 2 diabetes, without distinguishing between men and women.[58] This study showed differences in trajectories between people with type 2 diabetes and controls in systolic blood pressure and high-density lipoprotein cholesterol, but not in body mass index. For a better understanding of the course of risk factors before the onset of type 2 diabetes, besides confirming previous findings, we investigated trajectories of other factors associated with type 2 diabetes, including markers of liver fat,[59, 60] chronic inflammation,[47] oxidative stress[59-61] and reduced kidney function.[62] Comparing trajectories of metabolic risk factors and biochemical markers for type 2 diabetes with those for CVD may also improve our understanding of the differences and similarities in the development of these diseases.

Objectives and outline of this thesis

The central theme of this thesis is changes in lifestyles and metabolic risk factors in young and middle-aged adults, and their effect on CVD. Two 'dimensions' of change are studied. First, changes across generations, that is, higher or lower age-specific levels of risk factors in successive generations, are studied in **Part I**. Second, changes with age in lifestyle, metabolic risk factors and biochemical markers within individuals is studied in **PART II** and **III**. In this thesis, gamma glutamyltransferase, alanine aminotransferase, C-reactive protein, uric acid, creatinine and cystatin C are referred to as 'biochemical markers'.

Part I – Metabolic risk factors across generations

The first objective of this thesis is to determine the age-specific levels and prevalences of metabolic risk factors across four generations (i.e. 10-year age groups). **Chapter 2** describes the age-specific prevalence of overweight, obesity, hypertension, hypercholesterolemia, low HDL-cholesterol and type 2 diabetes in those four generations. In **chapter 3**, the age-specific levels of body mass index and biomarkers of pathophysiological processes that are believed to mediate the effects of risk factors (i.e. markers of oxidative stress and chronic inflammation) in those four generations are presented. The age-specific increase in the prevalence of obesity may have an unfavourable effect on the levels of biochemical markers. Therefore, the effect of changes in body mass index on age-related changes in markers of oxidative stress and inflammation is specifically addressed.

Part II – Cardiovascular risk factor profiles and risk of cardiovascular disease

The second objective is to determine the full benefit of medium-term favourable 'lifestyle profiles' and long-term favourable 'metabolic risk profiles' (as defined above). **Chapter 4** describes the association between maintenance or, in contrast, improvement or deterioration in lifestyle profiles over a five-year period and risk of CVD and all-cause mortality. **Chapter 5** describes the association between maintenance or, in contrast,

improvement or deterioration in metabolic risk profiles over an 11-year period and risk of CVD. **Chapter 6** describes determinants of medium-term changes in metabolic risk profiles.

Part III – Trajectories of metabolic risk factors and biochemical markers preceding cardiovascular disease and type 2 diabetes

The third objective is to determine the course of metabolic risk factors and biochemical markers preceding CVD and type 2 diabetes. In **chapter 7** and **8**, differences in long-term trajectories of metabolic risk factors and biochemical markers are identified between people who develop CVD or type 2 diabetes and people who do not.

To address these objectives, data from the Doetinchem Cohort Study was used in all studies described in this thesis. The Doetinchem Cohort Study is an ongoing prospective population-based cohort study of almost 7,800 men and women aged 20-59 years. Extensive information about demographics, lifestyle and risk factors was obtained from 1987-1991 onwards, with measurements every five years over a 20-year period. Data about non-fatal and fatal CVD events was obtained through linkage with the Dutch Hospital Discharge Registry and Statistic Netherlands respectively.

In **chapter 9**, the general discussion, the main findings of this thesis and their implications for public health are discussed.

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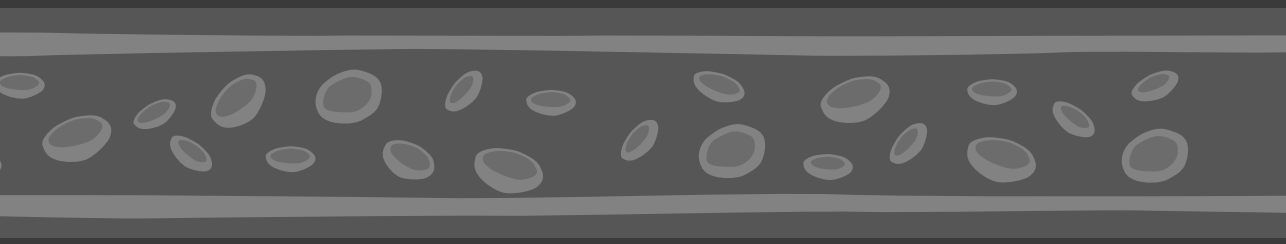

Part I

Metabolic risk factors across generations

Chapter 2

Today's adult generations are less healthy than their predecessors: generation shifts in metabolic risk factors

Gerben Hulsegge, H Susan J Picavet, Anneke Blokstra, Astrid CJ Nooyens, Annemieke MW Spijkerman, Yvonne T van der Schouw, Henriëtte A Smit, WM Monique Verschuren

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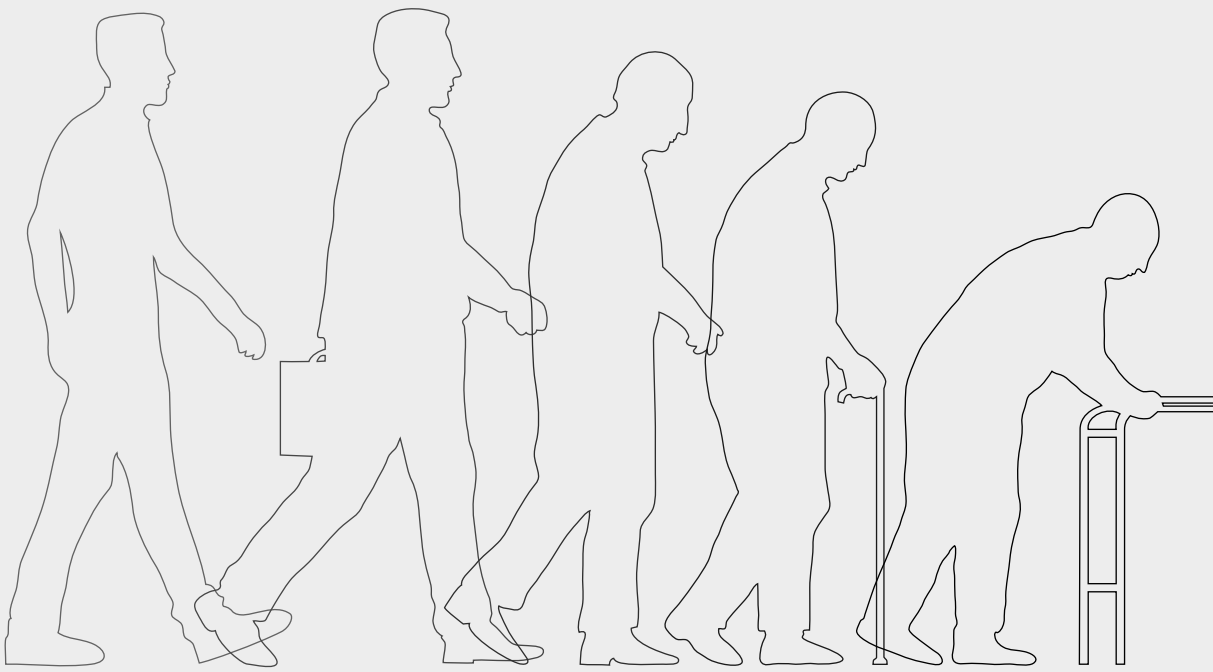

Abstract

Background: The health of the elderly of the future is partly determined by their exposure to metabolic risk factors during their life course. Our aim is to study generation shifts in metabolic risk factors.

Methods: We used data of the Doetinchem Cohort Study, that started in 1987–1991 and had follow-up examinations after 6, 11, and 16 years (n=6,377). The analyses were stratified by sex and generation, i.e. 10-year age groups (20–29, 30–39, 40–49, and 50–59 years) at baseline. Whether a generation had, at a similar age, a different risk profile compared to a generation born 10 years earlier (i.e. generation shift) was tested by means of generalized estimation equations.

Results: The prevalence of overweight, obesity, and hypertension increased with age within all generations, but in general more recently born generations had, at a similar age, a higher prevalence of these risk factors than generations born 10 years earlier ($p < 0.05$). Unfavourable generation shifts were most pronounced for overweight/obesity, present in men between every generation while in women especially present between the most recently born generations. We observed unfavourable generation shifts in diabetes among men but not among women. No generation shifts for hypercholesterolaemia were observed and favourable generation shifts for low high-density lipoprotein cholesterol between the oldest two generations only. In general, the pattern of generation shifts did not differ according to socioeconomic status.

Conclusions: The lifelong exposure to especially obesity will increase. As a consequence, more elderly of the future will develop overweight-related diseases such as diabetes and cardiovascular disease.

Introduction

It is estimated that in the year 2040, more than one in every four Europeans and more than one in every five North Americans will be 65 years or older.[1] Whether the health of the elderly of the future differs from today's elderly depends among others on differences in exposure to metabolic risk factors during the life course of the current adult generations. Metabolic risk factors including obesity, hypertension, and dyslipidaemia substantially increase the risk of chronic and in particular cardiovascular disease (CVD),[2-6] which is the main cause of disability and death in most high-income countries.[3, 7]

Data on time trends in cardiovascular risk factors among the adult population of high-income countries have shown increases in the prevalences of overweight, obesity, and diabetes in the last two decades.[8-14] For the prevalence of hypertension and hypercholesterolaemia, most studies showed a decline over time, though stable and increasing prevalences were also observed.[8, 9, 12, 15-19] Although some information about the development of metabolic risk factors is available for adult populations, little attention has been paid to whether or not there are differences in these time trends and levels between the younger and older adult generations. Data from the USA showed that more recently born generations were doing worse, e.g. the prevalence of obesity among 20-39-year-old men was 15% in 1988-1994 and 24% in 1999-2000.[10] The prevalence of hypertension among 18-29-year-old men was 4% in 1988-1994 and 6% in 1999-2004,[17] findings that were based on repeated cross-sectional data. A longitudinal study from Austria showed in general a higher mean body mass index (BMI) but lower mean total cholesterol and mean blood pressure at a similar age among more recently born generations compared to generations born 10 years earlier.[20]

Knowledge about risk profiles of today's generations is essential since it determines the disease burden later in life. Over time, generations (i.e. 10-year age groups) will reach a similar age as their preceding generation. Therefore, generation shifts can be determined by comparing the prevalence of risk factors, at a similar age, across consecutive generations. We were able to analyse this in a large-scale longitudinal study that started in 1987-1991 among Dutch adults between 20 and 59 years of age. Our aim is to study generation shifts in metabolic risk factor prevalences and means, by describing changes within four 10-year generations of Dutch adults during 16 years of follow-up.

Methods

Population

Participants were randomly selected based on an age- and sex-stratified sample from the civil registries of Doetinchem, a small town in the Netherlands (46,967 inhabitants in

2000). At baseline (1987-1991: wave 1), 20,155 people aged 20-59 years were invited to visit the municipal health centre to participate in the 'Monitoring Project on Cardiovascular Disease Risk Factors'. From the participants in wave one (n=12,405, participation rate 62%), a random sample of 7,768 was invited for a second examination (1993-1997: wave 2). The total random sample was invited again in 1998-2002 (wave 3, n=6,579) and 2003-2007 (wave 4, n=5,783), except for those who did not give permission to retrieve their information from the municipal administration, missed two examinations in a row, emigrated, actively withdrew from the study, or died. The response rates for all follow-up measurements varied between 75% and 80%, resulting in 6,113, 4,916, and 4,520 participants for wave 2, 3, and 4 respectively. The study design of the Doetinchem Cohort study is extensively described elsewhere.[21] For the present analyses, we excluded pregnant women only for that specific wave (n=140) and included all participants who took part in at least two waves. Most of these participants completed all four waves (61.1%), three examinations were completed by 19.4%, and two by 19.5% of the participants. This resulted in a total of 6,308, 6,070, 4,898, and 4,517 participants in wave 1, 2, 3 and 4 respectively. In total, 21,786 examinations from 6,377 participants were included for the present study. All participants gave written informed consent and the study was approved according to the guidelines of the Helsinki Declaration by the external Medical Ethics Committee of the Netherlands Organization for Applied Scientific Research.

Measures

Trained staff completed standardized measurements of cardiovascular risk factors (i.e. overweight, obesity, hypertension, hypercholesterolaemia, and low high-density lipoprotein (HDL) cholesterol) during a visit to the municipal health service. These included anthropometric measurements, blood pressure measurement, and blood sampling. Demographic characteristics, medical history of chronic diseases, use of medication, and lifestyle factors were collected using standardized questionnaires. Body weight and height were measured with participants wearing light indoor clothing with emptied pockets and without shoes. Body weight was measured to the nearest 0.1 kg on calibrated scales and height to the nearest 0.5 cm. BMI was calculated as weight, minus 1 kg to adjust for clothing, divided by height squared (kg/m^2). Anyone with a BMI ≥25 kg/m^2 was classified as overweight, and those with a BMI ≥30 kg/m^2 as obese. Systolic and diastolic blood pressure were measured with a random zero sphygmomanometer (Hawksley and Sons, Lancing, UK) in wave 1 to 3. Participants were measured twice in each wave in sitting position after 2 minutes of rest. Systolic blood pressure was recorded at the appearance of sounds (first-phase Korotkoff) and diastolic blood pressure was recorded at the disappearance of sounds (fifth-phase Korotkoff). The mean value of two measurements was used in the analyses. Hypertension was defined as a systolic blood pressure ≥140 mmHg, and/or diastolic blood

pressure ≥90 mmHg, and/or use of antihypertensive medication (according to World Health Organization definition).[22] In wave 4, blood pressure was measured with a different measuring device (Speidel Keller) and participants sat in a slightly different position during the measurement. Blood pressure measurements are sensitive to small changes in the methodology and results were not as expected. Therefore, we only included data on blood pressure of the first three waves. Total and HDL cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory (LRL), using standardized enzymatic methods. Hypercholesterolaemia was defined as total cholesterol ≥6.5 mmol/l and/or use of cholesterol-lowering medication and low HDL cholesterol as HDL cholesterol <0.9 mmol/l. Type 2 diabetes cases were defined on basis of self-report. Most self-reported diabetes cases were verified with information from the general practitioner or pharmacist (86%). Of the identified cases, 20 were type 1 diabetes and eight unknown/other diabetes type and were excluded for the analysis on diabetes. The highest level of completed education during follow-up was used as a proxy for socioeconomic status (SES) and classified into three categories: low (intermediate secondary education or less), intermediate (intermediate vocational or higher secondary education), and high (higher vocational education or university).

Statistical analyses

The developments in the prevalence of metabolic risk factors over time in four 10-year generations are described. The generations are defined on the baseline age of the participants: 20-29, 30-39, 40-49, and 50-59 years, further referred to as those who were in their 20 s, 30 s, 40 s, and 50 s respectively. In Figure 2.1, the prevalence of metabolic risk factors is plotted against the mean age of these generations at the time of measurement, for men and women. At baseline, those who were in their 20 s were on average 25 years, 31 years in wave 2, 36 years in wave 3, and 41 years in wave 4. Means and standard deviations were calculated for all continuous outcome variables (i.e. BMI, systolic blood pressure, diastolic blood pressure, total cholesterol, and HDL cholesterol) at each wave for every generation and both sexes.

A generation shift refers to a difference in the prevalence or mean of a metabolic risk factor between generations when they have attained a similar age. From the figure, this can be derived by determining whether the lines of consecutive generations overlap or not. Overlap of the lines implies no generation shift. An unfavourable generation shift is present if the line of the more recently born generation is above that of a generation born 10 years earlier, a favourable generation shift if the line is below that of a generation born 10 years earlier. To test whether the generation shifts were statistically significant, logistic regression for dichotomous outcomes and linear regression for continuous outcomes were used. To take the correlations amongst repeated observations on the same participants into account

generalized estimating equations (GEE) with auto-regressive structure were performed. At waves 3 and 4, the average age of a generation was approximately the same as the average age of a generation born 10 years earlier at wave one and two respectively. Therefore, the prevalence or mean of a metabolic risk factor (dependent variable) at waves 3 and 4 of a generation (independent variable) was compared to the prevalence or mean of a generation born 10 years earlier at waves 1 and 2 respectively. GEE was not used for hypertension and mean blood pressure, since only wave 1 was compared to wave 3. The analyses were adjusted for age and a p-value <0.05 was considered statistically significant. To determine whether generation shifts were present among different socioeconomic classes, the plots were also stratified by SES. In addition, to statistically test whether the generation shifts differed by SES, we entered SES and an interaction term of generation and SES into all regression models. All analyses were performed in SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

At baseline, the age range was 20-59 years and 47.5% were men (Table 2.1). Slightly more than half of the population (51.5%) had a low educational level. At baseline, participants belonging to older generations were more often low educated, smokers, and had unfavourable levels of the risk factors studied.

The prevalence of metabolic risk factors increased over the 16-year follow-up period within all generations, except for low HDL cholesterol (Table 2.2-2.3 and Figure 2.1). In general, more recently born generations had, when reaching a similar age as the generation born 10 years earlier, a statistically significant higher prevalence of overweight, obesity, and hypertension. Among men, such unfavourable generation shifts in the prevalence of obesity were present between all generations. For example, 40% of the males who were in their 30 s at baseline (average age 35 years) were overweight. At 11-year follow-up, the prevalence was 52% among men who were then in their 30 s (those who were in their 20 s at baseline) (p <0.01). Unfavourable generation shifts of overweight and obesity among women were only evident between the most recently born generations. Although the prevalence of obesity increased during follow-up within all generations, the prevalence among those who were in their 20 s at baseline was almost twice as high, when they reached a similar age as those who were in their 30 s at baseline (P <0.01). At the average age of 41 years, 15% of those who were in their 20 s at baseline and 8% of those who were in their 30 s at baseline had obesity. Unfavourable statistically significant generation shifts in hypertension were observed in both sexes between every consecutive generation, except for the two most recently born generations of men. Generation shifts were not present for

the prevalence of hypercholesterolaemia. For low HDL cholesterol, statistically significant favourable generation shifts were present between those who were in their 40 s and those who were in their 50 s at baseline only. The prevalence of type 2 diabetes was low during the complete follow-up period, especially among those who were in their 20 s and 30 s at baseline. Unfavourable generation shifts were observed between the two most recently born generations of men and between the two oldest generations of men (P <0.05). No generation shifts in diabetes were present among women.

Table 2.1. General baseline (1987-1991) characteristics of the Doetinchem Cohort Study (n=6,308).

Age at baseline	**20-29 years**		**30-39 years**		**40-49 years**		**50-59 years**	
	♂ n=467	♀ n=588	♂ n=940	♀ n=1,038	♂ n=928	♀ n=955	♂ n=659	♀ n=733
Age (years)	25.4± 2.9	25.3± 2.9	35.1± 2.9	35.0± 2.7	44.3± 2.6	44.2± 2.8	54.5± 2.8	54.5± 2.9
Education[a]								
Low	32.6	38.8	37.8	52.5	47.6	64.5	53.7	78.9
Intermediate	46.9	44.9	33.2	25.3	28.6	18.6	22.7	12.0
High	20.6	16.3	29.0	22.2	23.8	17.0	23.6	9.9
Smokers	39.2	39.8	36.9	38.1	33.8	33.5	30.7	24.5
BMI (kg/m^2)	23.4± 2.9	22.6± 3.4	24.5± 2.9	23.5± 3.5	25.6± 3.0	24.6± 3.7	26.0± 2.9	26.3± 4.0
BMI classes								
Normal	73.1	83.9	59.7	73.4	44.4	61.6	39.5	41.6
Overweight	24.1	13.0	36.2	21.9	48.0	30.2	52.4	43.5
Obesity	2.8	3.1	4.1	4.7	7.5	8.2	8.1	14.9
SBP	125± 12	114± 11	124± 12	113± 12	124± 14	118± 15	130± 15	127± 16
DBP	75±9	72±9	78±10	73±9	81±10	77±10	82±11	81±11
Hypertension[b]	14.1	4.6	17.9	6.3	23.7	16.1	35.3	32.4
Total cholesterol (mmol/l)	4.8± 0.9	5.0± 0.9	5.4± 1.1	5.0± 0.9	5.8± 1.1	5.4± 0.9	6.0± 1.0	6.2± 1.0
Hypercholesterolaemia[c]	3.6	4.6	15.0	6.9	23..8	13.3	29.3	33.9
HDL cholesterol (mmol/l)	1.13± 0.24	1.36± 0.29	1.12± 0.26	1.35± 0.30	1.12± 0.27	1.40± 0.32	1.09± 0.26	1.35± 0.32
Low HDL cholesterol[d]	14.8	2.7	17.6	4.6	18.2	3.6	22.9	4.7
Type 2 diabetes (%)	0.2	0.2	0.0	0.1	0.7	0.5	1.1	1.6

Values are mean ± SD or %; [a]Highest attained level during follow-up; [b]Systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥ 90mmHg, and/or on antihypertensive medication; [c]Total cholesterol ≥6.5 mmol/l and/or on cholesterol-lowering medication; [d]HDL cholesterol < 0.9 mmol/l; BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; SBP, systolic blood pressure.

The observed generation shifts in obesity, hypertension, and low HDL cholesterol were also reflected in the changes for mean BMI, blood pressure (diastolic and systolic), and HDL cholesterol respectively (Table 2.2-2.3). Statistically significant favourable generation shifts for mean total cholesterol were observed between those who were in their 40 s compared to those who were in their 50 s at baseline, among men and women. Generation shifts for the use of blood pressure and cholesterol-lowering medication were present i.e. the use of medication was, at a similar age, higher among more recently born generations compared to generations born 10 years earlier.

A SES gradient was observed for every risk factor: participants with a low SES had higher prevalences of metabolic risk factors compared to those with intermediate or high SES. However, in general the pattern of generation shifts was similar for different socioeconomic classes (P >0.05). As an example, this is illustrated for the prevalence of obesity among men (Figure 2.2).

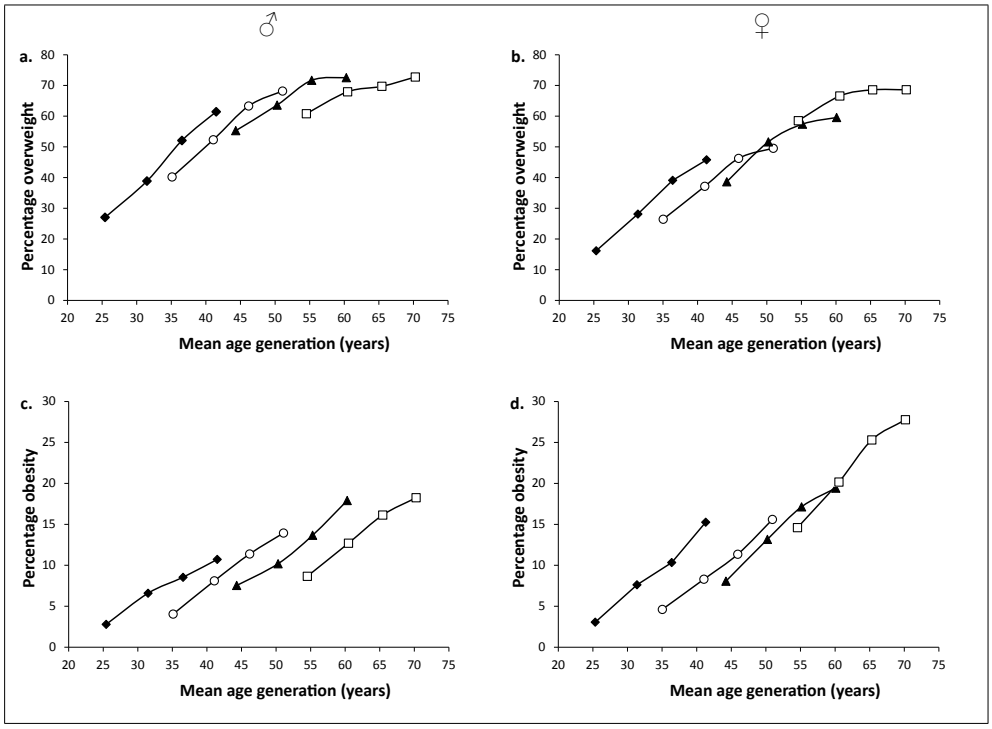

Figure 2.1 continues.

Figure 2.1 continued.

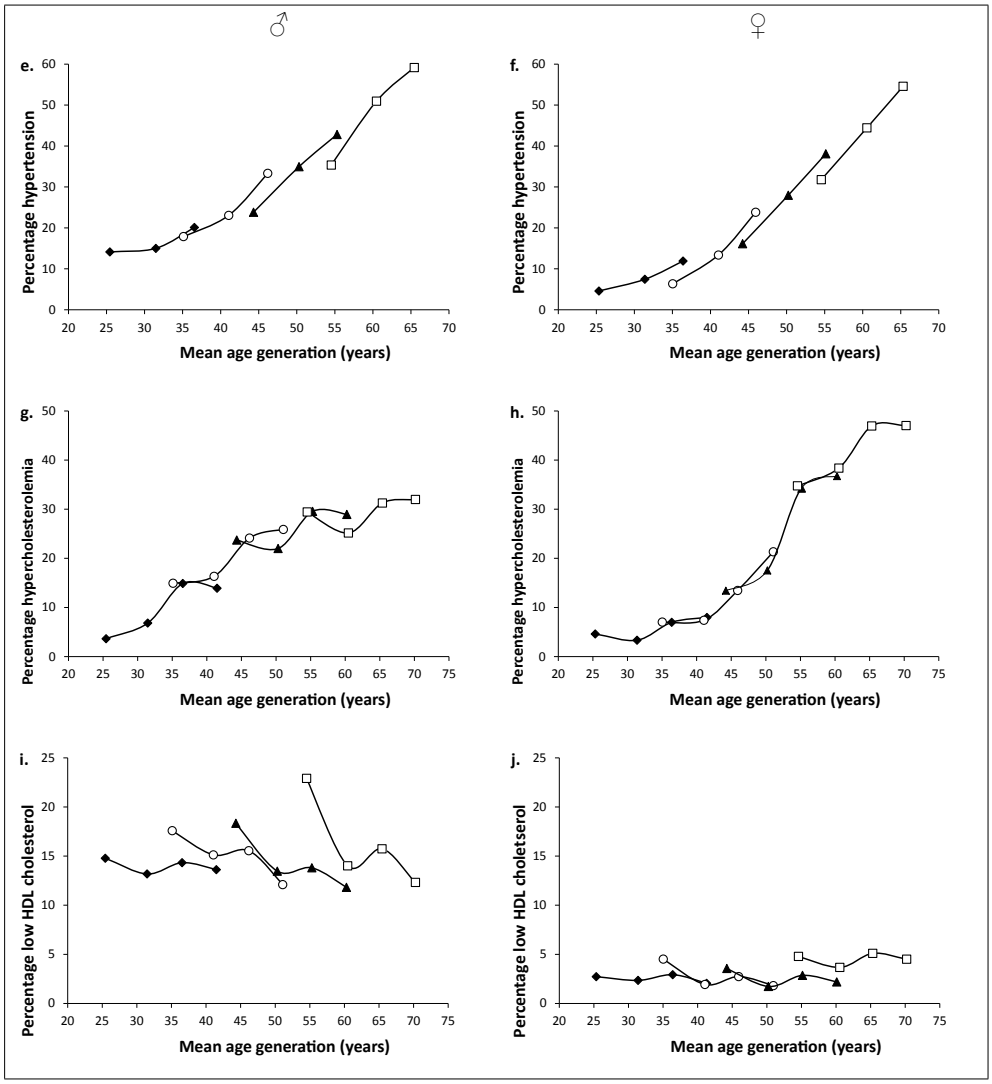

Figure 2.1 continues.

Figure 2.1 continued.

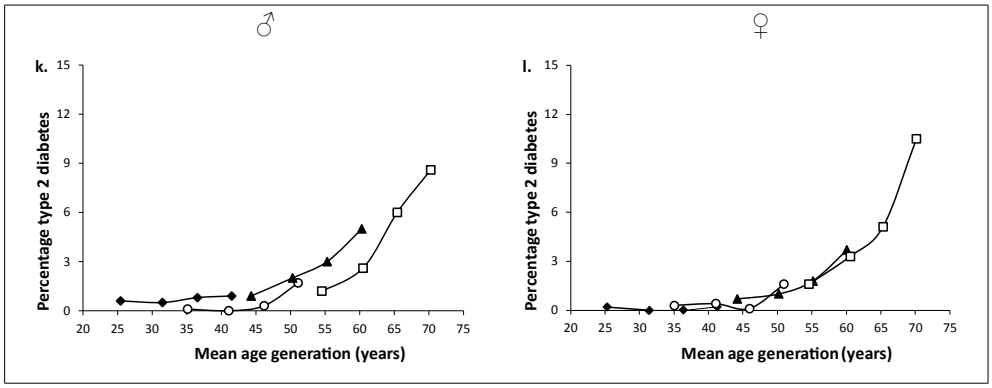

Figure 2.1. Age-specific prevalence of risk factors over 16 years follow-up (4 waves) in those who were in their 20 s (-♦-), 30 s (-○-), 40 s (-▲-), and 50 s (-□-) at baseline, stratified by gender: for overweight (BMI ≥25 kg/m^2) (a,b), obesity (BMI ≥30 kg/m^2) (c, d), hypertension (systolic blood pressure ≥140 mmHg, and/or diastolic blood pressure ≥90 mmHg, and/or on antihypertensive medication) (e, f), hypercholesterolaemia (total cholesterol ≥6,5 mmol/l and/or on cholesterol-lowering medication) (g, h), low HDL cholesterol (HDL cholesterol <0.9 mmol/l) (i, j), and type 2 diabetes (k,l).

Discussion

Our results suggest a clear generation shift in the prevalence of overweight, obesity and hypertension, with more recently born adult generations doing worse than their predecessors. Generation shifts were not found for hypercholesterolaemia and low HDL cholesterol, except a favourable generation shift in HDL cholesterol between the two oldest generations. The prevalence of type 2 diabetes was low in the present study and unfavourable generation shifts were observed among men but not among women. The unfavourable generation shifts observed in the total population were consistent across different SES strata and do not lead to increasing or decreasing socioeconomic differences.

The unfavourable generation shifts in metabolic risk factors observed in our study are partly in line with previous studies. For BMI an increase in high-income countries over the past two decades is well known, as indicated by repeated cross-sectional studies.[9, 10, 12, 23-25] These studies did however not separate the analyses by age group at all[9, 12, 23, 24] or only by two age groups of adults.[10, 25] Therefore, these studies were unable to show which age groups specifically had higher BMI levels over time. In the present study, unfavourable generation shifts were present in men between every generation and in women especially present between the most recently born 10-year generations. For example, in 10 years the prevalence of obesity doubled among young women (mean baseline age was 25 years). This seems to be in line with results from the Nutrition Examination Survey (NHANES) in the USA that found an increase of 9% and 10% in the prevalence of obesity between 1988-

1994 and 1999-2000 among 20-39 year old men and women respectively.[10] The observed unfavourable generation shifts for overweight and obesity are, in theory, a result of an unfavourable imbalance between energy intake and energy expenditure. Trends in energy intake and physical activity in the Netherlands during this period are unclear. Evidence indicates that physical inactivity increased over time and energy intake slightly decreased with the note that underreporting of energy intake probably also increased.[26]

For blood pressure, most previous studies showed decreasing levels over the last decades,[9, 12, 20, 23, 27, 28] which is in contrast to our findings. However, the NHANES showed also an increase in the prevalence of hypertension.[17, 29] Differences between studies may be due to differences between countries/cultures in for example lifestyle patterns, and primary prevention and treatment guidelines.[30] The inclusion of blood pressure-lowering medication in the definition of hypertension may also lead to different results compared to other studies. When different studies do include medication in their definition, results can also be influenced by differences in prescribing habits between countries. The present study also found unfavourable generation shifts in mean diastolic and systolic blood pressure, so the generation shifts are not only due to the inclusion of hypertensive medication in the definition. Blood pressure is also partly determined by BMI.[31] As an explanatory analysis, the GEE analyses were also adjusted for BMI to investigate whether the observed generation shifts in hypertension could be explained by unfavourable generation shifts in BMI. The large unfavourable generation shifts in BMI among the two most recently born generations of women is reflected in an attenuation of the unfavourable generation shift in hypertension after adjustment for BMI, i.e. odds ratio decreased from 1.69 (95% confidence interval: 1.14-2.50) to 1.41 (95% confidence interval: 0.94-2.11). BMI could not explain the other generation shifts of hypertension among women and attenuated the odd ratios among men somewhat (data not shown). This is in line with the observed generation shifts in overweight and obesity.

The impact of including medication in the definition is reflected in the cholesterol figures. The prevalence of hypercholesterolaemia was similar for the different generations at a similar age, although we observed favourable generation shifts for total cholesterol levels between the two oldest generations, i.e. the more recently born generation is doing better. In line with this last finding, previous studies generally found decreasing total cholesterol levels over time.[8, 9, 12, 20, 23, 32, 33] The present study suggests that this is mainly a result of generation shifts among people in late adulthood (50-59 years). A repeated cross-sectional study that observed declines in cholesterol over time indicated that the increased use of medication explained the observed decline in cholesterol in part.[15] The present study also observed that more recently born generations were, at a similar age, more often on cholesterol-lowering medication than generations born 10 years earlier. The increased use of cholesterol-lowering medication of the last decades may have had a substantial favourable impact on population total cholesterol levels.

Table 2.2. Metabolic risk factors at four waves of men who were in their 20 s, 30 s, 40 s, and 50 s at baseline.

	1987-1991 Wave 1	1993-1997 Wave 2	1998-2002 Wave 3	2003-2007 Wave 4	P-value[a]
Overweight (%)			[b]		
20 s	27.0	38.9	52.1	61.5	<0.01
30 s	40.2	52.3	63.3	68.2	0.05
40 s	55.3	63.6	71.7	72.5	<0.01
50 s	60.8	67.9	69.7	72.8	-
Obesity (%)					
20 s	2.8	6.6	8.5	10.7	<0.05
30 s	4.0	8.1	11.4	13.9	0.02
40 s	7.5	10.2	13.7	17.9	<0.01
50 s	8.7	12.7	16.1	18.3	-
Body mass index (kg/m²)					
20 s	23.4±2.9	24.6±3.1	25.5±3.2	26.2±3.6	<0.01
30 s	24.5±2.9	25.4±3.1	26.2±3.2	26.7±3.3	0.03
40 s	25.6±3.0	26.3±3.1	26.8±3.4	27.1±3.6	<0.01
50 s	26.0±2.9	26.5±3.1	26.7±3.2	27.1±3.3	-
Hypertension (%)[c]					
20 s	14.1	15.0	20.1	-	0.48
30 s	17.9	23.1	33.3	-	<0.01
40 s	23.8	34.9	42.8	-	<0.01
50 s	35.4	51.0	59.2	-	-
Systolic blood pressure (mm Hg)[c]					
20 s	125±12	124±12	125±13	-	0.13
30 s	124±12	125±13	128±14	-	<0.01
40 s	125±14	129±16	133±18	-	<0.01
50 s	130±15	137±18	141±20	-	-
Diastolic blood pressure (mm Hg)[c]					
20 s	75±9	78±9	80±10	-	0.02
30 s	78±10	81±10	83±11	-	<0.01
40 s	81±10	83±10	85±11	-	<0.01
50 s	82±11	84±10	84±11	-	-
Hypercholesterolaemia (%)					
20 s	3.6	6.8	14.9	13.9	0.34
30 s	14.9	16.3	24.1	25.9	0.25
40 s	23.7	22.0	29.6	28.9	0.24
50 s	29.4	25.2	31.3	32.0	-
Total cholesterol (mmol/l)					
20 s	4.8±0.9	5.0±1.0	5.4±1.0	5.5±0.9	0.86
30 s	5.4±1.1	5.5±1.0	5.7±1.0	5.6±1.0	0.15
40 s	5.8±1.1	5.7±1.0	5.8±1.0	5.5±1.0	<0.01
50 s	6.0±1.0	5.8±0.9	5.8±0.9	5.4±1.0	-

Table 2.2 continues.

Table 2.2 continued.

Low HDL cholesterol (%)					
20 s	14.8	13.2	14.3	13.6	0.29
30 s	17.6	15.1	15.6	12.1	0.44
40 s	18.3	13.5	13.8	11.8	<0.01
50 s	22.9	14.0	15.7	12.3	-
HDL cholesterol (mmol/l)					
20 s	1.13± 0.24	1.21± 0.29	1.20± 1.32	1.21± 0.29	0.03
30 s	1.12± 0.26	1.19± 0.29	1.20± 0.31	1.24± 0.33	<0.01
40 s	1.12± 0.27	1.21± 0.31	1.22± 0.35	1.29± 0.37	<0.01
50 s	1.09± 0.26	1.20± 0.31	1.20± 0.33	1.26± 0.33	-
Antihypertensive medication (%)					
20 s	0.2	0.5	1.1	3.5	0.34
30 s	1.2	2.2	4.6	10.1	<0.01
40 s	2.1	5.3	10.1	18.0	0.04
50 s	8.5	13.1	19.0	30.9	-
Cholesterol-lowering medication (%)					
20 s	0.0	0.5	0.6	1.8	0.04
30 s	0.0	0.6	3.1	8.6	<0.01
40 s	0.2	2.6	7.3	14.4	<0.01
50 s	0.5	4.2	12.5	23.0	-
Type 2 Diabetes (%)					
20 s	0.6	0.5	0.8	0.9	0.01
30 s	0.1	0.0	0.3	1.7	0.11
40 s	0.9	2.0	3.0	5.0	<0.01
50 s	1.2	2.6	6.0	8.6	-

[a] Logistic and linear regression using generalized estimation equations, adjusted for age, were used to statistically test whether a generation was, at a similar age, statistically significant different compared to the consecutive generations born 10 years earlier; [b] The rectangles and the arrows show how the table should be read. Difference in cardiovascular risk factor at wave 3 and 4 of a generation were compared to the generation born 10 years earlier at wave 1 and 2 respectively. Consecutive generations had approximately a similar age at those moments; [c] Wave 3 was compared to wave 1 of a 10-year older generation using logistic or linear regression.

Table 2.3. Metabolic risk factors at four waves of women who were in their 20 s, 30 s, 40 s, and 50 s at baseline.

	1987-1991 Wave 1	1993-1997 Wave 2	1998-2002 Wave 3	2003-2007 Wave 4	P-value[a]
Overweight (%)			[b]		
20 s	16.2	28.1	39.1	45.8	<0.01
30 s	26.4	37.2	46.3	49.5	0.70
40 s	38.6	51.6	57.4	59.6	0.11
50 s	58.5	66.6	68.6	68.6	-
Obesity (%)					
20 s	3.1	7.6	10.3	15.3	<0.01
30 s	4.6	8.3	11.4	15.6	0.14
40 s	8.1	13.2	**17.1**	19.4	0.33
50 s	14.6	20.2	25.3	27.8	-
Body mass index (kg/m²)					
20 s	22.6±3.4	23.8±4.0	24.9±4.2	25.5±4.7	<0.01
30 s	23.5±3.5	24.6±3.9	25.3±4.1	25.9±4.2	0.04
40 s	24.6±3.7	25.7±4.2	26.4±4.4	26.5±4.5	0.03
50 s	26.3±4.0	27.0±4.3	27.2±4.4	27.6±4.9	-
Hypertension (%)[c]					
20 s	4.6	7.4	11.9	-	<0.01
30 s	6.4	13.4	23.8	-	0.04
40 s	16.1	28.0	38.1	-	0.65
50 s	31.8	44.4	54.6	-	-
Systolic blood pressure (mm Hg)[c]					
20 s	114±11	114±12	115±14	-	0.05
30 s	113±12	118±14	122±16	-	<0.01
40 s	118±15	125±17	130±18	-	<0.01
50 s	127±16	133±19	138±19	-	-
Diastolic blood pressure (mm Hg)[c]					
20 s	72±9	75±9	75±10	-	0.02
30 s	73±9	77±10	80±10	-	<0.01
40 s	77±10	81±11	82±11	-	0.03
50 s	81±11	81±11	82±10	-	-
Hypercholesterolaemia (%)					
20 s	4.6	3.3	7.0	8.0	0.91
30 s	7.0	7.4	13.5	21.3	0.41
40 s	13.4	17.5	34.3	36.7	.72
50 s	34.8	38.4	46.9	47.0	-
Total cholesterol (mmol/l)					
20 s	5.0±0.9	4.9±0.8	5.1±0.9	5.1±0.9	0.40
30 s	5.0±0.9	5.1±0.9	5.4±0.9	5.6±1.0	0.19
40 s	5.4±0.9	5.7±1.0	6.1±1.0	6.0±1.0	<0.01
50 s	6.2±1.0	6.2±1.0	6.2±1.1	5.9±1.1	-

Table 2.3 continues.

Table 2.3 continued.

Low HDL cholesterol (%)					
20 s	2.7	2.4	2.9	2.1	0.53
30 s	4.5	1.9	2.7	1.8	0.73
40 s	3.6	1.7	2.9	2.2	0.05
50 s	4.8	3.7	5.1	4.5	-
HDL cholesterol (mmol/l)					
20 s	1.36± 0.29	1.50± 0.35	1.45± 0.33	1.54± 0.35	0.05
30 s	1.35± 0.30	1.53± 0.36	1.52± 0.38	1.62± 0.40	<0.01
40 s	1.40± 0.32	1.58± 0.40	1.56± 0.40	1.61± 0.42	<0.01
50 s	1.35± 0.32	1.47± 0.37	1.46± 0.39	1.55± 0.42	-
Antihypertensive medication (%)					
20 s	0.3	1.2	3.3	6.9	<0.01
30 s	1.6	2.6	6.6	12.0	<0.01
40 s	3.2	6.7	12.5	22.8	0.19
50 s	13.1	20.1	25.6	32.1	-
Cholesterol-lowering medication (%)					
20 s	0.0	0.0	0.2	1.2	0.02
30 s	0.0	0.0	1.2	3.8	<0.01
40 s	0.0	0.7	3.2	11.7	<0.01
50 s	0.6	3.4	9.5	18.7	-
Type 2 Diabetes (%)					
20 s	0.2	0.0	0.0	0.2	0.33
30 s	0.3	0.4	0.1	1.6	0.85
40 s	0.7	1.0	1.8	3.7	0.46
50 s	1.6	3.3	5.1	10.5	-

[a] Logistic and linear regression using generalized estimation equations, adjusted for age, were used to statistically test whether a generation was, at a similar age, statistically significant different compared to the consecutive generations born 10 years earlier; [b] The rectangles and the arrows show how the table should be read. Difference in cardiovascular risk factor at wave 3 and 4 of a generation were compared to the generation born 10 years earlier at wave 1 and 2 respectively. Consecutive generations had approximately a similar age at those moments; [c] Wave 3 was compared to wave 1 of a 10-year older generation using logistic or linear regression.

Previous studies have shown, in line with our results among men, an increase in diabetes over time.[13, 14, 34] Unfavourable generation shifts in BMI explained these shifts in diabetes to some extent, i.e. explanatory analyses in which the GEE analyses were adjusted for BMI, decreased the odds ratios of diabetes to some extent (data not shown). In line with the observed absent of generation shifts in overweight/obesity among the older generations of women, we did also not observe shifts in diabetes among these women. The large unfavourable generation shift in obesity among the most recently born generations of women is not reflected in the diabetes figures since the prevalence of diabetes is too low at that age. However, taken the large unfavourable generation shifts in obesity among these young women, it is very likely that unfavourable generation shifts in diabetes will in the future also occur among women.

While interpreting the results also some limitations of the study should be taken into account. First, respondents to long-lasting prospective studies are usually slightly healthier and higher educated. Although response rates during follow-up were good (75-80%), this does not necessarily exclude bias. Baseline characteristics of participants who participated in all waves (complete cases) were compared to baseline characteristics of those with only two or three measurements. Those who participated in only two or three waves were more often low educated and smokers, and had more often overweight, obesity, hypertension, hypercholesterolaemia, and low HDL cholesterol. In addition, we also performed complete case analyses i.e. a subgroup analyses in which only participants were included who had complete follow-up data of the risk factors studied (n=3,875). The complete case analyses did not lead to notable different results than the current analyses. In addition, we studied the development of metabolic risk factors excluding those who reported to have (had) diabetes, myocardial infarction, stroke, or cancer. And again, there were no notable differences. Taken together, this means that bias due to selective dropout is probably limited but the actual Dutch prevalence of metabolic risk factors is probably even slightly higher than presented in this study.

The strength of the present study is that we were able to examine the development of several important metabolic risk factors over a long follow-up period. The study consisted of adults encompassing a wide age range (20-59 years at baseline) who were followed for 16 years, including four measurements. Another advantage is that the same group of trained workers objectively measured data on body weight, height, blood pressure, and cholesterol with standardized protocols and instruments.

We found generation shifts with more recently born adult generations doing worse. This indicates that the prevalence of metabolic risk factors and the lifelong exposure to them has increased and probably will continue to increase. These findings seem to be similar across socioeconomic classes and among all ages, though for women they were present especially among the most recently born generations. With more recently born generations having a worse risk factor profile than older generations, this may have a major impact on public health as 'metabolic risk factor'-related diseases such as diabetes, but also diseases such as musculoskeletal disorders, will significantly increase and start at a younger age, resulting in an increased risk of cardiovascular and other chronic diseases. This strengthens the evidence for the need of stimulating a healthy weight, both in general practice and by preventive interventions. This should especially be targeted at young generations. It is essential to keep monitoring cardiovascular risk factors, as changes in these risk factors influences the chronic disease burden of the future. As the lifelong exposure to risk factors, for example obesity, is increasing, future research should take the cumulative effect of exposures to risk factors into account.

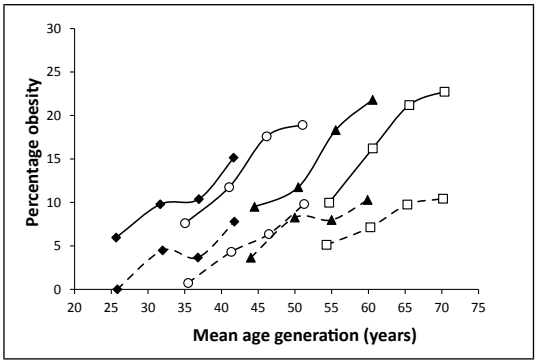

Figure 2.2. Age-specific prevalence of obesity (BMI ≥30 kg/m^2) over 16 years follow-up (4 waves) in those who were in their 20 s (-♦-), 30 s (-○-), 40 s (-▲-), and 50 s (-□-) at baseline, among men with a low socioeconomic status (solid line) and high socioeconomic status (dotted line).
Note; Intermediate socioeconomic class not presented, but the generation shifts were also observed between all generations in the intermediate socioeconomic class.

Acknowledgement

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Chapter 3

Obesity and age-related changes in markers of oxidative stress and inflammation in four generations

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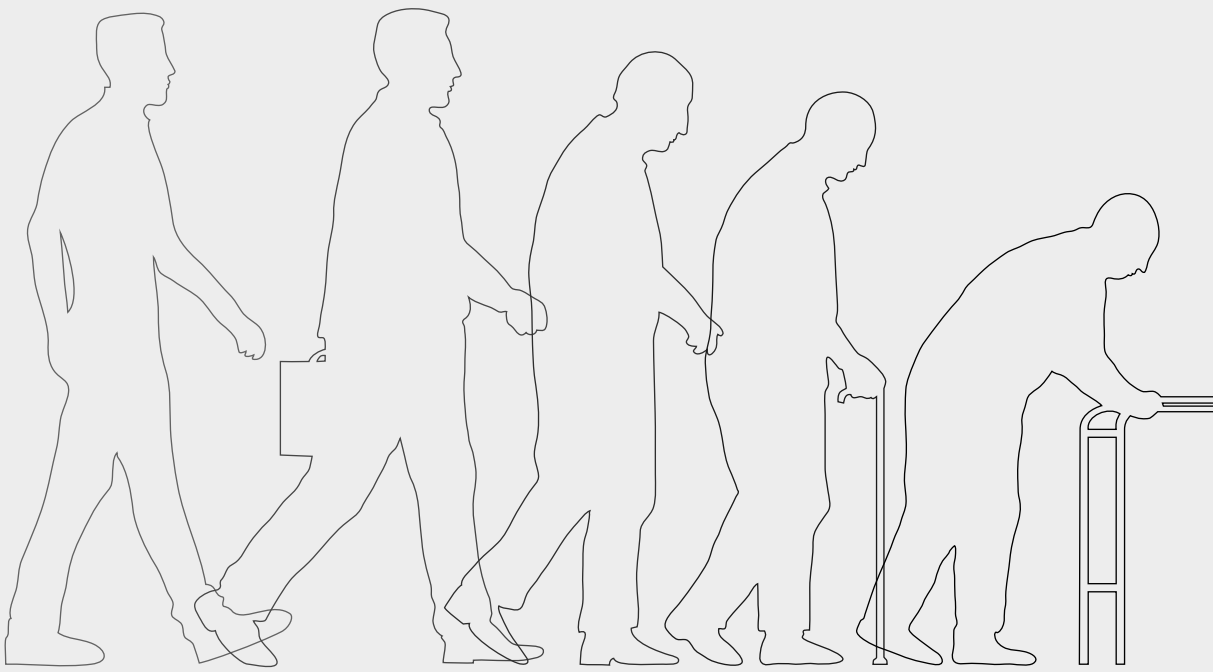


Abstract

Background: The prevalence of obesity increases with age and is higher in each younger generation. This may influence age-specific levels of oxidative stress and inflammation. We aimed to investigate age-related changes in markers of oxidative stress and inflammation in four generations, specifically addressing the role of age-related increasing body mass index (BMI).

Methods: Four generations (26–35, 36–45, 46–55 and 56–65 years at baseline) in a population-based study of 2,453 men and 2,702 women were examined every five years for 15 years between 1993 and 2012. Random coefficient analyses were used to study age-related changes and generation shifts in BMI, gamma glutamyltransferase (GGT), uric acid (UA) and C-reactive protein (CRP). Analyses were also stratified by stable BMI (change≤1 kg/m^2/15 year) and increasing BMI (increase>1 kg/m^2/15 year).

Results: Levels of BMI, UA and CRP increased with age in all generations up to 75 years. GGT increased up to 55 years, after which it remained stable or decreased. Younger generations had, at the same age, more unfavourable levels of BMI than 10-year older generations ($P<0.05$), but no consistent generation shifts were observed for GGT, UA and CRP. Compared to participants with increasing BMI, participants with a stable BMI had either no increases with age in GGT, UA and CRP, or increases that were 2-4 times smaller (P-value interaction <0.01).

Conclusion: The unfavourable age-related changes in obesity-related biochemical markers, particularly among individuals with increasing BMI, stress the importance of maintaining a healthy weight to improve population levels of oxidative stress and chronic inflammation.

Introduction

Knowledge about levels and the development over time of risk factors in current generations is essential as it partly determines the future disease burden. Differences in risk factors across generations (i.e. generation shifts) can be determined over time, indicating whether the future health of younger generations will be different from that of their predecessors. One of the most striking developments in risk factors in recent decades is the increase in obesity prevalence with age in all adult generations [1] and the unfavourable generation shifts in obesity,[1, 2] whereby obesity at a given age is more prevalent among younger generations than older generations. These changes in obesity affect developments in other risk factors. The unfavourable effect of obesity on major cardiovascular risk factors such as blood pressure and cholesterol is well known. However, it is unclear to what extent the increase in obesity prevalence is reflected in patterns of markers of oxidative stress and chronic inflammation, such as gamma glutamyltransferase (GGT), uric acid (UA) and high-sensitive C-reactive protein (CRP).[3-7] These markers are strongly associated with obesity [8-10] and with chronic diseases such as gout, type 2 diabetes, cardiovascular disease, and with mortality.[11-14]

In a few longitudinal studies that investigated age-related changes, UA [15] and GGT [16, 17] have been found to increase with age, while less is known about changes of CRP with age. With regard to trends and differences across generations, two of these studies also showed that GGT [16] and UA [15] levels were higher in subsequent younger generations. Likewise, results from the U.S. National Health and Nutrition Examination Survey (NHANES) indicated that the prevalence of elevated UA increased between 1988 and 2008,[18] while the prevalence of elevated CRP decreased between 1999 and 2010.[19] These earlier studies however were conducted only in Asian [15, 16] or US [17-19] study populations, had a limited follow-up duration (six-nine years),[15, 16] were based on cross-sectional data only [18, 19] and/or only included men up to the age of 25 years [17] or 45 years.[16] Importantly, none of these studies investigated the extent to which age-related changes in obesity prevalence influenced patterns of markers of oxidative stress and inflammation in different adult generations. We expect a detrimental impact of the age-related increase in body mass index (BMI), which reflects the increase in obesity prevalence, on developments in oxidative stress and inflammation. Therefore, the aim of the present study is to examine changes with age and differences between generations in GGT, UA and CRP among men and women who were aged 26-65 at baseline and who were followed up for 15 years, specifically addressing the role of age-related increases in BMI.

Methods

Population

The Doetinchem Cohort Study is an ongoing prospective population-based study that started in 1987-1991, involving men and women aged 20-59 at the start of the study from Doetinchem, a town in the eastern part of the Netherlands. Adults who participated in 1987-1991 (N=7,768, participation rate: 62%) were re-invited for measurements in 1993-1997 (wave 2, N=6,117), 1998-2002 (wave 3, N=4,918), 2003-2007 (wave 4, N=4,520) and 2008-2012 (wave 5, N=4,018), with response rates of 75% or higher. Details of the study design have been described elsewhere.[20] GGT, UA and CRP were assessed from wave two onwards, therefore the second examination wave was considered as baseline for the present analyses. Pregnant women were excluded from the wave in which they were pregnant. Of the 6,390 participants who attended one or more of the waves 2-5, we excluded 1,235 participants who had fewer than two measurements of the biochemical markers, leaving data for 5,155 participants eligible for the present analyses. All participants gave written informed consent for each wave and the study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Measurements

At each wave, anthropometry, blood pressure and blood samples were taken according to standard protocol.[20] Body weight was measured to the nearest 0.1 kg on calibrated scales and 1 kg was subtracted to adjust for clothing. Height was measured to the nearest 0.5 cm. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured twice to the nearest 0.5 cm, at a level midway between the lowest rib and the iliac crest at the end of expiration, with participants in a standing position. The mean of two measurements was used for analysis. Total cholesterol and HDL cholesterol were measured with standardised enzymatic methods. In 2013-2014, standardised enzymatic methods were used to retrospectively determine GGT, UA, CRP, creatinine and cystatin C in waves 2-5 using non-fasting plasma samples that had been stored at -20 degree Celsius until June 1995 and at -80 degree Celsius from July 1995 onwards. GGT and UA were measured using a colorimetric method (Roche/Hitachi Modular P analyser, Mannheim, Germany). GGT values greater than three times the upper normal reference were recoded to 'missing' for that wave since this may indicate liver problems (N≤58 at each wave).[21] High sensitivity CRP was measured based on the principle of particle-enhanced immunological agglutination (Tina-quant CRP). CRP values above 10 mg/L were recoded to 'missing' for that wave because this may indicate an acute-phase response to infection for example or physical injury (N≤195 at each wave).[22] Cystatin C measurement was based on a particle-enhanced turbidimetric immunoassay using reagents from Gentian (Gentian, Moss, Norway) and creatinine was

measured with a Creatinine Plus assay (IDMS traceable). Estimated glomerular filtration rate (eGFR) was estimated with the Chronic Disease Epidemiology Collaboration (CKD-EPI) equation using a combination of cystatin C and creatinine.[23] Data on educational attainment, use of oral contraceptives, hormone replacement therapy, cholesterol-lowering medication, anti-hypertensive medication, smoking status (yes or no) and alcohol intake were obtained by questionnaire. Type 2 diabetes was defined based on self-reported history and/or non-fasting blood glucose concentrations of 11.1 mmol/L or more.

Data analysis

Modelling age-related changes and generation shifts

A linear random effect model that accounts for repeated observations on the same participant was used to model means of each of BMI and the three biochemical markers as a function of age and generation (i.e. aged 26–35, 36–45, 46–55 and 56–65 at baseline). The explanatory variables included the generation, linear and quadratic age terms, and the interactions between the generation and the age terms. The quadratic age term was included to allow for a potentially non-linear relationship between age and outcomes. All analyses were stratified by sex. We log-transformed GGT and CRP and report back-transformed (geometric) means since these biochemical markers were not normally distributed. The models with CRP as the dependent variable were additionally adjusted for use of oral contraceptives and hormone replacement therapy as both have been reported to have a substantial impact on CRP levels in women, probably due to a direct effect of hepatocyte CRP synthesis rather than inflammation or endothelial activation.[24-26]

In Figure 3.1, the model estimates for the means/geometric means of BMI, GGT, UA and CRP are plotted against the mean age of the generations at the time of measurement. A line linking the means/geometric means of BMI and the biochemical markers for each generation shows the change with age. If the lines of consecutive generations overlap, there is no generation shift. A generation shift is defined as a significant difference in mean/geometric mean between generations at a similar age. An unfavourable generation shift exists if the mean/geometric mean of a younger generation is higher than that of the generation ten years ahead. Generation shifts were tested statistically by contrasting the outcomes at predefined ages, and were considered statistically significant ($P<0.05$) if consecutive generations differed at two matching age points, e.g. at ages 40 and 45 for the youngest generation (at the 10- and 15-year follow-up waves) versus the second-youngest generation (at baseline and the five-year follow-up wave).

The association of age-related changes in BMI with age-related changes in biochemical markers

To study the associations between age-related increase in BMI and age-related changes in GGT, UA and CRP, the change in BMI was dichotomised as 'stable BMI' and 'increasing BMI'. A stable BMI was defined as a change of ≤1 kg/m^2 between baseline and the 15-year follow-up wave. Increasing BMI was defined as an increase of >1 kg/m^2 in that same period. Participants who lost >1 kg/m^2 in BMI were excluded from these analyses (N=483). Missing values for BMI at baseline (4%) or the 15-year follow-up wave (14%) were replaced by the observed value of the next wave or preceding wave respectively, if available. We tested two model interactions: 1) an interaction between age and the dichotomised BMI variable tested whether age-related changes in biochemical markers differed for participants with a stable BMI versus participants with increasing BMI; 2) a 3-way interaction between age, generation and the dichotomised BMI variable tested whether the first interaction differed between generations. A p-value for interaction of <0.05 was considered statistically significant. All analyses were performed using SAS 9.3 software.

Sensitivity analyses

In sensitivity analyses to further explain the observed patterns, all analyses were additionally adjusted for antihypertensive medications, cholesterol-lowering medications, smoking status and alcohol intake. UA was also adjusted for eGFR. We also stratified the analyses by changes in waist circumference (≤4 cm change over 15 years and >4 cm increase over 15 years) to investigate whether changes in general and intra-abdominal adiposity were differently related to patterns in markers of oxidative stress and inflammation.

Results

The mean age at baseline was 46 (range: 26-65) and 48% of the participants were men (Table 3.1). More than half of the population (53%) had a low level of educational attainment. Men had received more education and had less favourable levels of metabolic risk factors than women.

Table 3.1. Population characteristics (1993-1997) of the Doetinchem Cohort Study, by sex.

	Men (n=2,453)	Women (n=2,702)
Age (years)	46.5 ±9.8	45.5 ±10.0
Low educational level	1,044 (44%)	1,585 (61%)
Smoking		
Currently	701 (30%)	747 (29%)
Ex	983 (42%)	881 (34%)
Alcohol intake (g/day)	11 [3-26]	0 [0-9]
Use of oral contraceptives	NA	549 (21%)
Antihypertensive medication	117 (5.0%)	168 (6.5%)
Cholesterol-lowering medication	41 (1.7%)	23 (0.9%)
Body mass index (kg/m^2)	25.7 ±3.1	25.1 ±4.1
Waist circumference (cm)	95 ±9	86 ±11
Diastolic blood pressure (mm Hg)	81 ±11	78 ±11
Systolic blood pressure (mm Hg)	128 ±15	121 ±17
Total cholesterol (mmol/L)	5.5 ±1.0	5.4 ±1.0
HDL cholesterol (mmol/L)	1.21 ±0.30	1.54 ±0.37
Type 2 diabetes	30 (1.3%)	25 (1.0%)
Gamma glutamyltransferase (U/L)	27 [19-40]	15 [12-22]
Uric acid (mmol/L)	0.33 ±0.06	0.24 ±0.06
C-reactive protein (mg/L)	0.9 [0.5-1.9]	1.1 [0.5-2.4]
Estimated glomerular filtration rate (ml/min/1.73 m^2)	105 ±13	103 ±15

Values represent either means ±standard deviations, numbers and (percentages), or medians and [interquartile ranges].

Total population

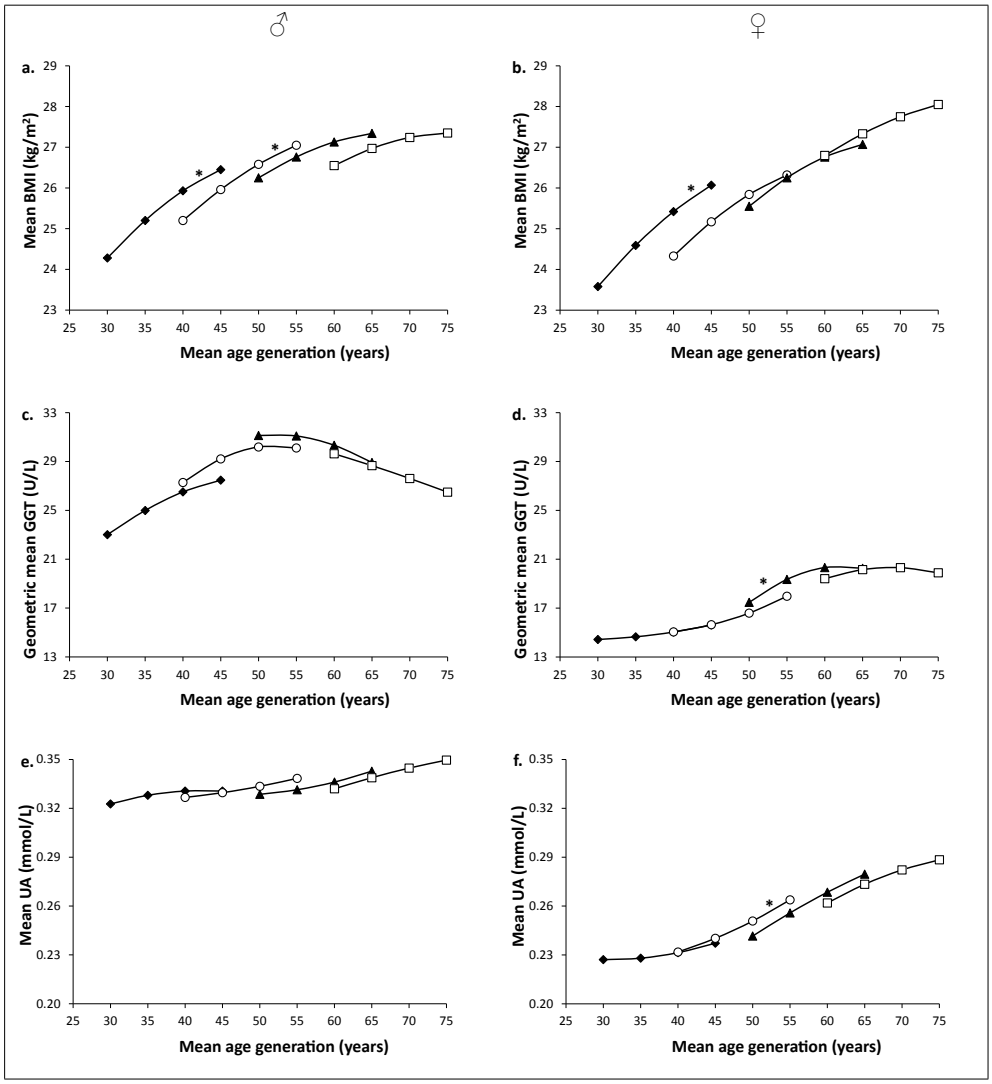
Age-related changes within generations

The mean levels of BMI increased with age within all generations of men and women (Figure 3.1). UA and CRP also increased with age within all generations until the end of follow-up, when the oldest generation was on average 75 years old. Except for CRP, in the oldest generation of women levels remained stable between the ages of 70 and 75. For GGT we observed a different pattern: in the two youngest generations of both men and women, GGT increased up to about 55, whereas in the two oldest generations GGT decreased among men between the ages of 55 and 75 and remained relatively stable among women.

Generation shifts

Younger generations of men had mean BMI levels that were 0.6-1.2 kg/m^2 higher than generations ten years ahead when at the same age ($P<0.05$) (Figure 3.1). Among women, mean BMI levels were 2.0 kg/m^2 higher in the youngest generation than in the second-youngest generation between the ages of 40 and 45 years ($P<0.01$), while no differences in BMI were observed between other generations ($P\geq0.05$).

Regarding GGT, UA and CRP, different generations had similar levels when reaching the same age, indicating the absence of generation shifts (Figure 3.1). Two exceptions were observed among women: geometric mean levels of GGT were 0.9-1.4 U/L lower and mean levels of UA were 0.008-0.009 mmol/L higher in the second-youngest compared with the second-oldest generation between the ages of 50 and 55 (P<0.05).



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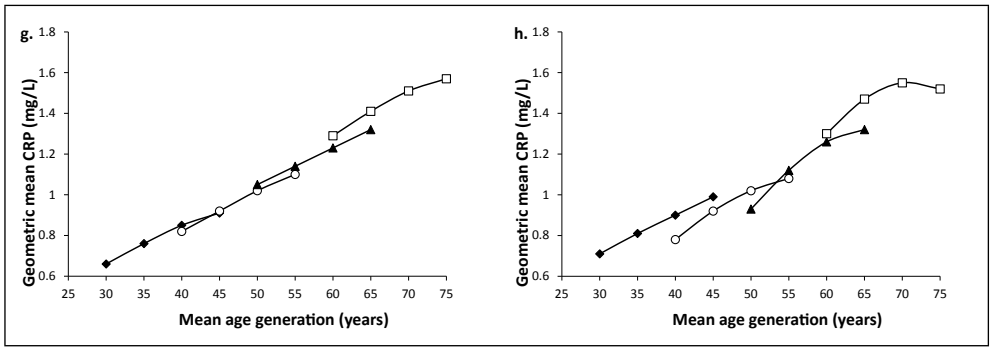


Figure 3.1. Age-specific means/ geometric means of BMI (A,B), GGT (C, D), uric acid (E, F) and CRP (G,H) over 15-year follow-up period in participants who were aged 26-35 (-♦-), 36-45 (-○-), 46-55 (-▲-) and 56-65 (-□-) at baseline, stratified by sex.
Abbreviations: BMI, body mass index; GGT, gamma glutamyltransferase; UA, uric acid; CRP, C-reactive protein; An asterisk (*) indicates a statistically significant difference (P<0.05) in mean/ geometric mean between consecutive generations at two matching age points, e.g. 40 and 45 years for the youngest versus the second-youngest generation. CRP was adjusted for oral contraceptives and hormone replacement therapy.

The association of age-related changes in BMI with age-related changes in biochemical markers

The proportion of men and women with increasing BMI was higher in each successive generation. At baseline, no or only small differences in mean BMI were observed between the stable BMI and increasing BMI group (Table 3.2).

The age-related changes in all three biochemical markers were more unfavourable in participants whose BMI increased than in participants whose BMI remained stable over 15 years (P<0.0001 for the interaction between age and BMI change) (Tables 3.3). This is illustrated in Figure 3.2: in men with a stable BMI the geometric mean of GGT decreased by 2.0 U/L (Figure 3.2a), whereas GGT increased in men with increasing BMI by 3.7 U/L on average over the 15-year period (Figure 3.2b). In women with a stable BMI, GGT increased by 1.1 U/L on average and in women with increasing BMI by 3.4 U/L, three times as much (Table 3.3, Figure 3.2c-d). Similar differences were observed for UA and CRP: compared with participants whose BMI remained stable over the time period, participants whose BMI increased had a 0.017-0.018 mmol/L larger increase (2-4 times larger) in UA and a 0.25-0.43 mg/L larger increase (3.2-3.3 times larger) in CRP during follow-up (Table 3.3, Figure 3.2e-l). There was no significant difference between the generations in the associations between changes in BMI during follow-up and age-related changes in all three biochemical markers (P≥0.10 for 3-way interactions).

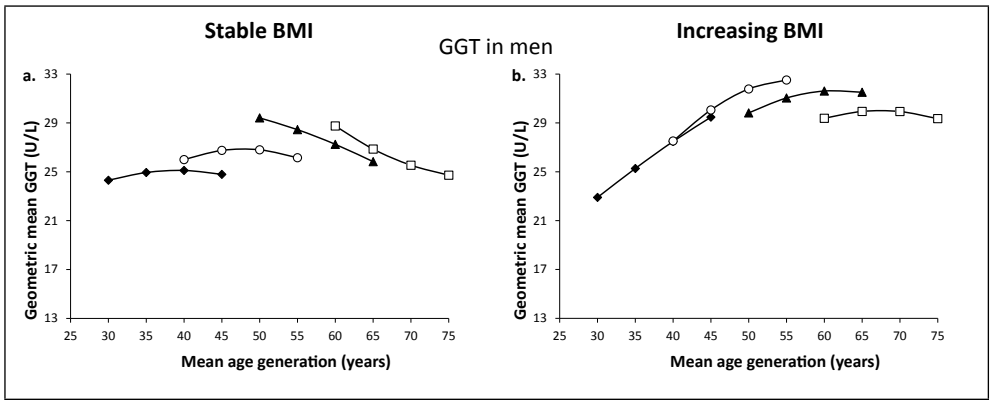
Sensitivity analyses

Adjustment for antihypertensive and cholesterol-lowering medications, smoking status, alcohol intake and eGFR had no effect on the results (data not shown). The results stratified by changes in waist circumference were also essentially the same as the results stratified by changes in BMI (Supplementary Figure 3.1).

Table 3.2. BMI (mean ±standard deviation) at waves 2 and 5, stratified by sex, generation and changes in BMI.[a,b]

	Men			Women		
	N	**BMI wave 2 (kg/m²)**	**BMI wave 5 (kg/m²)**	**N**	**BMI wave 2 (kg/m²)**	**BMI wave 5 (kg/m²)**
Stable BMI[a]						
Generation by baseline age						
26-35 yr	90 (31%[c])	24.0 ±2.7	24.3 ±2.7	99 (28%)	23.2 ±2.6	23.3 ±2.6
36-45 yr	189 (32%)	25.2 ±2.9	25.4 ±2.8	213 (32%)	23.7 ±3.2	23.8 ±3.2
46-55 yr	216 (43%)	25.7 ±2.5	25.8 ±2.5	185 (36%)	24.9 ±3.5	25.1 ±3.5
56-65 yr	150 (54%)	26.0 ±2.3	26.0 ±2.3	110 (40%)	25.6 ±3.6	25.6 ±3.8
Increasing BMI[b]						
Generation by baseline age						
26-35 yr	199 (69%[d])	24.4 ±2.8	27.4 ±3.5	250 (72%)	23.3 ±3.6	27.1 ±2.5
36-45 yr	405 (68%)	25.3 ±3.0	28.1 ±3.6	446 (68%)	24.4 ±3.6	27.7 ±4.5
46-55 yr	292 (57%)	25.9 ±3.2	28.4 ±3.7	323 (64%)	25.2 ±3.9	28.3 ±4.4
56-65 yr	129 (46%)	26.4 ±3.1	28.9 ±3.5	167 (60%)	27.8 ±4.5	30.8 ±5.5

Abbreviation: BMI, body mass index; [a] Stable BMI: BMI changed ≤1 kg/m² over 15 years follow-up; [b] Increasing BMI: BMI increased >1 kg/m² over 15 years follow-up; [c] Proportion of participants with a stable BMI; [d] Proportion of participants with increasing BMI; Note: data shown for participants without missing BMI data at waves 2 and 5.



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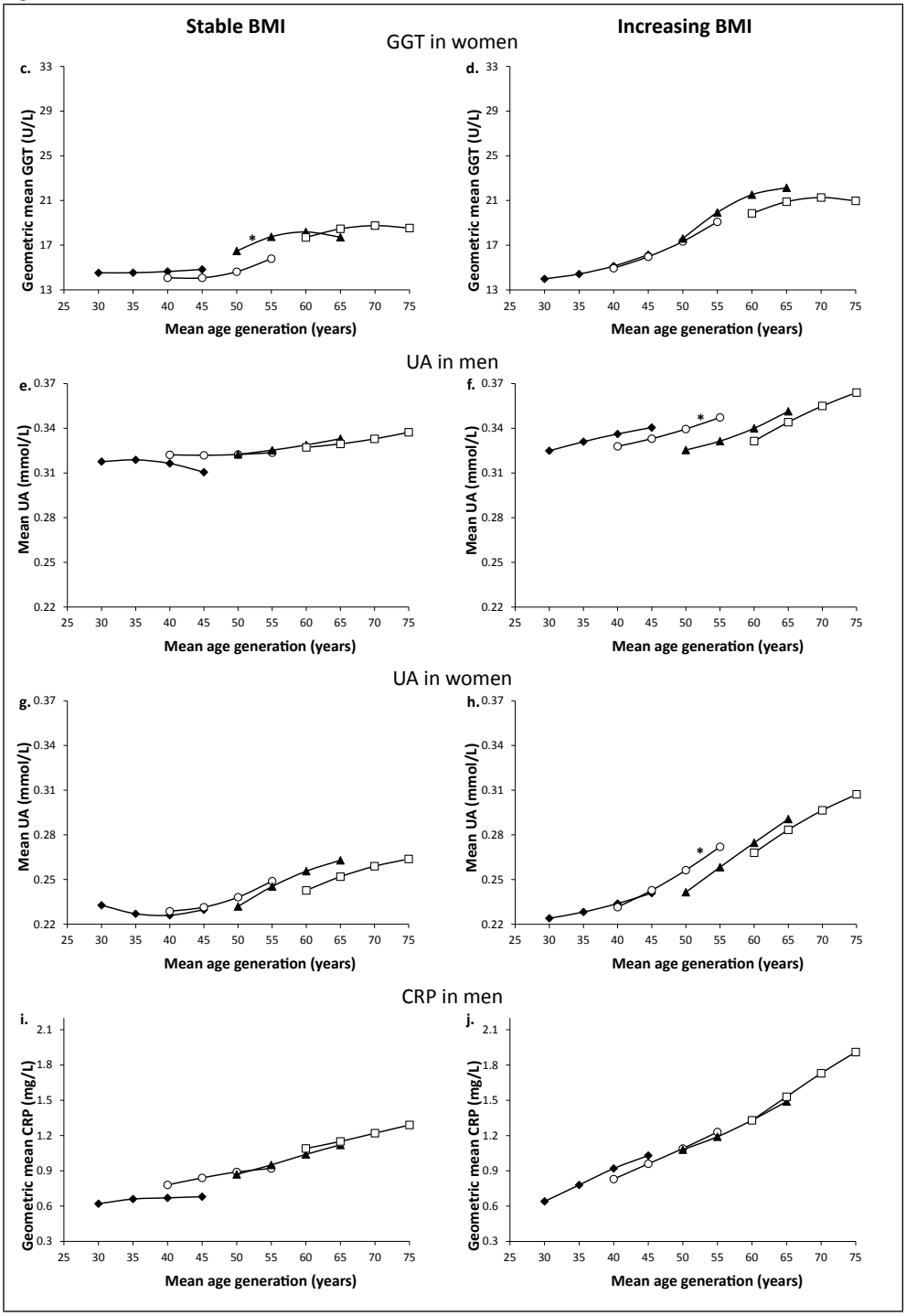

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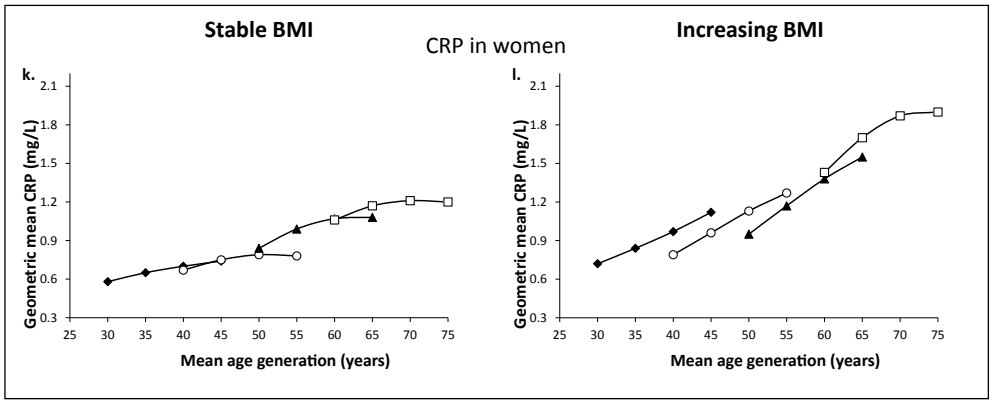

Figure 3.2. Stratification by changes in BMI. Age-specific geometric mean of GGT (a-d), UA (e-h) and CRP (i-l) over 15-year follow-up period in participants aged 26-35 (-♦-), 36-45 (-○-), 46-55 (-▲-) and 56-65 (-□-) at baseline, stratified by sex and participants with a stable BMI [a] and increasing BMI [b]. Abbreviations: BMI, body mass index, GGT, gamma glutamyltransferase; UA, uric acid; CRP, C-reactive protein; [a] Stable BMI: BMI changed ≤1 kg/m^2 over 15-year follow-up period; [b] Increasing BMI: BMI increased >1 kg/m^2 over 15-year follow-up period; An asterisk (*) indicates a statistically significant difference (P<0.05) in mean/ geometric mean between consecutive generations at two matching age points, e.g. 40 and 45 years for the youngest versus the second-youngest generation. CRP was adjusted for oral contraceptives and hormone replacement therapy.

Discussion

In the total study population, UA and CRP increased with age within all generations over the 15-year follow-up period. GGT increased up to age 55, after which it decreased among men and remained stable among women. All generations had, at the same age, a similar level of GGT, UA and CRP, indicating an absence of generation shifts in men and women aged 25 and older. The large potential impact of the increase in BMI on developments in oxidative stress and inflammation in the general population makes it particularly important to investigate associations between age-related increases in BMI and age-related changes in GGT, UA and CRP. We found that in all generations, individuals with a stable BMI showed little or no increase in levels of GGT, UA and CRP during follow-up, while individuals with increasing BMI had much larger increases with age in the biochemical markers. These findings suggest that unfavourable patterns of oxidative stress and inflammation in the population are at least partly driven by increasing BMI.

The strength of the present study is that we were able to objectively examine the development of several biochemical markers over a follow-up period of 15 years that included four measurements, with a large number of participants at each wave (N>3,950 at each wave). The examination of all available blood samples from consecutive waves in

one assay run reduced the risk of measurement error to an absolute minimum.[27] The study consisted of adults encompassing a wide age range who are representative for the general Dutch population. One limitation of our study might be that participants who participated in only two or three waves were more likely to be less educated, smoker, and have slightly less favourable levels of GGT, UA and CRP compared with the individuals who participated in all four waves. Age-related changes and generation shifts may therefore be slightly less favourable than shown in the present study.

We have extended earlier findings about increases in GGT in men up to the age of 45 [16, 17] by showing that GGT decreases in men and remains stable in women between the ages of 55 and 75. Earlier findings of increasing UA levels from a Japanese longitudinal study [15] were similar to the present findings using participants from the general Dutch population. We also extended the cross-sectional findings of the NHANES and EPIC NORFOLK studies [19, 28] by showing that not only was CRP higher in each successive five-year or 10-year older age group but CRP levels also increase with age within all generations of men and women during follow-up.

Our results suggest an effect of increasing BMI on the age-related increases in oxidative stress and inflammation: participants with a stable BMI had no or only small increases in markers of oxidative stress and inflammation, while participants with increasing BMI had substantial increasing levels of the biochemical markers in all generations of men and women. Our sensitivity analyses indicated that unfavourable changes in general and intra-abdominal adiposity have similar effects on patterns of oxidative stress and inflammation. BMI and waist circumference increased with age in more than half of the study population, highlighting the importance of maintaining a healthy weight for the future health of the population due to the association of GGT, UA and CRP with gout, diabetes and cardiovascular disease.[11-14, 29] Several plausible mechanisms may explain our findings of the associations of BMI with biochemical markers: obesity is associated with a higher rate of hepatic fatty acid uptake from plasma, and the imbalance between fatty acid synthesis and the rate of fatty acid oxidation and export leads to higher GGT.[30] Obesity has also been associated with overproduction and with impaired renal clearance of UA;[31, 32] and with the increased expression of tumour necrosis factor-α, circulating tumour necrosis factor-α, circulating interleuking-6 and insulin resistance which can promote the production of CRP.[33-36]

Table 3.3. Average age-related change in GGT, UA and CRP over 15 years stratified by sex, generation and changes in BMI.[a,b]

Generation by baseline age	All men	26-35 yr	36-45 yr	46-55 yr	56-65 yr	All women	26-35 yr	36-45 yr	46-55 yr	56-65 yr
Stable BMI[a]										
GGT, U/L	-2.0 (-7%)	0.5 (2%)	0.2 (1%)	-3.6 (-12%)	-4.0 (-14%)	1.1 (7%)	0.3 (2%)	1.7 (12%)	1.2 (7%)	0.8 (5%)
UA, mmol/L	0.005 (2%)	-0.007 (-2%)	0.001 (0%)	0.011 (3%)	0.010 (3%)	0.020 (8%)	-0.003 (-1%)	0.020 (9%)	0.031 (13%)	0.021 (9%)
CRP, mg/L	0.18 (21%)	0.06 (10%)	0.14 (18%)	0.25 (29%)	0.20 (18%)	0.16 (21%)	0.16 (28%)	0.11 (16%)	0.24 (29%)	0.14 (13%)
Increasing BMI[b]										
GGT, U/L	3.7 (13%)	6.6 (29%)	5.0 (18%)	1.7 (6%)	0.0 (0%)	3.4 (21%)	2.2 (15%)	4.2 (28%)	4.5 (26%)	1.1 (6%)
UA, mmol/L	0.022 (7%)	0.016 (5%)	0.019 (6%)	0.026 (8%)	0.033 (10%)	0.038 (16%)	0.017 (8%)	0.041 (17%)	0.049 (20%)	0.039 (15%)
CRP, mg/L	0.43 (46%)	0.39 (61%)	0.40 (48%)	0.41 (38%)	0.58 (44%)	0.49 (54%)	0.40 (56%)	0.48 (61%)	0.60 (63%)	0.47 (33%)

Abbreviations: BMI, body mass index; GGT, gamma glutamyltransferase; UA, uric acid; CRP, C-reactive protein; [a] Stable BMI: BMI changed ≤1 kg/m^2 over 15 years follow-up; [b] Increasing BMI: BMI increased >1 kg/m^2 over 15 years follow-up; Notes: Values represent mean change and (percentage change) over 15 years follow-up; All slopes of age-related changes in biochemical markers were statistically significantly worse for participants whose BMI increased over time compared with participants whose BMI remained stable (P<0.0001 for interaction).

We observed no generation shifts in any of the biochemical markers across all generations of men and most generations of women in the total population. One reason why the unfavourable generation shifts in BMI (Figure 3.1A-B) and obesity [1] were not reflected in shifts in related biochemical markers may be that, on a population level, the effects of shifts in BMI and obesity have been counteracted by concomitant improvements in smoking rates and the increased prevalence of statin use in the present study population.[1, 37] It is well established that both not smoking and statin use are associated with lower levels of GGT [38, 39] and CRP.[40] However, findings from our sensitivity analyses showed that adjusting for current smoking, use of cholesterol-lowering medication and other factors that influence levels of the biochemical markers did not alter the results. The unfavourable shifts in BMI and obesity may also have been too small to be reflected in shifts in the biochemical markers. The age-related increases in BMI within generations were about two times larger than the shifts in BMI across generations, which may partly explain the finding that unfavourable changes in BMI were reflected in age-related increases in obesity-related markers but not in generation shifts. Nevertheless, a continuation of unfavourable trends in obesity might ultimately lead to higher mean levels of markers of oxidative stress and chronic inflammation in young generations compared with their predecessors in the general adult population.

In conclusion, levels of GGT, UA and CRP were similar across generations but increased substantially with age in men and women over the 15-year follow-up period, particularly in participants whose BMI increased. These findings therefore reinforce the importance of maintaining a stable weight to improve population levels of markers of oxidative stress and chronic inflammation.

Acknowledgement

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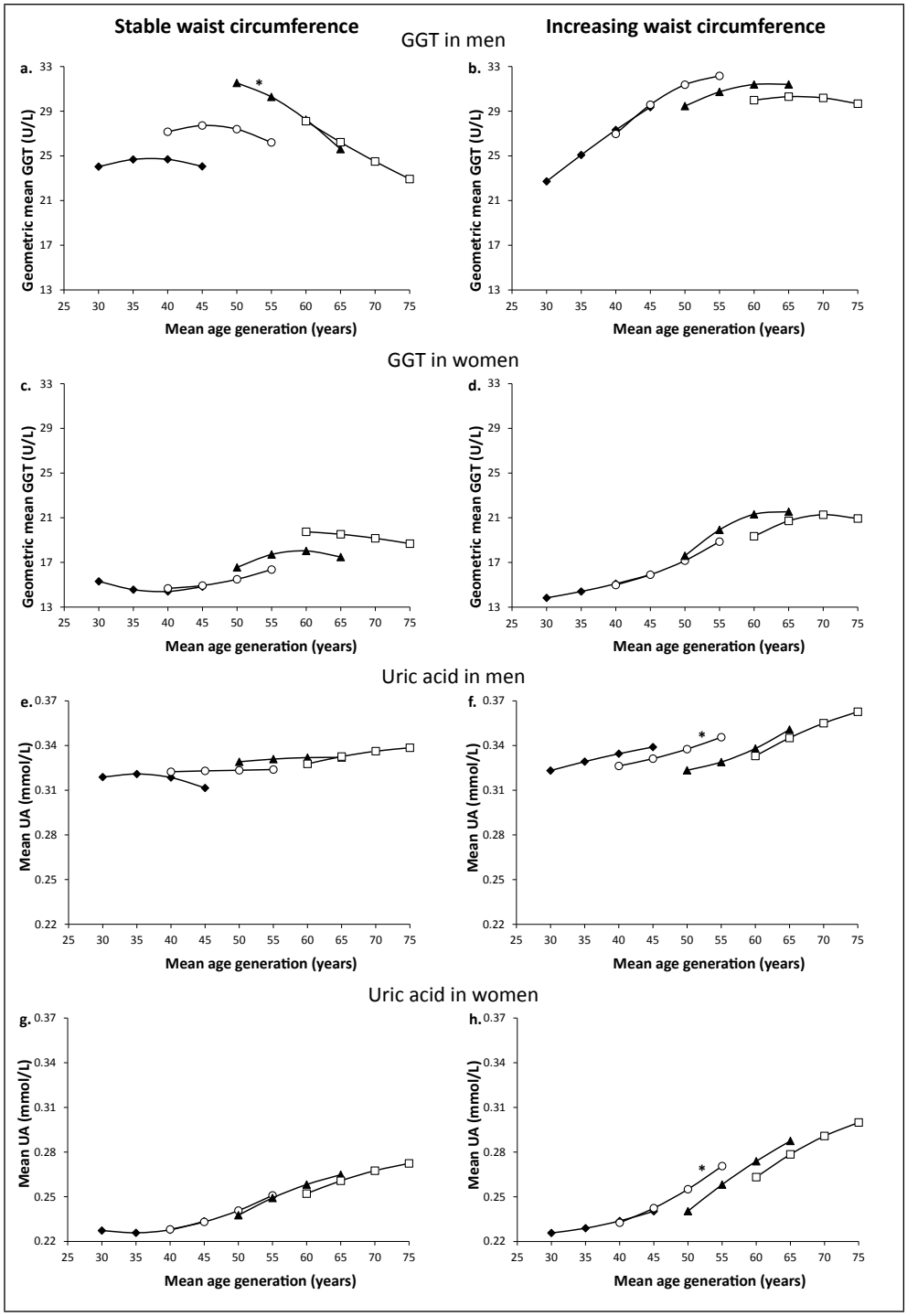

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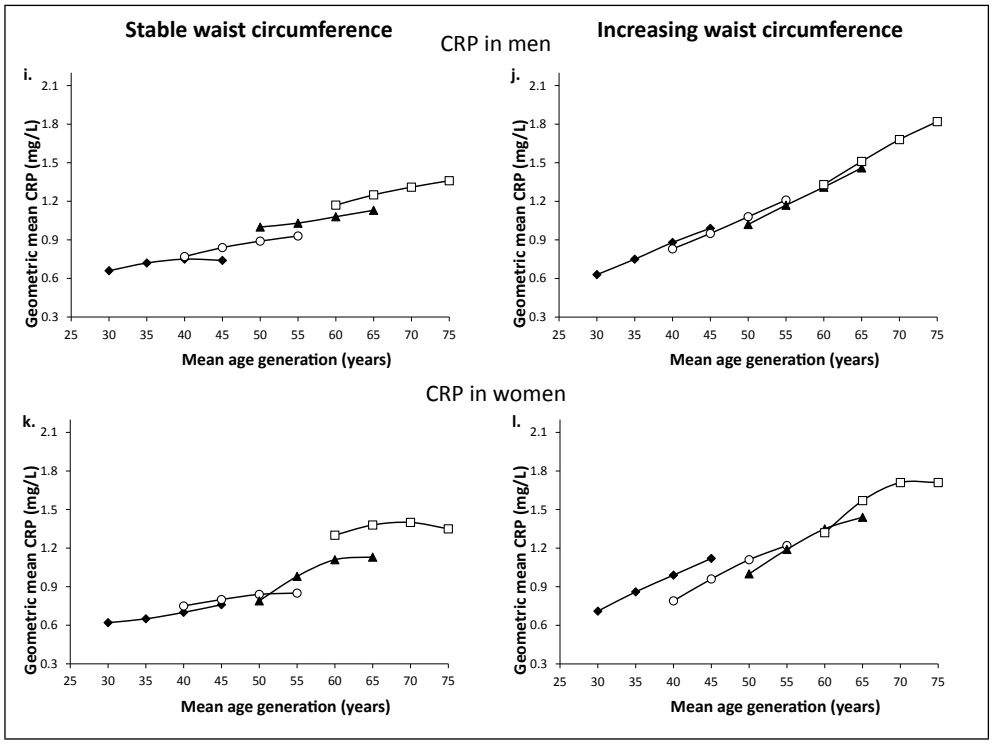
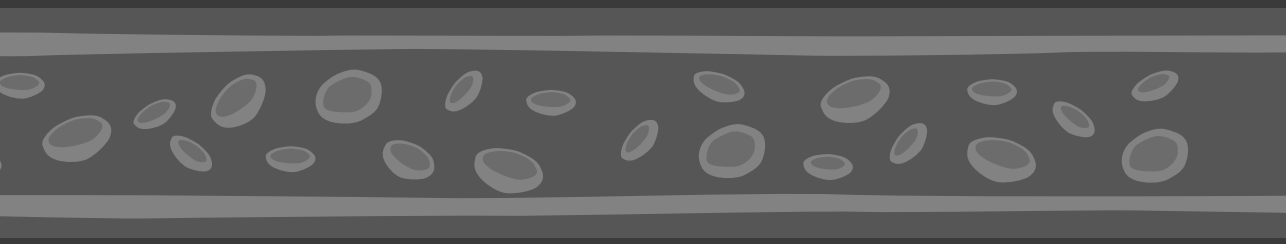


Supplemental Figure 3.1. Stratification by changes in waist circumference. Age-specific geometric mean of GGT (a-d), uric acid (e-h) and CRP (i-l) over 15-year follow-up period in participants aged 26-35 (-♦-), 36-45 (-○-), 46-55 (-▲-) and 56-65 (-□-) at baseline, stratified by sex and participants with a stable waist circumference [a] and increasing waist circumference [b].
Abbreviations: GGT, gamma glutamyltransferase; CRP, C-reactive protein; [a] Stable waist circumference: waist circumference changed ≤ 4 cm over 15-year follow-up period; [b] Increasing waist circumference: waist circumference increased > 4 cm over 15-year follow-up period. An asterisk (*) indicates a statistically significant difference (P<0.05) in mean/ geometric mean between consecutive generations at two matching age points, e.g. 40 and 45 years for the youngest versus the second-youngest generation; Note: CRP was adjusted for oral contraceptives and hormone replacement therapy.



Part II

Cardiovascular risk factor profiles and risk of cardiovascular disease

Chapter 4

Lifestyle changes in young adulthood and middle age and risk of cardiovascular disease and all-cause mortality

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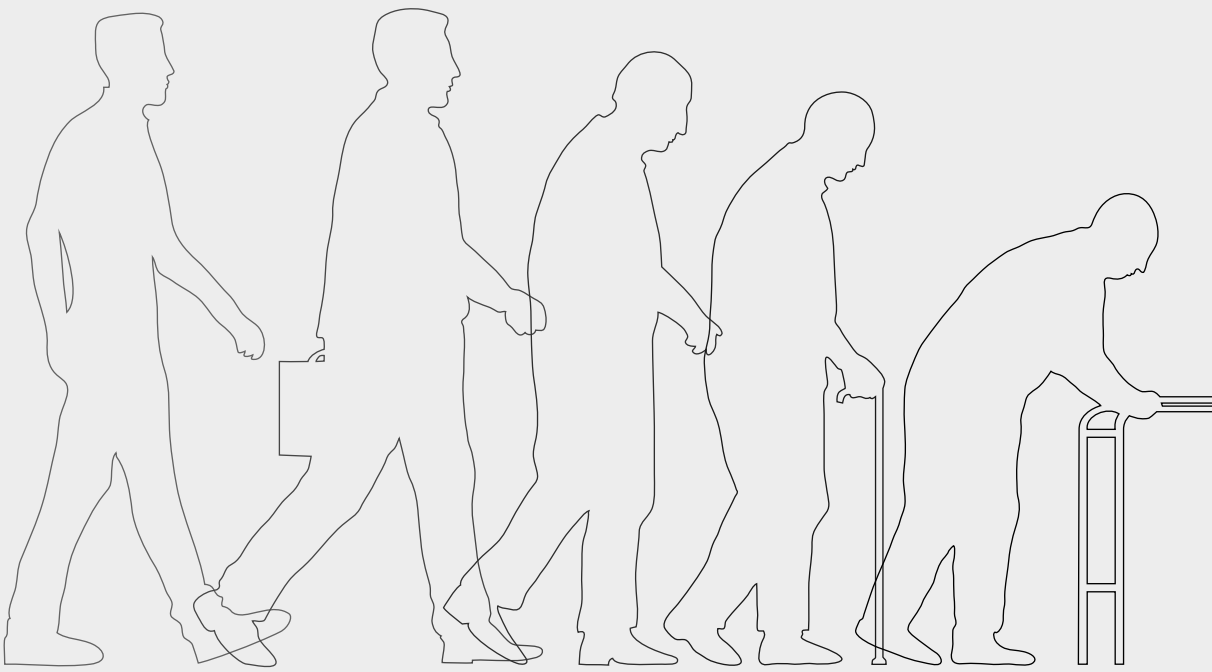

Abstract

Background: It is unclear to what extent changes in lifestyle in young adulthood/middle age can alter risk of cardiovascular disease (CVD) and death. We aimed to quantify the association of maintenance of and changes in lifestyle profiles over a period of five years with risk of CVD and all-cause mortality.

Methods: Lifestyle factors -- i.e. diet, physical activity, smoking, alcohol consumption -- and body mass index were assessed and dichotomised as healthy or unhealthy among 5,263 adults aged 26-66 years in 1993-1997 and five years later (1998-2002). The number of lifestyle risk factors was used as the independent variable. Fatal and non-fatal CVD and all-cause mortality during 8-15 years follow-up was the dependent variable. Multivariable-adjusted hazard ratios (HRs) and corresponding 95% confidence intervals (95%CIs) were estimated to quantify associations of change in the number of healthy lifestyle factors with CVD and all-cause mortality.

Results: Independent of the number of healthy lifestyle factors at baseline, each decrement in the number of healthy lifestyle factor over the five-year period was associated with on average a 35% higher risk of CVD incidence (HR: 1.35, 95%CI: 1.12-1.63) and a 37% higher risk of all-cause mortality (HR: 1.37, 95%CI: 1.10-1.70). In contrast, no association was observed with increase in the number of healthy lifestyle factors over the five-year period (P>0.5). Sixteen percent of the population maintained a healthy lifestyle profile (i.e. adherence to 4-5 healthy lifestyle factors) over five years. This part of the study population had a 2.5 times lower risk of CVD (HR: 0.43, 95%CI: 0.25-0.63) and all-cause mortality (HR: 0.40, 95%CI: 0.22-0.73) than those who maintained only 0-1 healthy lifestyle factor (i.e. unhealthy lifestyle profile during the whole five-year period).

Conclusions: Our findings indicate that the benefits of lifestyle are easier lost than gained over a five-year period. This underscores the need for the maintenance of healthy lifestyles throughout the life course.

Introduction

Although it is well-established that a healthy lifestyle is associated with a lower risk of cardiovascular disease (CVD) and premature mortality,[1-9] only a small fraction of the adult population has a healthy lifestyle.[6, 10] Adhering to a healthy diet, being physically active, no smoking, drinking alcohol in moderation, and having a normal weight are important factors in preventing CVD and premature mortality.[11, 12] The few studies that focused on overall lifestyle profiles (i.e. combination of lifestyle factors) showed that adherence to a higher number of healthy lifestyle factors, measured at a single point in time, was associated with a lower risk of CVD and all-cause mortality.[1-9] However, while lifestyle habits tend to change over time,[10, 13-15] little is known about the magnitude of health effects associated with changes in overall lifestyle profile over time. In addition, the long-term benefits of a healthy lifestyle profile can only become apparent when looking at lifestyles that are maintained over time. Quantifying the extent to which maintenance of and changes in lifestyles in young adulthood and middle age alter risk of CVD and premature mortality can provide insight on the potential effects of population-level lifestyle modification, and may serve as an incentive for adults to adopt and maintain an overall healthy lifestyle.

A study from the U.S. indicated that adults with unhealthy lifestyles who subsequently adopted healthier lifestyles had a 35% lower risk of CVD and a 40% lower risk of all-cause mortality over four years compared to those who maintained unhealthy lifestyles.[13] The Coronary Artery Risk Development in Young Adults study also showed that healthy or unhealthy changes in lifestyle over a 20-year period were associated with a lower or higher risk, respectively of coronary artery calcification and carotid intima-media thickness.[16] However, both studies were conducted in U.S populations and did not investigate the benefits of maintaining healthy lifestyles,[13, 16] only broadly compared changes in lifestyle profiles (four healthy lifestyle factors versus less than four),[13] or were limited to intermediate endpoints of CVD (e.g. coronary artery calcification) instead of CVD and all-cause mortality.[16] Therefore, we examined maintenance of and changes in overall lifestyle profiles over five years in a large prospective cohort, and investigated the association of these profiles with subsequent CVD and all-cause mortality risk over 8-15 years of follow-up.

Methods

Study population

The Doetinchem Cohort Study is an ongoing study that started in 1987-1991 with an age- and sex-stratified random sample of men and women aged 20-59 years living in Doetinchem, a provincial town in the Netherlands. Those who participated in 1987-1991

(N=7,768, participation rate: 62%) were re-invited for a second examination in 1993-1997 (wave two, N=6,117, participation rate: 79%) and a third examination in 1998-2002 (wave three, N=4,918, participation rate: 75%). Details of the study design have been described elsewhere.[17] Diet and physical activity were assessed from wave two onwards. Therefore, the second examination wave was considered as baseline for the present analyses. We excluded 854 participants due to: prevalent CVD or cancer at waves two or three based on hospital discharge data and self-report (N=676); lack of informed consent for linkage with Statistics Netherlands or the Dutch Hospital Discharge Registry (N=156); censoring before wave three (N=8); and lack of follow-up information on vital status or on CVD (N=14). Thus, these analyses are based on data from 5,263 participants (2,416 men; 2,847 women). All participants gave written informed consent and the study was approved according to the guidelines of the Helsinki Declaration by the Netherlands Organisation for Applied Scientific Research.

Measures

At each examination, participants underwent a physical examination, and information on demographic characteristics, lifestyle and medical history was obtained by self-administered questionnaires. We investigated four lifestyle factors (diet, physical activity, cigarette smoking and alcohol consumption) and body mass index (BMI), using similar methods to define healthy lifestyle as in previous studies.[3-5, 7-9, 13, 18] Dietary intake was assessed using a validated 178-item semi-quantitative food frequency questionnaire.[19, 20] A healthy diet was operationalised with the modified Mediterranean Diet Score (MDS) as described by Trichopoulou et al.[21] This score assigned values of 0 to 1 to each of the following nine nutritional components: alcohol, vegetables, fruits, legumes and nuts, grains, fish and seafood, meat products, unsaturated to saturated fatty acid ratio, and dairy products. Intakes equal or above the sex-specific median in the study population were assigned a value of 1, and intakes below that median a value of 0. For dairy and meat products, the scoring was inverted, as the traditional Mediterranean diet is characterised by low dairy and meat intake. Similar to other studies,[4, 5, 8, 9] alcohol consumption was included separately in the analysis and was, therefore, not included in the MDS. Thus, the MDS ranged from zero (minimal adherence) to eight (maximal adherence). Physical activity was assessed with a validated questionnaire developed for the 'European Prospective Investigation into Cancer and Nutrition' (EPIC). For the Doetinchem study, the questionnaire was extended with two-open ended questions on the type, frequency and duration of sports per week.[22] Sports, cycling, housekeeping, gardening and jobs that require heavy physical work were assessed as the number of hours per week, separately for summer and winter. The smallest number of hours per week reported for either summer or winter was used to ensure a conservative estimate. Hours per week spent on the various physical activities were summed. Only

physical activities with a Metabolic Equivalent of Task value of 4.0 or higher as reported by Ainsworth and colleagues were included,[23] which is in line with the Dutch physical activity guidelines.[24] Body weight was measured to the nearest 0.1 kg on calibrated scales and 1 kg was subtracted to adjust for clothing. Height was measured to the nearest 0.5 cm. BMI was calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Definition of lifestyle profiles

Each lifestyle factor was defined as a dichotomous variable, with healthy and unhealthy states. Similar to other studies,[2-3, 13, 18] lifestyle factors were classified as healthy as follows: healthy diet, MDS ≥ 5, healthy physical activity level: ≥3.5 hours per week spent on physical activities; not currently smoking; BMI lower than 30 kg/m^2; and moderate alcohol consumption, between 1 drink (i.e. 10 grams of alcohol/glass) per month and 2 drinks per day for men, and between 1 drink per month and 1 drink per day for women.[25]

Healthy lifestyle factor score

The number of healthy lifestyle factors present was summed to compute an aggregate 'healthy lifestyle factor score' ranging from 0 (none) to 5 (all), at both baseline and at the five-year follow-up wave. A 'change score' was constructed by subtracting the healthy lifestyle factor score at baseline from the score at the five-year follow-up wave (observed range: -4 to 4).

Lifestyle profiles

To examine the associations of maintenance of and changes in lifestyles, seven five-year lifestyle profiles were constructed (Figure 4.1). Both for the baseline and for the five-year follow-up wave, participants were categorised into one of three lifestyle categories based on the healthy lifestyle factor scores as: 'unhealthy': score 0-1; 'moderately healthy': score 2-3; and 'healthy': score 4-5. Participants could remain in the same lifestyle category in which they started at baseline, or could adopt a more healthy or unhealthy lifestyle over the five-year period.

Covariates

Highest educational level achieved was categorised as low (lower vocational training or primary school i.e. <10 years education), medium (secondary school and intermediate vocational training i.e. 11-14 years education) or high (higher vocational training or university i.e. >15 years education). Employment status was categorised as currently employed, homemaker, or unemployed/retired/unfit for work. At each examination wave, systolic and diastolic blood pressure were measured with a random zero sphygmomanometer (Hawksley and Sons, Lancing, UK). Blood pressure was measured twice after 2 minutes

of rest with participants in a seated position. Systolic blood pressure was recorded at the appearance of sounds (first-phase Korotkoff) and diastolic blood pressure was recorded at the disappearance of sounds (fifth-phase Korotkoff). The mean value of these two measurements was used for analyses. Hypertension was defined as a systolic blood pressure ≥ 140 mmHg, diastolic blood pressure ≥ 90 mmHg, and/or using blood pressure-lowering medication. Total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory, using standardised enzymatic methods. The TC/HDL-ratio was calculated by dividing the TC level by the HDL cholesterol level. Hypercholesterolemia was defined as having a TC/HDL-ratio ≥ 6.0 and/or taking cholesterol-lowering medication. Diabetes was defined based on self-reported history and/or non-fasting blood glucose concentration of 11.1 mmol/L or more.[26] Ninety percent of the cases were also verified using information from the participant's general practitioner or pharmacist.[27] Of the verified self-reported cases, 13 had type 1 diabetes, five an unknown/other type and four did not have diabetes, and were classified as being free of type 2 diabetes.

Outcome

Non-fatal and fatal cardiovascular events that occurred after the five-year follow-up wave were ascertained until January 1, 2011. In order to evaluate all-cause mortality, vital status was verified until June 1, 2013, using the municipal population register. Cause of death was ascertained through linkage with Statistics Netherlands, and morbidity data were obtained through probabilistic linkage with the Dutch Hospital Discharge Registry. In the Netherlands, 88% of hospital admissions can uniquely be linked to an individual based on date of birth, sex and postal code.[28] We defined fatal CVD cases (where CVD was the primary or secondary cause of death) and non-fatal CVD cases according to ICD-9[29] codes 410–414, 415.1, 427.5, 428, 430–438, 440-442, 443.9, 444, 798.1, 798.2, 798.9 and corresponding ICD-10 codes.[30]

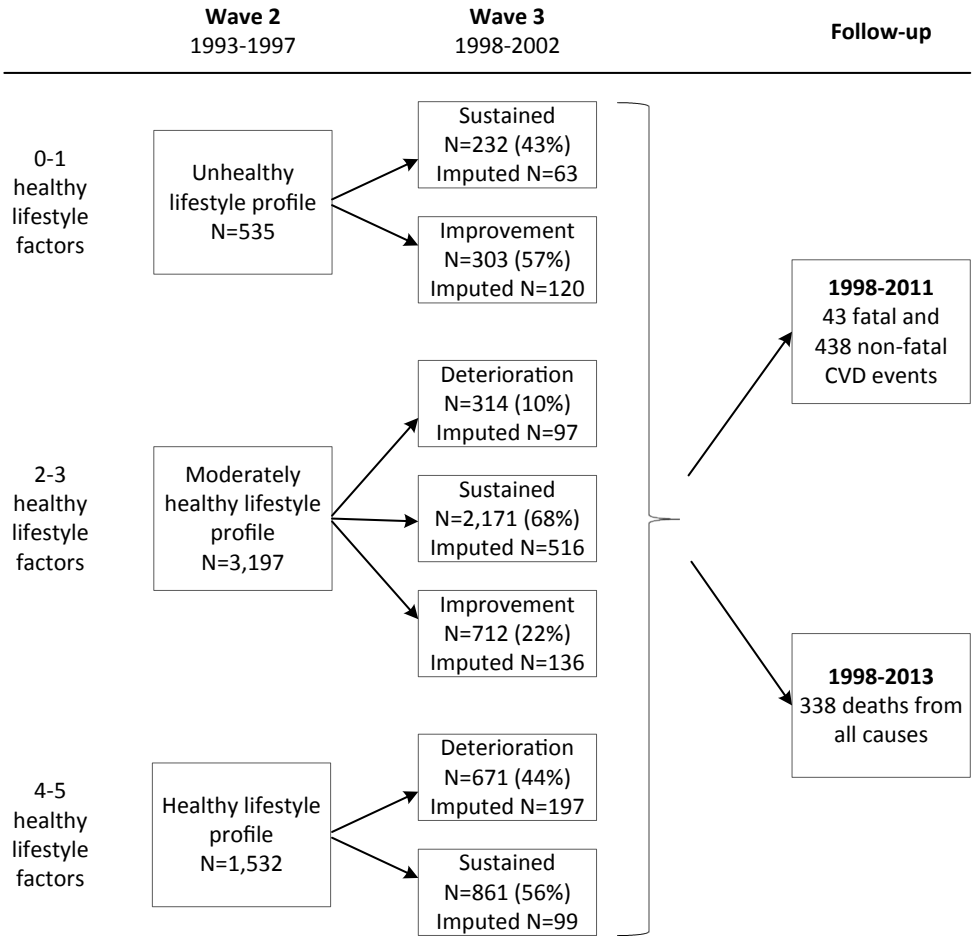

Figure 4.1. Overview of baseline and five-year lifestyle profiles.
Note: The number of participants in each lifestyle profile represents the average number of participants of the 20 imputed datasets. The categories do not add up to 5,263 due to rounding.

Data analyses

Of the study population, 0.4% had some missing data on lifestyle or covariates at baseline and 22.7% had some missing exposure data mainly due to non-response at the five-year follow-up wave. Exclusion of participants with missing data may lead to biased results and loss of precision.[31, 32] Therefore, missing values for all determinants and covariates were multiple-imputed using the 'multivariate imputation by chained equations' method in the statistical program R (version 3.1.0).[33, 34]

Age- and sex-adjusted event rates per 10,000 person-years of follow-up were estimated for the five-year lifestyle profiles. Hazard ratios (HRs) and corresponding 95% confidence intervals (95%CIs) from Cox proportional hazards regression were used to assess associations

of changes in single lifestyle factors, healthy lifestyle factor change scores, and lifestyle profiles (unhealthy lifestyle as reference) with CVD and all-cause mortality. The analyses of the healthy lifestyle factor change score were stratified by 'improvement' (score 0 to 4) and 'deterioration' (score 0 to -4), with the stable lifestyle as reference in both scores. The latter score was inverted. These analyses were also performed using an aggregate weighted healthy lifestyle score (range 0-5), which allocated points for each lifestyle factor based on their strength of associations with outcomes.

All analyses were adjusted for baseline age, sex, highest educational level achieved during follow-up and employment status in model 1. To investigate whether associations of lifestyle with outcomes were independent from intermediate metabolic risk factors, the analyses were additionally adjusted for hypertension, hypercholesterolemia and diabetes in model 2. To also take into account change over the five-year period for the intermediate variables, the additional variables in model 2 had four categories, e.g. hypertension at both waves of data collection, no hypertension at both waves, and change from non-hypertensive to hypertensive, or vice versa. The healthy lifestyle factor change scores were additionally adjusted for the baseline healthy lifestyle factor score in both models. Interaction terms between the determinants of interest and follow-up time were not statistically significant ($p>0.15$) indicating that the proportional hazards assumption was not violated. Results for men and women were similar (p-values for interaction with sex >0.10). In sensitivity analyses, all analyses were performed using complete data only. All analyses were performed using SAS 9.3 software and a two-sided P-value<0.05 was considered statistically significant.

Results

Participants were on average 46 (SD: 10) years of age at baseline and 46% were male (Table 4.1). At baseline, 29% of the participants had a healthy lifestyle, 61% a moderately healthy lifestyle and 10% an unhealthy lifestyle. Participants with a healthy lifestyle were more likely to have a higher educational level, and to be currently employed, and tended to have more favourable levels of the major biological CVD risk factors compared to those with less healthy lifestyles (Table 4.1). Over the five-year follow-up period, 62% of the participants maintained the same lifestyle profile, whereas 19% improved their lifestyles and 19% adopted unhealthier lifestyles (Figure 4.1). Improvement or deterioration was more likely to be observed in diet score, physical activity and alcohol consumption, than in smoking status or BMI (Table 4.2). During an average of 9.8 years follow-up, 481 CVD events occurred; there were 338 deaths after 12.2 years of follow-up.

Table 4.1. Baseline characteristics of the Doetinchem Cohort Study (1993-1997) according to baseline lifestyle profiles[a].

	Total population	**Healthy lifestyle profile**	**Moderately healthy lifestyle profile**	**Unhealthy lifestyle profile**
	N=5,263	N=1,532	N=3,197	N=535
Age (years), mean (sd)	45.7 (9.9)	45.7 (9.6)	45.7 (10.0)	46.4 (9.9)
Women, n (%)	2,847 (54%)	719 (47%)	1,819 (57%)	309 (58%)
Low educational attainment, n (%)	2,633 (50%)	617 (40%)	1,670 (52%)	345 (65%)
SBP (mmHg), mean (sd)	125 (16)	124 (16)	124 (16)	127 (18)
DBP (mmHg), mean (sd)	80 (11)	79 (10)	80 (11)	81 (11)
TC (mmol/L), mean (sd)	5.5 (1.0)	5.4 (1.0)	5.5 (1.0)	5.6 (1.0)
HDLc (mmol/L), mean (sd)	1.38 (0.37)	1.42 (0.37)	1.38 (0.38)	1.27 (0.36)
BMI (kg/m^2), mean (sd)	25.7 (3.7)	24.9 (2.6)	25.8 (3.8)	27.8 (5.1)
Type 2 diabetes mellitus[b], n (%)	65 (1.2%)	11 (0.7%)	43 (1.3%)	12 (2.3%)
Healthy lifestyle factors				
BMI < 30kg/m^2 (%)	4,639 (88%)	1,508 (98%)	2,810 (88%)	321 (60%)
Healthy diet MDS ≥ 5 (%)	1,987 (38%)	1,128 (74%)	843 (26%)	16 (3%)
Physical active ≥ 3.5h/week (%)	2,983 (57%)	1,340 (87%)	1,600 (50%)	43 (8%)
Not smoking (%)	3,660 (70%)	1,439 (94%)	2,118 (66%)	103 (19%)
Moderate alcohol consumption (%)	1,891 (36%)	1,048 (68%)	829 (26%)	14 (3%)

Abbreviation: SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDLc, HDL cholesterol; BMI, body mass index; MDS, Mediterranean diet score; [a] Healthy lifestyle profile was defined as having 4-5 of the following healthy lifestyle factors: Mediterranean diet score ≥5, ≥ 3.5 hours per week spent on moderate-to-vigorous intensity physical activities, not currently smoking, moderate alcohol consumption and a BMI lower than 30 kg/m2. Participants with a moderately healthy lifestyle profile adhered to 2-3 healthy lifestyle factors and participants with an unhealthy lifestyle profile to one or less factors; [b] Self-reported diabetes and/or non-fasting blood glucose concentration ≥ 11.1 mmol/L.

Change in individual lifestyle factors and risk of CVD and all-cause mortality

For most individual lifestyle factors, improvement and deterioration over five years tended to be associated with a lower and higher risk, respectively, of CVD and all-cause mortality, although associations were not statistically significant (Table 4.3). For example, change from being obese to overweight/normal weight was (not statistically significantly) associated with a 48% lower risk of CVD (HR: 0.52, 95%CI: 0.21-1.31) compared to staying obese over time. Similarly, change from a healthy weight to being obese was (not statistically significantly) associated with a 34% higher risk of CVD (HR: 1.34, 95%CI: 0.92-1.94) compared to maintaining a healthy weight.

Table 4.2. Means of each lifestyle factor for participants who maintained the same, improved or deteriorated in that lifestyle factor.

	Sustained health[a] N≥1282	**Deteriorated healthy**[a] N≥126	**Improved unhealthy**[a] N≥74	**Sustained unhealthy**[a] N≥549
Body mass index	< 30 kg/m² at both waves	< 30 kg/m² at baseline and ≥30 kg/m² at five-year follow-up	≥30 kg/m² at baseline and <30 kg/m² at five-year follow-up	≥30 kg/m² at both waves
N, (%)	4318 (82%)	321 (6%)	74 (1%)	549 (10%)
Mean at baseline	24.5±2.5 kg/m²	28.6±1.1 kg/m²	31.0±0.9 kg/m²	33.1±3.0 kg/m²
Mean at five-year follow-up	25.2±2.6 kg/m²	31.3±1.2 kg/m²	28.6±1.3 kg/m²	34.1±3.2 kg/m²
Smoking	Not smoking at both waves	Not smoking at baseline and smoking at five-year follow-up	Smoking at baseline and not smoking at five-year follow-up	Smoking at both waves
N, (%)	3533 (67%)	126 (2%)	297 (6%)	1306 (25%)
Mediterranean diet score	≥5 at both waves	≥5 at baseline and <5 at five-year follow-up	<5 at baseline and ≥5 at five-year follow-up	<5 at both waves
N, (%)	1218 (23%)	768 (15%)	999 (19%)	2278 (43%)
Mean at baseline	5.7±0.8	5.4±0.7	3.4±0.9	2.9±1.0
Mean at five-year follow-up	5.7±1.0	3.5±1.0	5.3±0.9	3.0±1.1
Physical activity	≥3.5 h/wk at both waves	≥3.5 h/wk at baseline and <3.5 h/wk at five-year follow-up	<3.5 h/wk at baseline and ≥3.5 h/wk at five-year follow-up	<3.5 h/wk at both waves
N, (%)	1910 (36%)	1073 (20%)	864 (16%)	1416 (27%)
Mean at baseline	12.0±13.2 h/wk	10.0±11.3 h/wk	1.8±1.1 h/wk	1.4±1.1 h/wk
Mean at five-year follow-up	11.6±12.5 h/wk	1.6±1.2 h/wk	8.9±9.6 h/wk	1.4±1.1 h/wk
Alcohol consumption	At both waves moderately	Moderately at baseline and not moderately at five-year follow-up	Not moderately at baseline and moderately at five-year follow-up	Not moderately at both waves
N, (%)	1282 (24%)	623 (12%)	768 (14%)	2634 (50%)
Median at baseline	0.7 [0.6-1.1] glasses/day	0.8 [0.4-1.0] glasses/day	0.0 [0.0-2.1] glasses/day	0.0 [0.0-2.3] glasses/day
Median at five-year follow-up	0.9 [0.6-1.1] glasses/day	1.1 [0.0-2.1] glasses/day	0.7 [0.0-2.1] glasses/day	0.0 [0.0-2.3] (glasses/day

[a] The number of participants in each category differs per lifestyle factor. Values represent either means ± standard deviations, numbers and (percentages), or medians and [interquartile ranges].

Changes in the number of healthy lifestyle factors and risk of CVD and all-cause mortality

Regardless of the number of healthy lifestyle factors at baseline, each healthy lifestyle factor lost during follow-up was associated with a 35% higher risk of CVD (HR: 1.35, 95%CI: 1.12-1.63) and a 37% higher risk of all-cause mortality (HR: 1.37, 95%CI: 1.10-1.70) (Table 4.4). Improvement in lifestyle was not associated with risk of CVD (HR: 0.95, 95%CI: 0.80-1.14) and all-cause mortality (HR: 0.96, 95%CI: 0.76-1.21). Analyses using the weighted scores gave similar results as those based on unweighted scores (Table 4.4). Further adjustment for presence of diabetes, hypertension and hypercholesterolemia at baseline or the five-year follow-up wave only slightly attenuated the associations for all analyses.

Table 4.3. Hazard ratios and 95% confidence intervals for the associations between change in single lifestyle factors and cardiovascular disease and all-cause mortality.

	HR and 95%CI of fatal and non-fatal cardiovascular disease		HR and 95%CI of all-cause mortality	
	Improved[a]	deteriorated[a]	Improved	deteriorated
Body mass index				
Model 1[b]	0.52 (0.21-1.31)	1.34 (0.92-1.94)	0.83 (0.31-2.22)	1.14 (0.74-1.77)
Model 2[c]	0.52 (0.09-3.04)	1.59 (0.94-2.68)	1.24 (0.37-4.20)	1.00 (0.48-2.06)
Smoking				
Model 1[b]	0.71 (0.45-1.11)	1.56 (0.81-3.02)	0.78 (0.47-1.28)	0.83 (0.21-3.21)
Model 2[c]	0.76 (0.44-1.32)	1.50 (0.57-3.95)	0.73 (0.37-1.46)	0.73 (0.11-4.71)
Physical activity				
Model 1[b]	0.86 (0.67-1.11)	1.17 (0.88-1.56)	0.87 (0.58-1.35)	1.31 (0.95-1.82)
Model 2[c]	1.05 (0.72-1.54)	1.03 (0.74-1.45)	1.04 (0.63-1.71)	1.42 (0.96-2.11)
Mediterranean Diet Score				
Model 1[b]	1.01 (0.77-1.31)	1.14 (0.80-1.62)	0.93 (0.66-1.32)	1.37 (0.90-2.08)
Model 2[c]	1.17 (0.84-1.61)	1.16 (0.75-1.79)	1.09 (0.73-1.63)	1.19 (0.72-1.96)
Alcohol consumption				
Model 1[b]	0.82 (0.59-1.13)	1.19 (0.85-1.68)	0.93 (0.65-1.32)	1.11 (0.73-1.71)
Model 2[c]	0.75 (0.50-1.13)	1.32 (0.85-2.05)	0.96 (0.60-1.53)	1.10 (0.63-1.93)

Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval. [a] Change in single healthy lifestyle factors from unhealthy to healthy (improved) or vice versa (deteriorated) over a five-year period. [b] Cox proportional hazard models adjusted for age, sex, educational level and occupation. [c] Analyses additionally adjusted for (other) lifestyle factors.

Table 4.4. Hazard ratios and 95% confidence intervals for the healthy lifestyle factor change score and change in single healthy lifestyle factors.

	HR and 95%CI of fatal and non-fatal cardiovascular disease		HR and 95%CI of all-cause mortality	
	Improved	**deteriorated**	**Improved**	**deteriorated**
HLF change score[a]				
Model 1[b]	0.95 (0.80-1.14)	1.35 (1.12-1.63)	0.96 (0.76-1.21)	1.37 (1.10-1.70)
Model 2[c]	0.96 (0.81-1.15)	1.31 (1.08-1.58)	0.98 (0.77-1.24)	1.36 (1.09-1.69)
Weighted HLF change score[d]				
Model 1[b]	0.88 (0.70-1.10)	1.27 (1.05-1.54)	0.94 (0.74-1.20)	1.41 (1.13-1.76)
Model 2[c]	0.88 (0.70-1.10)	1.22 (1.00-1.48)	0.96 (0.75-1.23)	1.40 (1.12-1.76)

Abbreviations: HR, hazard ratio; 95%CI, 95% confidence interval; HLF, healthy lifestyle factor.
[a] Change in risk for each healthy lifestyle factor gained (improved) or lost (deteriorated) over a five-year period. [b] Cox proportional hazard models adjusted for age, sex, educational level, occupation and the number healthy lifestyle factors at baseline. [c] Analyses additionally adjusted for hypertension, hypercholesterolemia and diabetes. [d] Change in risk for each point gained (improved) or lost (deteriorated) in aggregate weighted healthy lifestyle score between baseline and the five-year follow-up wave. This score was based on the strength of associations between each individual lifestyle factor and outcomes.

Associations of baseline and five-year lifestyle profiles with outcomes

Participants who maintained a healthy lifestyle profile over the five-year period had a 57% lower risk of CVD (HR: 0.43, 95%CI: 0.26-0.70) and a 60% lower risk of all-cause mortality (HR: 0.40, 95%CI: 0.22-0.73) compared to those who maintained an unhealthy lifestyle (Figure 4.2 and Table 4.5). We compared the risks of improvement and deterioration in each lifestyle category (healthy, moderately healthy and unhealthy) with maintenance of the same lifestyle profile (Figure 4.2 and Tables 4.5). Improvement resulted in similar or only slightly lower HRs for CVD and all-cause mortality and deterioration in higher HRs. The HR for CVD and all-cause mortality was lower for those with a sustained healthy lifestyle (HR: 0.43 and 0.40) compared to those with healthy lifestyles at baseline who changed to an unhealthy lifestyle over the five-year period (HR: 0.56, 95%CI: 0.36-0.87 and HR: 0.54, 95%CI: 0.31-0.97 respectively). On the other hand, HRs for CVD (HR: 0.97, 95%CI: 0.56-1.66) and all-cause mortality (HR: 0.98, 95%CI: 0.49-1.95) were similar for those with an unhealthy lifestyle at baseline who improved over five years compared to those who maintained an unhealthy lifestyle profile (HR: 1.00).

Sensitivity analysis

The complete case analysis returned similar results as the results based on multiple imputed data.

Table 4.5. Hazard ratio and 95% confidence interval of cardiovascular disease events and all-cause mortality in five-year lifestyle profiles compared to sustained unhealthy lifestyle profile.

Baseline	**Healthy lifestyle profile[a]**		**Moderately healthy lifestyle profile[a]**			**Unhealthy lifestyle profile[a]**	
Five-year follow-up	**Sustained**	**Deteriorate**	**Improve**	**Sustained**	**Deteriorate**	**Improve**	**Sustained**
Fatal and non-fatal cardiovascular disease							
Person-years of follow-up (no. of events)	8,537 (56)	6,732 (55)	7,146 (52)	21,492 (199)	3,064 (44)	2,875 (42)	2,201 (52)
Age- and sex-adjusted event rate (/10,000 person-years)	48	66	59	77	131	118	121
Hazard ratio (95% confidence interval)[b]	0.43 (0.26-0.70)	0.56 (0.35-0.92)	0.50 (0.30-0.83)	0.65 (0.43-0.98)	1.06 (0.63-1.78)	0.97 (0.56-1.66)	1.00 (ref)
Hazard ratio (95% confidence interval)[c]	0.50 (0.31-0.81)	0.63 (0.38-1.02)	0.56 (0.37-0.94)	0.70 (0.46-1.06)	1.10 (0.65-1.86)	1.00 (0.58-1.71)	1.00 (ref)
All-cause mortality							
Person-years of follow-up (no. of events)	10,610 (35)	8,287 (36)	8,834 (41)	26,499 (142)	3,762 (31)	3,616 (31)	2,750 (22)
Age- and sex-adjusted event rate (/10,000 person-years)	21	29	29	36	60	53	54
Hazard ratio (95% confidence interval) [b]	0.40 (0.22-0.73)	0.54 (0.31-0.97)	0.55 (0.31-0.97)	0.66 (0.39-1.10)	1.10 (0.60-2.02)	0.98 (0.49-1.95)	1.00 (ref)
Hazard ratio (95% confidence interval) [c]	0.45 (0.24-0.83)	0.60 (0.33-1.07)	0.60 (0.33-1.08)	0.70 (0.41-1.17)	1.16 (0.62-2.14)	1.03 (0.51-2.06)	1.00 (ref)

[a] Healthy lifestyle profile was defined as having 4-5 of the following healthy lifestyle factors: Mediterranean diet score ≥5, ≥ 3.5 hours per week spent on moderate-to-vigorous intensity physical activities, not currently smoking, moderate alcohol consumption and a BMI lower than 30 kg/m^2. Participants with a moderately healthy lifestyle profile adhered to 2-3 healthy lifestyle factors and participants with an unhealthy lifestyle profile to one or less factors. [b] Model 1: adjusted for age, sex, educational attainment and employment status.; [c] Model 2: model 1 + adjusted for hypertension, hypercholesterolemia and diabetes.; ref.: reference.

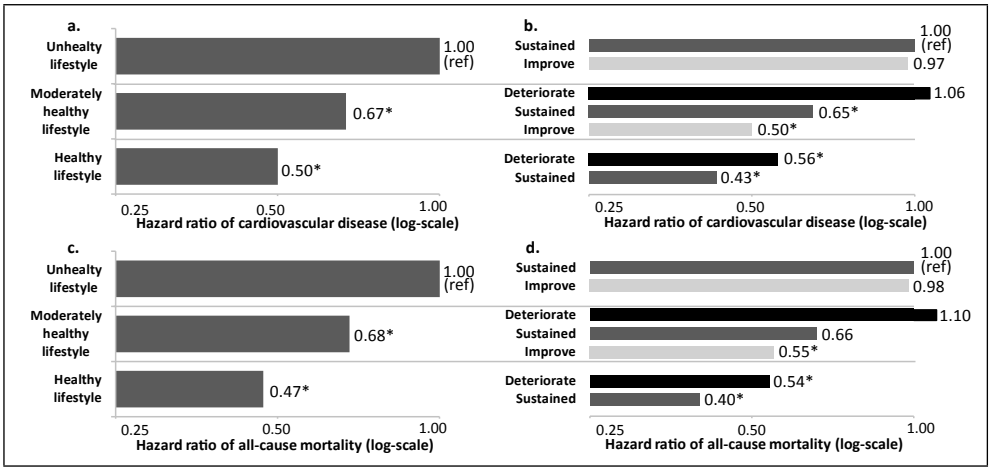

Figure 4.2. Multivariable adjusted hazard ratios of incident total cardiovascular disease (a, b) and all-cause mortality (c, d). For baseline lifestyle profiles (a, c) [a] and five-year lifestyle profiles (b, d) including adults who maintained that same lifestyle profile (grey)[b], improved (light grey)[c] or deteriorated (black) [d] during five-year follow-up.
An asterisk (*) indicates a statistically significant different from (sustained) unhealthy lifestyle profile, P< 0.05; [a] Healthy lifestyle profile was defined as having 4-5 of the following healthy lifestyle factor: Mediterranean diet score ≥5, ≥ 3.5 hours per week spent on moderate-to-vigorous intensity physical activities, not currently smoking, moderate alcohol consumption and a BMI lower than 30 kg/m^2. Participants with moderately healthy lifestyle profile adhered to 2-3 healthy lifestyle factors and participants with an unhealthy lifestyle profile to one or less factors; [b] Sustained: lifestyle profile remains steady over five years; [c] Improve: lifestyle profile improved over five years; [d] Deteriorate: lifestyle profile deteriorated over five years.

Discussion

The current study found that deterioration of lifestyle habits over five years was associated with a higher risk of CVD and all-cause mortality independent of baseline lifestyle status, while improvement in lifestyle was not statistically significantly related to these outcomes. Each healthy lifestyle factor lost over time resulted on average in a one-third higher risk of CVD and all-cause mortality over the following 8-15 years. Furthermore, compared to those who maintained an unhealthy lifestyle, adults who maintained a healthy lifestyle over five years had a 57-60% lower risk of CVD and all-cause mortality, while adults with a healthy lifestyle at baseline who turned to a less healthy lifestyle.

Our findings of the association of baseline lifestyle profiles with risk of CVD and all-cause mortality are consistent with previous studies, which reported that healthy lifestyles were associated with 46-68% lower risk for CVD[1, 3, 4, 7, 8] and 32-87% lower risk for all-cause mortality[3, 6] compared to unhealthy lifestyles. However, these previous studies did not examine changes in lifestyle over time. In our study, 38% of the adults changed their

lifestyle over a period of five years, which is in line with an earlier study in the Netherlands investigating the stability of lifestyle behaviour.[15] The fact that such a large proportion of adults changed their behaviour over time underscores the importance of quantifying the impact of changes in lifestyle on risk of CVD and all-cause mortality in a healthy young adult/ middle-aged population, especially when considering the fact that many epidemiological studies only take into account baseline lifestyle profiles.

King et al., reported that among adults ages 45-64 years, improvement from <4 to 4 healthy lifestyle factors over a four-year period was associated with a lower risk of CVD and death over the following four years.[13] In contrast, although a strong graded association was observed between baseline lifestyle profiles and CVD and all-cause mortality in the current study, improvement in lifestyle was, contrary to our expectations, not associated with these outcomes. While significant associations of baseline physical activity[35] and the MDS[36] with incident CVD have been previously reported in the MORGEN and EPIC-NL studies, respectively (of which the Doetinchem Cohort Study was a part), the current analyses showed that improvement in physical activity and the MDS over a five-year period were not associated with risk reduction. The absence of such associations and the fact that changes from unhealthy to healthy were most often due to changes in physical activity and the MDS likely contributed to the absence of associations of improvement in overall lifestyle with CVD and death. The absence of associations was likely not attributable to the magnitude of changes, since improvements were substantial, i.e. participants increased their amount of physical activity on average by 7.1 hours/week and their MDS by 1.9 units over the five-year follow-up period.

In addition, improvement in obesity status was not significantly associated with CVD and all-cause mortality, most likely due to the limited number of people who improved in obesity status (N=74). This may also have contributed to the absence of statistically significant associations between improvement in overall lifestyle and CVD and death. Reverse causation does not appear to explain the absence of associations because exclusion of events in the first two years of follow-up did not change risk estimates (data not shown). Lifelong unhealthy lifestyles up to young adulthood/ middle age may have resulted in damage which cannot be compensated by lifestyle improvements over a period as short as five years. However, we did not have information on lifestyle history before the five-year period, and thus it was not possible to determine whether absence of associations could be explained by lifelong unhealthy lifestyles. Finally, we cannot exclude the possibility that improvement in lifestyle over a five-year period has a very small impact on CVD and mortality risks, which requires a larger sample size to demonstrate.

Our results indicate that more effort is needed to increase the proportion of young/ middle-aged adults who maintain a healthy lifestyle since each healthy lifestyle factor lost over just a five-year period resulted on average in a one-third higher risk of CVD and all-

cause mortality. We also showed that maintaining an overall healthy lifestyle was associated with the lowest risk of CVD and all-cause mortality, i.e. a 57-60% lower risk compared to those with an unhealthy lifestyle. While prevention and control of CVD has traditionally focused primarily on those with high CVD risk profiles and/or unhealthy lifestyles, our findings indicate that attention is needed to the maintenance of healthy lifestyles since lifestyle habits of many adults deteriorate over time with a sizeable impact on their CVD and mortality risk.

We previously demonstrated that maintenance of a low CVD risk profile based on major CVD risk factors (ideal levels of blood pressure, cholesterol, BMI, no smoking and no diabetes) was associated with seven times lower risk of CVD compared to having long-term high risk profile.[37] Our findings also suggested that improvement and deterioration in CVD risk profile resulted in twofold lower and higher risk of subsequent CVD incidence respectively. Risk differences associated with changes in lifestyle profile observed in the present study were smaller, which was to be expected since lifestyle factors are more distal risk factors. However, the current analyses indicate that deterioration in lifestyle directly influence risk of CVD and all-cause mortality independent of major CVD risk factors such as hypertension, hypercholesterolemia or diabetes since adjustment for these factors only slightly altered risk estimates.

This study has several strengths, including the prospective design, high participation rate, long follow-up period, and the extensive information about lifestyle and risk factors. Some limitations include the small number of participants with zero healthy lifestyle factors in the unhealthy lifestyle profile group, i.e. most participants in this group had 1 healthy lifestyle factor. The exclusion of participants who had a CVD event and/or cancer between baseline and the five-year follow-up wave (N=332) further mitigated the true difference between the healthy and unhealthy groups, which may have resulted in somewhat conservative estimates. Furthermore, we have no data on cycling in lifestyle profile during the five-year period. Cycling might have occurred in part of the population, and particularly weight cycling may have led to some underestimation of the associations of improvement and deterioration in lifestyle with outcomes. In addition, we used the Mediterranean diet score because this has commonly been used in previous cardiovascular research and it includes several important foods/food groups. As with all diet scores, some important nutrients are not included in the Mediterranean diet score such as salt intake. This may have slightly underestimated the benefits of a healthy diet and consequently underestimated the associations between change in diet and cardiovascular disease and death. Although a substantial proportion of the data at the five-year follow-up wave (1998-2002) was multiple-imputed (22.7%), complete-case analysis yielded similar results. The use of self-reported lifestyle data may possibly have led to misclassification, resulting in underestimation of the associations. Finally, our results were obtained in a relatively healthy population, which has

most likely led to underestimation of the strength of associations due to underestimation of the number of participants with CVD and those who died.

In conclusion, having and maintaining an overall healthy lifestyle profile – i.e. non-smoking, a healthy diet, adequate physical activity, moderate alcohol consumption and a healthy BMI -- is associated with the lowest risk of CVD and all-cause mortality, i.e. 57-60% lower risks compared to maintaining an unhealthy lifestyle profile. However, few adults have a healthy lifestyle and even fewer are able to maintain this healthy lifestyle over time. Independent of lifestyle behaviour at young adulthood/ middle age, deterioration in lifestyle over a five-year period may lead to an approximate one-third higher risk of CVD incidence and all-cause mortality, whereas improvement in lifestyle over the same period did not reduce those risks in this cohort. Thus, at young adulthood/ middle age, the benefits of a healthy lifestyle are easily lost by deterioration in lifestyle. These findings underscore the need to focus CVD prevention efforts not only on adults with unhealthy lifestyles or at high risk of CVD, but also on adults with healthy lifestyles by promoting the maintenance of healthy lifestyles throughout the life course.

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Chapter 5

Quantifying the benefits of achieving and maintaining long-term low risk profile for cardiovascular disease

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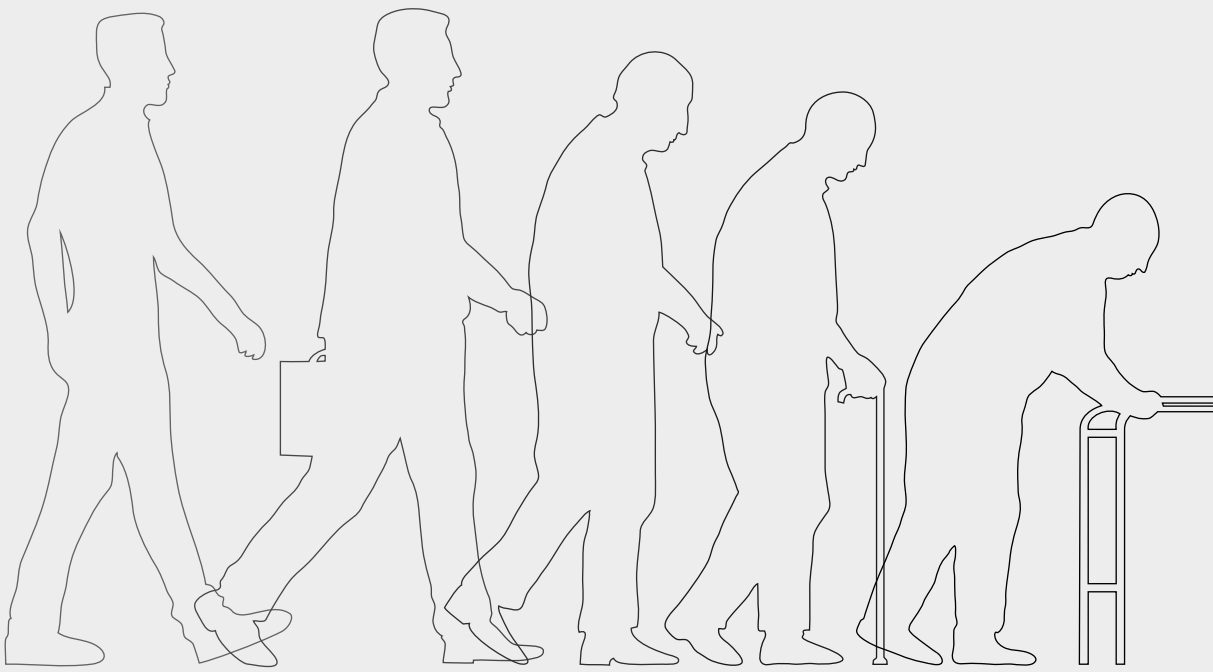

Abstract

Background: Studies investigating the relation between risk profiles and cardiovascular disease (CVD) have measured risk at baseline only. We investigated maintenance of and changes in risk profiles over time and their potential impact on incident CVD.

Methods: In a population-based cohort study, we measured risk factors among 5,574 CVD-free adults aged 20-59 years. They were classified into four risk categories according to smoking status, presence of diabetes and widely accepted cut-off values for blood pressure, total cholesterol/HDL-ratio, and body mass index. Categories were subdivided (maintenance, deterioration, improvement) based on risk factor levels at 6 and 11 years of follow-up. Multivariable-adjusted hazard ratios (HRs) and 95% confidence intervals (95%CI) for CVD incidence 5-10 years following the risk-change period were fitted using Cox proportional hazards models.

Results: Only 12% of participants had a low risk profile (i.e. ideal levels of blood pressure, cholesterol and body mass index, non-smoking and no diabetes) at baseline, and only 7% maintained it. Participants who maintained a low risk profile over 11 years had 7 times lower risk of CVD (HR: 0.14, 95%CI: 0.05-0.40) than participants with long-term high risk profile, whereas those low risk at baseline whose profile deteriorated had 3 times lower risk (HR: 0.35, 95%CI: 0.18-0.69). Our results suggest that, within each baseline risk profile group, compared to a stable profile, improving profiles may be associated with up to twofold lower HRs, and deteriorating profiles with about twofold higher HRs.

Conclusions: Our study, using long-term risk profiles, demonstrates the full benefits of low risk profile. These findings underscore the importance of achieving and maintaining low risk from young adulthood onwards.

Introduction

In past decades, research focused on the impact of elevated levels of the major cardiovascular disease (CVD) risk factors, that is serum cholesterol, blood pressure, body mass index (BMI), smoking, and diabetes. However, in most developed countries, the prevalence of major CVD risk factors remains high.[1-3] As eloquently stated by Geoffrey Rose, the notion of what constitutes a healthy level for a risk factor is influenced by the mean population level of that risk factor.[4] From the perspective of optimal health and well-being, only a small proportion of the population has favourable levels of all major risk factors (i.e. a low risk profile) and an even smaller proportion maintains those low risk levels over time.[5, 6]

Previous studies have demonstrated the benefits of having low levels of all major CVD risk factors. They have consistently demonstrated a significantly lower risk of coronary heart disease,[7-10] stroke,[7, 9, 10] and total CVD[7-9, 11-13] at older ages among individuals who had low risk profile in young adulthood and middle-age compared with others. However, these earlier studies on benefits of low risk profile have relied on single measures of risk factors, obtained at baseline, without taking changes in risk profiles over time into account. The full impact of low levels of risk factors can only become apparent when looking at long-term low levels. In addition, the extent to which CVD risk differs between adults who maintain an unfavourable risk profile and those who experience improvement or deterioration in their risk profile over time is unknown, but risk profiles are likely to change and influence CVD risk. Therefore, in order to effectively demonstrate the importance of low risk and changes in risk profiles in CVD prevention, it is necessary to quantify the magnitude of the benefits of sustained low risk and the impact of changes in risk profiles over time.

We investigated the association of baseline risk profiles with CVD risk and compared it with association of long-term risk profiles (sustained, improved or deteriorated over an 11-year period) with 5-10 year CVD risk following the risk change-period. This study is unique as we were able to use three repeated measurements of CVD risk factor over an 11-year period to define the long-term exposure of risk profiles and relate this to risk of CVD.

Methods

Population

The Doetinchem Cohort Study is an ongoing study involving an age- and sex-stratified random sample of men and women aged 20-59 years. They are drawn from the civil registries of Doetinchem, a town in the eastern part of the Netherlands with 46,967 inhabitants in the year 2000. At baseline (1987-1991: wave one), we invited 20,155 men and women to undergo a clinical examination. Of the 62% who participated (N=12,405), a random sample

of 7,768 participants was invited for a second examination (1993-1997: wave two) of whom 79% participated (N=6,117). All participants invited to participate in wave two were also re-invited for a third examination (1998-2002: wave three) except for those who, at any point, did not give permission to retrieve their information from the municipal administration, emigrated or otherwise withdrew from the study. This resulted in the invitation of 6,579 participants for wave three of whom 75% (N=4,918) participated. Only participants who participated in two of the three waves were included (N=6,368) in the analyses, of which 4,661 participants attended all three examinations. The study design of the Doetinchem Cohort study has been described in detail elsewhere.[14] All participants gave written informed consent and the study was approved according to the guidelines of the Helsinki Declaration by the external Medical Ethics Committee of the Netherlands Organisation for Applied Scientific Research.

Measures

The major CVD risk factors (i.e. weight and height to calculate BMI, diastolic and systolic blood pressure, total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol, and blood glucose) were measured by trained staff according to a standardized protocol. Lifestyle factors (i.e. physical activity, diet, and alcohol intake), demographic characteristics, and medical history were collected with standardized questionnaires. Details of these measurements have been described elsewhere[14] and are available in Supplementary Methods.

Definition of risk profile

Baseline risk

Participants were categorized into four baseline risk profiles (low risk, medium-low risk, medium-high risk, and high risk) using smoking status, presence of diabetes and widely accepted cut-off values for blood pressure, TC/HDL-ratio, and BMI as described in Table 5.1.[7-11] The TC/HDL-ratio was used instead of TC, as it has been associated more strongly with risk of CVD.[15, 16]

Long-term risk

Each baseline risk profile was further categorised based on similarly defined risk-factor levels at six years (wave two) and 11 years (wave three) of follow-up, resulting in 11 distinct long-term risk profiles (Table 5.2):

1. Long-term low risk: favourable levels of all risk factors (i.e. low risk) at all three waves. Since only 154 participants were low risk at all waves, this profile also included persons low risk at two waves and medium-low risk at one;

2. Deteriorated low risk: low risk profile at baseline with worsening of risk profile during follow-up;
3-8. Medium-low or medium-high risk profile at baseline, with either maintenance of that profile at all waves or improvement/deterioration of risk profile over time;
9. Long-term high risk: high risk at all waves;
10. Improved high risk profile: high risk profile at baseline with improvement in risk profile over time.

We were interested only in participants who maintained a stable risk profile and those who experienced deterioration or improvement during follow-up. Therefore, participants with inconsistent long-term risk profiles (N=587) are included as a category in the analyses but, for simplicity, are not presented here.

Table 5.1. Definition of baseline risk profiles.

	Blood pressure	**Cholesterol**	**Body mass index**	**Smoking**	**Diabetes**
Low risk profile All risk factors with low values:	Untreated DBP<80 mm Hg, and SBP<120 mm Hg	Untreated TC/HDL<4.0	<25.0 kg/m^2	Former or never smoker	No history of diabetes
Medium-low risk profile At least one risk factor with suboptimal values:	Untreated DBP 80-89 mm Hg, and/or SBP 120-139 mm Hg	Untreated TC/HDL 4.0-5.9	25.0-29.9 kg/m^2	Former or never smoker	No history of diabetes
Medium-high risk profile One risk factor with high values:	DBP≥90, and/or SBP≥140 mm Hg, and/or taking antihypertensive medication	≥6.0 and/or taking cholesterol-lowering medication	≥30.0 kg/m^2	Currently smoking	History of diabetes
High risk profile	Two or more risk factors with high values as indicated at medium-high risk.				

Abbreviations: SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL, total cholesterol/HDL cholesterol ratio.

Outcomes

Outcomes were determined based on fatal and non-fatal CVD events that occurred after wave three (1998-2002) up to 2008, resulting in a maximum follow-up time of 10.0 years and an average of 7.2 years. CVD follow-up data was incomplete for 3% of the participants who were on average censored 3.8 years before the end date of follow-up. Vital status was identified using the municipal population register. Cause of death was ascertained through linkage with Statistics Netherlands, and morbidity data was obtained through linkage with the Dutch Hospital Discharge Diagnosis Database. We defined fatal cases (in which CVD was the primary or secondary cause of death) and non-fatal CVD cases according to ICD-9[17] codes 410–414, 415.1, 427.5, 428, 430–438, 440-442, 443.9, 444, 798.1, 798.2, 798.9 and

corresponding ICD-10 codes.[18] A validation study in the Netherlands compared the Dutch Hospital Discharge Diagnosis Database with a detailed clinical registry of CVD patients, showing a high sensitivity (72-84%) and positive predictive value (91-97%) of coronary heart disease and acute myocardial infarction.[19] The reliability of cause-of-death coding for major CVD events in Statistics Netherland is reportedly high (>90%).[20]

Data analysis

Since exclusion of participants with missing data would result in biased results and loss of precision,[21, 22] missing values for all determinants were multiple-imputed as 20 datasets, using the 'multivariate imputation by chained equations' method in the program R (version 2.15.0).[23] The imputation matrix consisted of the event indicator, the Nelson-Aalen estimate of cumulative hazard, and all covariates.[24, 25] After imputation, of the 6,368 participants who attended at least two of the three waves, we excluded 794 participants as follows: those who did not give informed consent for linkage with Statistics Netherlands data or the Dutch Hospital Discharge Diagnosis Database (N=92); all prevalent CVD cases at wave three (N=326); those who were censored before wave three (N=46) since the definition of long-term risk profiles was determined during the first three waves; those who did not participate in wave two and were therefore not linked with registry data for follow-up information on CVD (N=251); and participants for whom it was not possible to establish a linkage with registry data for other reasons (N=79). Thus, the analyses are based on data from 5,574 participants (3039 women and 2535 men). Of these, 0.3% had some missing exposure data in wave two and 22.4% had some missing data in wave three.

Age- and sex-adjusted CVD event rates per 10,000 person-years of follow-up were estimated across baseline and long-term risk categories. Cox proportional hazards regression models were fitted to estimate hazard ratios (HRs) and 95% confidence intervals (CIs) by baseline and long-term risk categories. None of the interaction terms between exposure and follow-up time was significant (P> 0.15), which indicates that the proportional hazards assumption was not violated. Results for men and women were the same (p-value for interaction terms > 0.55). We present age- and sex-adjusted analyses (model 1), and analyses adjusted for age, sex, attained education, occupation, cycling and/or sport activity, alcohol intake and Mediterranean diet score (model 2). Analyses of long-term risk profiles were adjusted for the same covariates as above, taking long-term exposure into account. That is, values were averaged over all waves, except for educational attainment, for which we used the highest level attained by wave three.

Table 5.2. Definition of long-term risk profiles.

	Wave 1	Wave 2	Wave 3
Long-term low risk profile,[b] N=384	Low risk[a]	Low risk	Low risk
Deteriorated low risk profile, N=318	Low risk	Any risk	Medium-low risk
	Low risk	Any risk	Medium-high risk
	Low risk	Any risk	High risk
Improved medium-low risk profile, N=67	Medium-low risk[a]	Any risk	Low risk
Long-term medium-low risk profile, N=697	Medium-low risk	Medium-low risk	Medium-low risk
Deteriorated medium-low risk profile, N=867	Medium-low risk	Any risk	Medium-high risk
	Medium-low risk	Any risk	high risk
Improved medium-high risk profile, N=385	Medium-high[a]	Any risk	Low risk
	Medium-high	Any risk	Medium-low risk
Long-term medium-high risk profile, N=844	Medium-high	Medium-high	Medium-high
Deteriorated medium-high risk profile, N=604	Medium-high	Any risk	High risk
Improved high risk profile, N=272	High risk[a]	Any risk	Low risk
	High risk	Any risk	Medium-low risk
	High risk	Any risk	Medium-high risk
Long-term high risk profile, N=549	High risk	High risk	High risk
Inconsistent risk profile, N=587	Low risk	Medium-high risk	Low risk
	Low risk	High risk	Low risk
	Medium-low risk	Low risk	Medium-low risk
	Medium-low risk	Medium-high risk	Medium-low risk
	Medium-low risk	High risk	Medium-low risk
	Medium-high risk	Low risk	Medium-high risk
	Medium-high risk	Medium-low risk	Medium-high risk
	Medium-high risk	High risk	Medium-high risk
	High risk	Low risk	High risk
	High risk	Medium-low risk	High risk
	High risk	Medium-high risk	High risk

[a]Low risk profile was defined as including all of the following: systolic blood pressure<120 mmHg, diastolic blood pressure<80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio<4.0, not taking cholesterol-lowering medication, body mass index<25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk at baseline were classified into three groups based on five risk factors: 1) systolic blood pressure≥140mm Hg, diastolic blood pressure≥90mm Hg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio≥6.0 or taking cholesterol-lowering medication; 3) body mass index_30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors; [b]'Long-term low risk' was defined as favourable levels of all risk factors (i.e. low risk) at all three waves, or low risk at two waves and medium-low risk at one wave (since only few participants were low risk at all three waves; N=154).

Table 5.3. Baseline characteristics of the Doetinchem Cohort Study (1987-1991) according to baseline risk profiles.[a]

	Total population	Low risk profile[a]	Medium-low risk profile[a]	Medium-high risk profile[a]	High risk profile[a]
Characteristic	(N=5,574)	(N=652)	(N=1,910)	(N=2,065)	(N=947)
Age (years), mean (SD)	40.1 (10.0)	36.0 (8.8)	39.8 (9.9)	40.1 (10.1)	43.5 (9.5)
Women (%)	3039 (55)	528 (81)	1025 (54)	1108 (54)	378 (40)
Educational attainment					
Low (%)	3444 (62)	313 (48)	1091 (57)	1342 (65)	698 (74)
Intermediate (%)	1219 (22)	194 (30)	438 (23)	431 (21)	157 (17)
High (%)	911 (16)	145 (22)	381 (20)	292 (14)	93 (10)
In the risk definition					
BMI (kg/m^2), mean (SD)	24.9 (3.5)	22.0 (1.7)	24.6 (2.5)	24.7 (3.4)	27.7 (4.3)
SBP (mm Hg), mean (SD)	121 (15)	107 (7)	119 (10)	122 (15)	132 (16)
DBP (mm Hg), mean (SD)	77 (10)	69 (6)	76 (7)	77 (11)	85 (11)
TC/HDL, mean (SD)	4.6 (1.6)	3.2 (0.5)	4.2 (0.9)	4.6 (1.4)	6.4 (1.9)
Currently smoking (%)	1851 (33)	0 (0)	0 (0)	1202 (58)	648 (68)
Diabetes mellitus (%)	21 (0.4)	0 (0)	0 (0)	9 (0.4)	12 (1.3)
Cycling and/or sport activity (hours/week), median (IQR) [b]	3 (1-6)	4 (2-7)	4 (2-6)	3 (1-6)	2 (0-5)
Mediterranean diet score (scale: 0-9), mean (SD) [b]	4.8 (1.6)	5.1 (1.6)	4.9 (1.6)	4.7 (1.5)	4.6 (1.6)
Alcohol intake (gr/day), median (IQR)	6 (0-14)	3 (0-9)	4 (0-11)	7 (0-16)	7 (0-20)

Abbreviations: SD, standard deviation; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL, total cholesterol/HDL-ratio; IQR, interquartile range; [a] Low risk profile was defined as including all of the following: systolic blood pressure<120 mmHg, diastolic blood pressure<80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio<4.0, not taking cholesterol-lowering medication, body mass index<25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk at baseline were classified into three groups based on five risk factors: 1) systolic blood pressure≥140 mmHg, diastolic blood pressure≥90 mmHg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio≥6.0 or taking cholesterol-lowering medication; 3) body mass index≥30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors; [b] Not assessed at baseline. Therefore, values of wave two are shown.

Results

The average age at baseline was 40.1 (range 20-59) years. At baseline, 12% of the participants were low risk, 34% were medium-low risk, 37% medium-high risk, and 17% high risk (Table 5.3). Women, younger participants, and participants with higher educational attainment were more likely to have more favourable baseline risk profiles (Table 5.3). During follow-up, about 7% (N=384) of the total study population maintained low risk at any two or all three examinations (i.e. long-term low risk) and about 10% (N=549) maintained a high risk profile

at all examinations (i.e. long-term high risk). In addition, about 32% (N=1,789) of the cohort experienced deterioration in risk profile, whereas 13% (N=724) experienced improvement in risk profile.

Table 5.4. Event rates, hazard ratio, and 95% confidence interval of incident total cardiovascular disease by baseline risk profiles.[a]

	Person-years of follow-up	No. of events	Age- and sex-adjusted event rate (/10,000 person-years)	Model 1[b]	Model 2[c]
Low risk profile[a]	4847	14	27	0.30 (0.17-0.52)	0.32 (0.18-0.57)
Medium-low risk profile[a]	13962	88	38	0.42 (0.32-0.55)	0.43 (0.33-0.58)
Medium-high risk profile[a]	14796	126	47	0.55 (0.43-0.71)	0.56 (0.44-0.72)
High risk profile[a]	6449	124	82	1.00 (ref)	1.00 (ref)

[a] Low risk profile was defined as including all of the following: systolic blood pressure<120 mmHg, diastolic blood pressure<80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio<4.0, not taking cholesterol-lowering medication, body mass index<25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk at baseline were classified into three groups based on five risk factors: 1) systolic blood pressure≥140 mmHg, diastolic blood pressure90 mmHg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio≥6.0 or taking cholesterol-lowering medication; 3) body mass index≥30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors; [b] Model 1: age- and sex-adjusted analyses; [c] Model 2: analyses adjusted for age at baseline, sex, attained education, occupation, cycling and/or sport activity, alcohol intake, and Mediterranean diet score.; ref.: reference.

Over an average of 7.2 years of follow-up, there were 204 coronary heart disease events, 64 strokes, and 84 other cardiovascular events (about two-thirds were peripheral vascular disease cases, one-quarter heart failure and one-tenth pulmonary embolism and infarction cases), for a total of 352 CVD events (40 fatal and 312 non-fatal). Absolute rates of CVD events per 10,000 person-years were lower with more favourable baseline and long-term risk profiles (Table 5.4 and Table 5.5). Figure 5.1 shows the HRs of (a) baseline and (b) long-term risk profiles for incident CVD. Compared with participants with a high risk profile at baseline, risk of CVD was three times lower among participants with a low risk profile at baseline (HR: 0.32, 95%CI: 0.18-0.57). It was 2.3 and 1.8 times lower among those with medium-low and medium-high risk profiles at baseline respectively (Figure 5.1(a) and Table 5.4).

Adults with long-term low risk profile had seven times lower risk of CVD compared with adults with long-term high risk profile (HR: 0.14, 95%CI: 0.05-0.41), while adults who were low risk at baseline but developed adverse risk profile over an 11-year period had less than 3 times lower risk of CVD (HR: 0.36, 95%CI: 0.18-0.71) (Figure 5.1b and Table 5.5). Only 15 CVD events per 10,000 person-years occurred among the former group, while 36 CVD events per

10,000 person-years occurred among the latter group. Our results suggest that, within each baseline risk profile group, compared with a stable profile, improvement in risk profiles may be associated with up to twofold lower HRs, and deteriorating profiles with an approximate twofold increase in risk.

Discussion

Participants who maintained a long-term low risk profile over a period of 11 years had much lower CVD risk than those who were low risk at only one point in time. Thus, participants who maintained a long-term low risk profile had seven times lower risk of CVD compared with those who maintained a long-term high risk profile, whereas participants who were low risk at baseline but did not maintain that status, had only a three times lower risk of CVD. On the other hand, adults with an unfavourable risk profile could gain large benefits by improving their risk profile during follow-up. Our analyses suggest that persons at unfavourable risk who improve their risk profile over 11 years may experience up to twofold lower HRs of incident CVD compared with those who maintain the unfavourable risk profile over time. Similarly, our results suggest that deterioration of baseline risk profiles may be associated with up to twofold higher risk of CVD compared with maintenance of the initial risk profile.

Prevention and control of CVD requires a focus on total risk profiles, including all major risk factors examined in the present study, and not on single factors.[26-28] Each major risk factors is interrelated with the others and stems from similar lifestyle behaviours.[5] Therefore, the present study investigated risk profiles and their changes in relation to CVD. Our findings of associations between baseline risk profiles and CVD are consistent with the majority of previous studies.[7, 8, 10] However, some studies observed no difference in associations between low and medium-low (unfavourable) risk profiles and CVD incidence.[8, 11] The present study showed a clear gradient, suggesting that the risk of CVD is indeed lower among adults with low risk profile compared with adults with medium-low risk profile. Likewise, a multicentre cohort study in Italy and the Atherosclerosis Risk in Communities Study in the USA found a clear gradient of event rates of coronary heart disease and stroke related to the number of risk factors.[10, 13] Thus, for effective CVD prevention, it is essential to aim for low-risk status, that is, simultaneous attainment of favourable levels of all major risk factors by adoption of healthy lifestyles, rather than simply the absence of adverse risk factors (which includes medium-low risk status).

To our knowledge, the association of long-term exposure to combinations of several major risk factors with subsequent incidence of fatal and non-fatal CVD has not been investigated. Taking long-term risk profiles into account, our analyses demonstrate greater benefits of a low risk profile that is maintained over a longer period of time compared with low risk at baseline only. These findings are readily explained by the fact that a substantial

proportion of participants (45%) experienced deterioration in risk profiles over an 11-year period, with a sizeable impact on their CVD risk. In addition, our results suggest that adults whose risk profile improved over time had lower CVD risks, comparable in magnitude to those with the next most favourable long-term risk profile. That is, participants with medium-high risk profile at baseline who improved over time attained HRs similar to those who maintained a medium-low risk profile. Thus, improvement of risk profiles in adulthood is of great importance. Given the current low prevalence of people at low risk, large gains in cardiovascular health can theoretically be achieved.

A large proportion of adults who were low risk in young adulthood did not maintain their favourable risk status over time. Similar findings were observed in the US CARDIA study, where only about half of participants aged 18-30 years who were low risk at baseline maintained their low risk status during 20 years of follow-up.[5] The low prevalence of long-term low risk profile among adults likely results from unhealthy lifestyles such as smoking, poor eating habits and lack of physical activity.[5] This finding underscores the importance of a national public health policy that stresses prevention and control of all major CVD risk factors by improving lifestyles starting from young adulthood onwards. Increasing the proportion of the population with a long-term low risk profile and encouraging improvement in all risk areas is crucial for improvement of cardiovascular health.

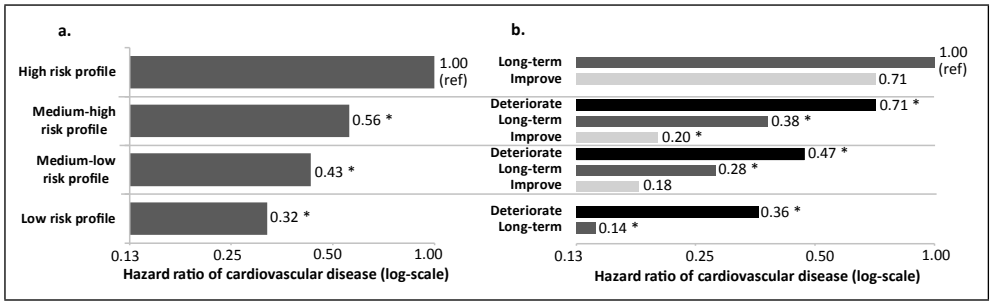

Figure 5.1. Multivariable-adjusted hazard ratios of incident total cardiovascular disease. For (a)[a] baseline risk profiles and (b) long-term risk profiles including adults who maintained that same risk profile (grey),[b] improved (light grey)[c] or deteriorated (black)[d] during follow-up.
An asterisk (*) indicates a statistically significant difference from (long-term) high risk profile, p<0.05.
[a]Low risk profile was defined as including all of the following: systolic blood pressure<120 mmHg, diastolic blood pressure<80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio<4.0, not taking cholesterol-lowering medication, body mass index<25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk at baseline were classified into three groups based on five risk factors: 1) systolic blood pressure≥140 mmHg, diastolic blood pressure≥90 mmHg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio≥6.0 or taking cholesterol-lowering medication; 3) body mass index≥30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors; [b] Long-term: risk profile remains steady at all measurements over 11 years; [c] Improve: risk profile improved from baseline over 11 years; [d] Deteriorate: risk profile deteriorated from baseline over 11 years.

Table 5.5. Event rates, hazard ratio, and 95% confidence interval of incident total cardiovascular disease by long-term risk profiles.[abcd]

	Low risk profile[a]			**Medium-low risk profile**[a]			**Medium-high risk profile**[a]			**High risk profile**[a]
Characteristic	**Long-term**[b] (N=384)	Deteriorate[c] (N=318)	Improve[d] (N=67)	**Long-term**[b] (N=697)	Deteriorate[c] (N=867)	Improve[d] (N=385)	**Long-term**[b] (N=844)	Deteriorate[c] (N=604)	Improve[d] (N=272)	**Long-term**[b] (N=549)
Person-years of follow-up	2851	2366	495	5096	6328	2823	6048	4286	1892	3677
No. of events	4	10	1	23	55	9	37	62	27	85
Age- and sex-adjusted event rate (/10,000 person-years)	15	36	18	28	49	20	39	73	68	95
Model 1[e]	0.14 (0.05-0.39)	0.34 (0.17-0.66)	0.17 (0.02-1.23)	0.27 (0.16-0.43)	0.46 (0.32-0.65)	0.19 (0.09-0.41)	0.37 (0.25-0.56)	0.71 (0.50-1.00)	0.69 (0.44-1.10)	1.00 (ref)
Model 2[f]	0.14 (0.05-0.41)	0.36 (0.18-0.71)	0.18 (0.02-1.28)	0.28 (0.17-0.45)	0.47 (0.33-0.67)	0.20 (0.09-0.43)	0.38 (0.25-0.58)	0.71 (0.50-1.00)	0.71 (0.44-1.13)	1.00 (ref)

Participants with an inconsistent varying long-term risk profile (N=587) had a 46% lower risk of cardiovascular disease compared with those with a long-term high risk profile (hazard ratio: 0.54, 95% confidence interval: 0.37–0.82). For simplicity, not presented in the table; [a] Low risk profile was defined as including all of the following: systolic blood pressure<120 mmHg, diastolic blood pressure<80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio<4.0, not taking cholesterol-lowering medication, body mass index<25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk at baseline were classified into three groups based on five risk factors: 1) systolic blood pressure≥140 mmHg, diastolic blood pressure≥90 mmHg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio≥6.0 or taking cholesterol-lowering medication; 3) body mass index≥30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors; [b] Long-term: risk profile remains steady at all measurements over 11 years; [c] Deteriorate: risk profile deteriorated from baseline over 11 years; [d] Improve: risk profile improved from baseline over 11 years; [e] Model 1: age- and sex-adjusted analyses; [f] Model 2: analyses adjusted for age at baseline, sex, attained education, occupation, cycling and/or sport activity, alcohol intake and Mediterranean diet score.

The strength of the present study is that extensive information about all major risk factors and other lifestyle factors was objectively obtained at three points in time over a long period of follow-up, with a consistent group of trained study personnel using standardized protocols and instruments. Therefore, the long-term exposure was consistently assessed and the analyses could be adjusted for many relevant covariates. Limitations of the present study include the small numbers of low-risk participants, especially low-risk men, which reflects the rarity of maintaining a low risk profile. Nevertheless, the low-risk subgroup in this cohort was relatively larger than that in studies from other countries.[6,7,9-11,13] Given the small number of low-risk participants at all three waves, our long-term low risk group included persons who were at low risk during two of the three waves (and not medium-high or high risk at any wave). CVD incidence among the long-term low risk group would likely have been even lower than what is reported here if this group had comprised only participants who were low risk at all three waves without inclusion of those who were low risk in at least two waves and medium-low risk at the third. Thus, our estimates of the risk difference between the lowest and highest risk groups may be somewhat conservative. Moreover, participants with a CVD event between waves one and three (N=175) were excluded in order to define the long-term risk profiles. Thus, adults with the highest CVD risk were not included in the analyses. In addition, another 27% of those examined at baseline were not included due to exclusion of those with a history of CVD at baseline, drop-out and unavailability of data on CVD morbidity and mortality. At baseline, these participants had worse levels of the CVD risk factors studied. Underestimation of the high risk group may further have led to underestimation of our results since the true risk difference between the lowest and highest category was mitigated. Finally, individuals who participate in cohort studies are generally healthier and better educated than non-participants. This potential selection bias may have resulted in a higher prevalence of low risk among study participants compared with the general population.[29]

To the best of our knowledge, this is the first study to show the large benefits of maintaining a low cardiovascular risk profile over 11 years. Furthermore, our results suggest that improvement in unfavourable risk profiles over time may be associated with up to twofold lower risk of CVD than with maintenance of the initial risk profile. These findings underscore the importance of efforts to achieve and maintain low risk from young adulthood onwards. The current low prevalence of low risk offers the potential for substantial improvements in cardiovascular health. Consequently, future research should establish which factors are associated with improvements in risk profiles and with maintaining a low risk profile over time.

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Supplementary Methods

Description of the measurements

Body weight and height were measured wearing light indoor clothing with emptied pockets and without shoes. Body weight was measured to the nearest 0.1 kg on calibrated scales and height to the nearest 0.5 cm. Body mass index was calculated as weight minus 1 kg to adjust for clothing, divided by height squared (kg/m^2). At each examination, systolic and diastolic blood pressure levels were measured twice after 2 minutes of rest with participants in a seated position. The average of these two measurements was used in the analyses. Total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory, using standardized enzymatic methods. The TC/HDL-ratio was calculated by dividing the TC level by the HDL cholesterol value. Diabetes was defined based on self-reported history and/or non-fasting blood glucose concentration of 11.1 mmol/L or more.[1] All cases were also verified using information from the participant's general practitioner or pharmacist.[2] Educational attainment was categorized as low (intermediate secondary education or less; i.e. about <10 years education), intermediate (intermediate vocational or higher secondary education; i.e. about 11-14 years education), and high (higher vocational education or university; i.e. about >15 years education). Occupation was categorized as: currently employed, homemaker, or unemployed/retired/unfit for work. Recreational sports activity and cycling has previously observed to be more strongly associated with CVD in this cohort than other general physical activities (such as walking and gardening) and was therefore included here as a covariate (dichotomized <3.5 or ≥3.5 hour/week of cycling and/or sport activity).[3] Self-reported weekly alcohol intake was categorized as: no alcohol consumption; 0-10 gram/day for women and 0-20 gram/day for men; or ≥10 gram/day for women and ≥20 gram/day for men. Dietary history was ascertained using a validated 178-item semi-quantitative food frequency questionnaire; a healthy diet was operationalized with the 9-scale modified Mediterranean diet score defined by Trichopoulou et al.,[4] This score assigned values of 0 to 1 to each nutritional component (i.e. vegetables, fruits, legumes and nuts, grains, fish and seafood, meat products, alcohol, fatty acid ratio, and dairy products) and was dichotomized at the median for this cohort.

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Chapter 6

Determinants of attaining and maintaining a low cardiovascular risk profile

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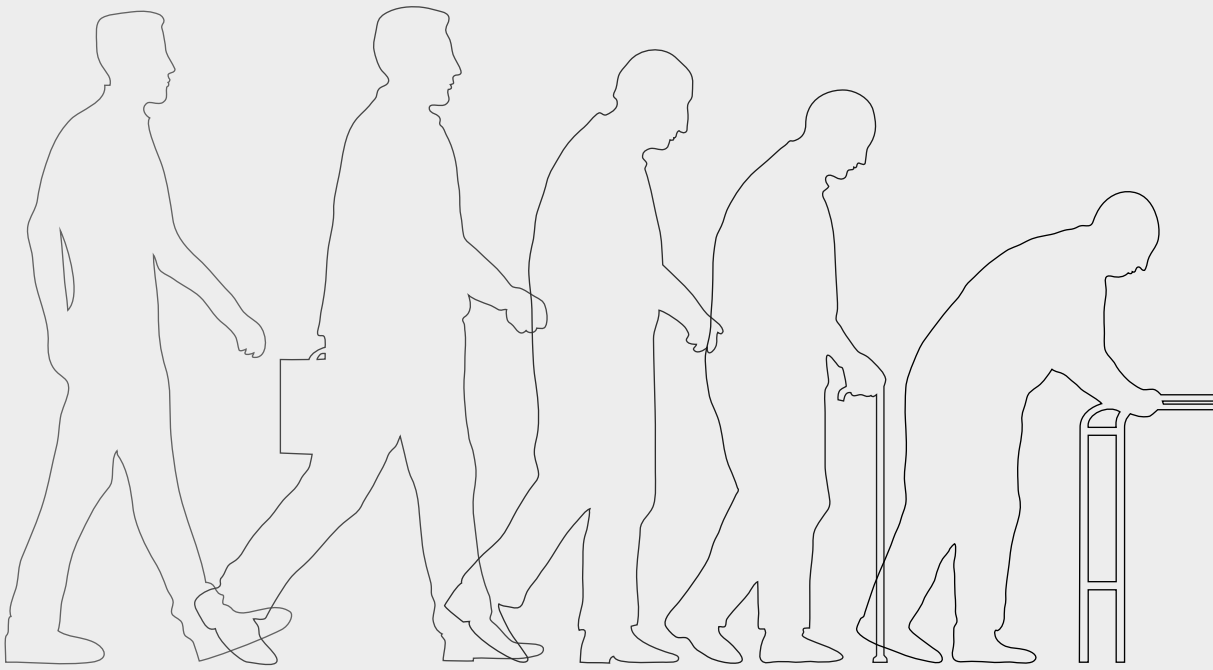

Abstract

Background: While maintenance of a low cardiovascular risk profile is essential for cardiovascular disease (CVD) prevention, few people maintain a low CVD risk profile throughout their life. We studied the association of demographic, lifestyle, psychological factors and family history of CVD with attainment and maintenance of a low risk profile over three subsequent 5-year periods.

Methods: Measurements of 6,390 adults aged 26-65 years at baseline were completed from 1993-1997 and subsequently at 5-year intervals until 2013. At each wave, participants were categorised into low risk profile (ideal levels of blood pressure, cholesterol, and body mass index, non-smoking, and no diabetes) and medium/high risk profile (all others). Multivariable-adjusted modified Poisson regression analyses were used to examine determinants of attainment and maintenance of low risk; risk ratios (RR) and 95% confidence intervals (95%CI) were obtained. Generalized estimating equations were used to combine multiple 5-year comparisons.

Results: Younger age, female gender, and high educational level were associated with higher likelihood of both maintaining and attaining low risk profile ($P<0.05$). In addition, likelihood of attaining low risk was 9% higher with each 1-unit increment in Mediterranean diet score (RR: 1.09, 95%CI: 1.02-1.16), twice as high with any physical activity versus none (RR: 2.17, 95%CI: 1.16-4.04), and 35% higher with moderate alcohol consumption versus heavy consumption (RR: 1.35, 95%CI: 1.06-1.73).

Conclusion: Healthy lifestyle factors such as adherence to a Mediterranean diet, physical activity and moderate as opposed to heavy alcohol consumption were associated with a higher likelihood of attaining a low risk profile.

Introduction

Maintenance of a low cardiovascular risk profile (i.e. ideal levels of blood pressure, cholesterol, and body mass index (BMI), non-smoking, and no diabetes) is essential for the effective prevention of cardiovascular disease (CVD). Several studies have demonstrated the benefits of a low risk profile, measured at a single point in time, in relation to the risk of coronary heart disease, stroke, and total CVD.[1-6] Recently, we indicated that adults who maintained a low risk profile over a period of 11 years had a 2.5 times lower risk of CVD when compared with adults who were low risk at baseline but deteriorated over time, emphasizing the importance of adults keeping their low risk status.[7]

Unfortunately, most adults 'lose' their low risk status during young adulthood or middle age, and for those not at low risk the likelihood of attaining a low risk profile is very low.[7-9] Little is known about determinants that influence the likelihood of losing and achieving this low risk status. Identification of modifiable factors associated with maintaining and achieving low risk is necessary for the development of effective preventive strategies to increase the proportion of adults with a low risk profile. In addition, it is important to characterize groups that face a higher likelihood of losing their low risk status and who may benefit from earlier and/or more intensive interventions. This study investigated the association of lifestyle, demographic, and psychosocial factors, history of CVD, and family history of diabetes and myocardial infarction with (1) maintaining a low risk profile versus losing a low risk status and (2) attaining a low risk profile versus remaining medium/high risk profile.

Methods

Population

The Doetinchem Cohort Study is an ongoing study which involves an age- and sex-stratified random sample of men and women aged 20-59 years in 1987-1991, drawn from the civil registries of Doetinchem, the Netherlands. From 1987-1991 (wave one), 20,155 men and women were invited to undergo a clinical examination, of whom 62% (N=12,405) participated. Of these, a two-third random sample of 7,768 participants was re-invited to be examined after a 6 year-interval in 1993-1997 (wave two, N=6,117), and subsequently at 5-year intervals in 1998-2002 (wave 3, N=4,918), 2003-2007 (wave 4, N=4,520), and 2008-2012 (wave 5, N=4,018). Details are described elsewhere.[10] As of the second wave, extensive information on diet, physical activity, and psychosocial factors were gathered. Therefore, data from waves 2-5 were used for the present study and wave 2 was considered to be the baseline examination. This resulted in 6,390 participants who attended at least one examination between waves 2-5.

Measures

Weight, height, diastolic and systolic blood pressure, total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, and blood glucose were measured by trained staff according to standardised protocols. Mean diastolic and systolic blood pressure levels measured at wave 4 were unexpectedly higher compared to the blood pressure values in the other waves. No causes could be identified: therefore the blood pressure values at wave 4 were statistically corrected. This is extensively described in Supplementary Methods. Lifestyle factors, demographic characteristics, psychosocial factors and medical history were collected with standardised questionnaires completed by the participants. Details of these measurements have been described elsewhere[10] and in Supplementary Methods.

Determinants

All determinants were assessed at waves 2-4. Educational status of the participant and his/her partner (if any) were categorised as low (intermediate secondary education or less), intermediate (intermediate vocational or higher secondary education) and high (higher vocational education or university). Occupation was categorised as currently employed, homemaker, or unemployed/retired/unfit for work. Marital status was categorised as married/civil union, unmarried, and widow/divorced/other. Self-reported weekly alcohol intake was categorised according to recommendations of the European guidelines on CVD prevention as no alcohol consumption, moderate alcohol consumption (1-10 g/day for women and 1-20 g/day for men) or heavy alcohol consumption (>10 g/day for women and >20 g/day for men).[11] Sleep duration was assessed as self-reported usual duration of sleep per 24-hour period and was categorised as short (≤6 h/day), intermediate (7-8 h/day) and long (≥9 h/day). Subjects were categorised into four groups according to the amount of physical activity performed at work and for recreational purposes using the validated index of Wareham et al.: inactive, low active, intermediate active, and high active.[12] A healthy diet was operationalised with an 8-scale modified Mediterranean diet score[13] that assigned values of 0 to 1 to eight nutritional components. Details are available in Supplementary Methods. All four psychosocial domains of the Dutch version of the RAND-36 were used to obtain scores for vitality, mental health, social role functioning, and emotional role functioning.[14, 15] Scores are summed and transformed to a 0-5 scale, with higher scores indicating better psychosocial wellbeing.

Definition of risk profile

At each wave, participants were categorised into low risk profile and medium/high risk profile (i.e. all others). In accordance with our previous work,[7] similar to other studies[1-6] and recent recommendations,[16] a low risk profile was defined as untreated systolic/diastolic blood pressure <120/<80 mmHg, untreated TC/HDL cholesterol ratio <4.0, BMI <25 kg/

m^2, currently non-smoking, and no diabetes. Diabetes was defined based on self-reported history (i.e. response to the question, "Do you have diabetes?" Yes/no) and/or a random glucose concentrations of ≥11.1 mmol/L. All other participants, i.e. those with intermediate or high levels of risk factors, were defined as having a 'medium/high risk profile'. Figure 6.1 schematically shows how four 5-year risk profiles were constructed: (1) 'maintained low risk profile': low risk two waves consecutively; (2) 'lost low risk status': low risk at one wave and medium/high risk at the following wave; (3) 'attained low risk profile': medium/high risk at one wave and low risk at the following wave and (4) 'remained medium/high risk profile': medium/high risk two waves consecutively.

	Low risk at wave$_x$ [a,b]	Not low risk at wave$_x$
Low risk at wave$_{x+1}$	**1)'Maintained low risk profile'**	**3)'Attained low risk profile'**
Not low risk at wave$_{x+1}$	2)'Lost low risk status'	4)'Remained medium/high risk profile'

Figure 6.1. Schematic representation of the four 5-year risk profiles.
[a] Low risk profile was defined as including all of the following: systolic blood pressure <120 mmHg, diastolic blood pressure <80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio <4.0, not taking cholesterol-lowering medication, body mass index <25 kg/m^2, not smoking, and no history of diabetes. People who were not low risk at baseline were classified as having a medium/high risk profile; [b] Wave$_x$: Wave 2, 3, or 4.

Data analyses

Since the exclusion of participants with missing data may lead to biased results and loss of precision,[17, 18] missing values were multiple-imputed as 20 datasets, using the 'multivariate imputation by chained equations' method in R (version 3.0.0).[19] Of the total population (N=6,390), 4%, 23%, 29% and 37% had missing data in wave 2, 3, 4 and 5 respectively. Missing outcome data were multiple-imputed since this may be superior over exclusion of the data due to high correlations between the outcome variables at consecutive waves.[20, 21] The transition from low risk to medium/high risk, and vice versa was determined over three consecutive 5-year periods, i.e. waves 2-3, waves 3-4 and waves 4-5. If participants did not participate in both waves at each end of a 5-year period, we excluded the multiple-imputed data of that period, excluding 2,813 of the 19,170 observations from the analyses.

A Poisson regression model using a sandwich variance estimator was used to obtain risk ratios (RRs) and 95% confidence intervals (CIs) adjusted for clustering for the associations of lifestyle, demographic and psychosocial factors, history of CVD and family history of diabetes and myocardial infarction with maintaining versus losing low risk profile and attaining low risk profile versus remaining medium/high risk profile (Figure 6.1). This method has been

developed for longitudinal studies with correlated binary outcomes.[22] To combine the three 5-year periods and take the correlations amongst repeated observations on the same participants into account, generalized estimating equations with exchangeable structure were performed. When analysing changes over a 5-year period, covariates measured at the beginning of that 5-year period were used. Analyses were adjusted for age and sex in model 1, and additionally for occupational status, marital status, attained education, history of CVD, Mediterranean diet score, physical activity, alcohol consumption, and sleep duration in model 2. Psychosocial factors were not adjusted for lifestyle factors, which are potential mediators in these associations. SAS software version 9.3 was used to perform all analyses. The analyses on determinants of maintaining a low risk profile were based on 852 participants who had a low risk profile at any of waves 2-4, resulting in 1,325 measurements. The analyses on determinants of attaining a low risk profile were based on 6,184 participants with 15,032 measurements who had a medium/high risk profile at any of waves 2-4.

As a sensitivity analysis, we categorised participants based on their cholesterol and blood pressure levels irrespective of the use of antihypertensive or cholesterol-lowering medication, because medication does lower cardiovascular risk although not to the extent of lifelong naturally low level.[23,24] Compared with the main analyses, in sensitivity analyses few participants (N≤26 each wave) were reclassified from medium/high to low risk profile and results were the same, and therefore not shown.

Results

The average age from 1993-1997 (wave 2) was 46.3 (range 26-65) years (Table 6.1). Participants with a low risk profile at baseline were more often women, younger, higher educated and had more favourable values for metabolic and lifestyle factors than those with a medium/high risk profile.

Table 6.1. Baseline characteristics (1993-1997) by risk status.

	Total population	Low risk profile[a]	Medium/high risk profile[b]
	n=6,368	n=601	n=5,767
Demographic factors			
Age (years), mean (SD)	46.3 (10.1)	41.0 (8.8)	46.9 (10.1)
Sex (women)	3,383 (53%)	483 (80%)	2,900 (50%)
Education (low)	3,597 (56%)	245 (41%)	3,352 (58%)
Occupation (employed)	3,871 (61%)	405 (67%)	3,466 (60%)
Civil status (married)	5,219 (82%)	487 (81%)	4,732 (82%)
In the risk definition			
BMI (kg/m^2), mean (SD)	25.9 (3.8)	22.4 (1.6)	26.2 (3.8)
SBP (mmHg), mean (SD)	125 (17)	108 (7)	127 (16)
DBP (mmHg), mean (SD)	80 (11)	70 (6)	81 (11)
TC/HDL, mean (SD)	4.3 (1.5)	3.0 (0.5)	4.5 (1.5)
Currently smoking	1,981 (31%)	0 (0%)	1,981 (34%)
Diabetes mellitus	106 (1.7%)	0 (0.0%)	106 (1.8%)
Lifestyle factors			
Physical activity (inactive)	730 (11%)	29 (5%)	702 (12%)
MDS (scale: 0-8), mean (SD)	4.0 (1.5)	4.2 (1.6)	4.0 (1.5)
Alcohol intake (gr/day), median (IQR)	6 (0-16)	3 (0-10)	6 (0-17)
Sleep duration, (≤6 hours/ day)	1,011 (16%)	79 (13%)	933 (16%)

Abbreviations: SD, standard deviation; IQR, Interquartile range; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC/HDL, total cholesterol/HDL-ratio, MDS, Mediterranean diet score; CVD, cardiovascular disease; MI, myocardial infarction; DM, diabetes mellitus; [a] Low risk profile was defined as including all of the following: systolic blood pressure <120 mmHg, diastolic blood pressure <80 mmHg, not taking antihypertensive medication, total cholesterol/HDL-ratio <4.0, not taking cholesterol-lowering medication, body mass index <25 kg/m^2, not smoking, and no history of diabetes; [b] Persons not low risk at baseline were classified as medium/high risk profile.

Factors associated with maintaining low risk profile

Of those having a low risk profile at any given wave, only 43% maintained that low risk profile, while 57% lost their low risk status by the following wave. Of those who were low risk at baseline, only 18% still had a low risk profile after 15 years of follow-up.

In multivariable adjusted analysis, age, gender, and education were the only factors significantly associated with maintaining a low risk profile ($p<0.05$) (Table 6.2); adults with high levels of education were 29% (RR: 1.29, 95%CI: 1.03-1.61) more likely to maintain a low risk profile compared with adults with low educational attainment. Women had a 38% higher likelihood of maintaining a low risk profile compared with men (RR: 1.38, 95%CI: 1.07-1.79) and with every 10-year increase in age, the likelihood of maintaining a low risk profile decreased by 26% (RR: 0.74, 95%CI: 0.65-0.84).

Table 6.2. Determinants of maintaining a low risk profile (maintained low risk profile versus lose low risk profile).

	Model 1[a]	Model 2[b]
	RR (95%CI)	**RR (95%CI)**
Demographic factors		
Age (per 10 years)	**0.74 (0.67-0.83)**	**0.74 (0.65-0.84)**
Sex (women)	**1.37 (1.07-1.75)**	**1.38 (1.07-1.79)**
Education attainment		
Low	ref (1.00)	ref (1.00)
Intermediate	1.12 (0.91-1.38)	1.12 (0.91-1.39)
High	**1.29 (1.04-1.59)**	**1.29 (1.03-1.61)**
Education attainment partner		
Low	ref (1.00)	ref (1.00)
Intermediate	1.10 (0.87-1.40)	1.07 (0.83-1.37)
High	1.24 (0.98-1.56)	1.13 (0.86-1.49)
Occupation		
Employed	ref (1.00)	ref (1.00)
Homemaker	1.01 (0.80-1.27)	1.01 (0.79-1.27)
Other	1.01 (0.71-1.45)	1.00 (0.70-1.43)
Civil status		
Married/civil union	ref (1.00)	ref (1.00)
Unmarried	0.97 (0.76-1.26)	0.97 (0.75-1.25)
Widow/divorced/other	1.01 (0.71-1.44)	0.98 (0.68-1.40)
Lifestyle factors		
MDS (per unit increase)	1.01 (0.96-1.07)	1.01 (0.95-1.07)
Physical activity		
Inactive	ref (1.00)	ref (1.00)
Low active	0.94 (0.63-1.41)	0.94 (0.62-1.43)
Intermediate active	0.85 (0.57-1.29)	0.86 (0.56-1.32)
High active	0.87 (0.59-1.28)	0.88 (0.59-1.32)
Alcohol intake		
None	1.10 (0.86-1.40)	1.12 (0.88-1.43)
Moderate[c]	1.10 (0.86-1.42)	1.10 (0.86-1.41)
Heavy[c]	Ref (1.00)	Ref (1.00)
Sleep duration		
Short (≤6 hours/ day)	Ref (1.00)	Ref (1.00)
Intermediate (7-8 hours/ day)	0.92 (0.73-1.15)	0.92 (0.74-1.15)
Long (≥9 hours/ day)	0.91 (0.58-1.44)	0.93 (0.58-1.48)
History		
CVD history	0.76 (0.16-3.73)	0.77 (0.15-3.95)
Parental history MI	0.94 (0.76-1.15)	0.96 (0.78-1.18)
Parental history DM	0.82 (0.63-1.07)	0.82 (0.63-1.07)
Psychosocial factors (range: 0-5)		
Mental health	1.11 (0.98-1.27)	1.11 (0.97-1.26)
Vitality	1.04 (0.93-1.16)	1.04 (0.93-1.16)
Social role functioning	1.00 (0.92-1.09)	1.00 (0.92-1.09)
Emotional role functioning	1.02 (0.96-1.09)	1.02 (0.96-1.09)

Abbreviations: 95%CI, 95% confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; MDS, Mediterranean diet score; MI, myocardial infarction; RR, risk ratio. Significant associations (at P<0.05) are printed in bold; [a] Model 1: adjusted for age and gender; [b] Model 2: adjusted for age, gender educational attainment, occupation, civil status, history of cardiovascular disease, and all lifestyle factors. Psychosocial factors were not adjusted for lifestyle factors since lifestyle factors might be an intermediate between the relation with change in risk profile; [c] Moderate alcohol intake: 1-10 gr/day for women and 1-20 gr/day for men; heavy alcohol intake: >10 gr/day for women and >20 gr/day for men.

Table 6.3. Determinants of attaining a low risk profile (attained low risk profile versus remained medium/high risk profile).

	Model 1[a]	Model 2[b]
	RR (95%CI)	**RR (95%CI)**
Demographic factors		
Age (per 10 years)	**0.57 (0.52-0.62)**	**0.58 (0.52-0.65)**
Sex (women)	**2.66 (2.12-3.33)**	**3.06 (2.38-3.93)**
Education attainment		
Low	ref (1.00)	ref (1.00)
Intermediate	**1.48 (1.16-1.89)**	**1.37 (1.07-1.75)**
High	**2.39 (1.87-3.04)**	**2.11 (1.63-2.74)**
Education attainment partner		
Low	ref (1.00)	ref (1.00)
Intermediate	**1.39 (1.07-2.80)**	1.19 (0.89-1.59)
High	**2.41 (1.85-3.12)**	**1.71 (1.24-3.35)**
Occupation		
Employed	ref (1.00)	ref (1.00)
Homemaker	**0.64 (0.48-0.86)**	0.80 (0.59-1.07)
Other	0.89 (0.66-1.21)	1.05 (0.77-1.42)
Civil status		
Married/civil union	ref (1.00)	ref (1.00)
Unmarried	1.00 (0.72-1.39)	0.95 (0.68-1.32)
Widow/divorced/other	1.09 (0.76-1.56)	1.12 (0.78-1.60)
Lifestyle factors		
MDS (per unit increase)	**1.13 (1.06-1.21)**	**1.09 (1.02-1.16)**
Physical activity		
Inactive	ref (1.00)	ref (1.00)
Low active	**2.40 (1.28-4.47)**	**2.17 (1.16-4.04)**
Intermediate active	**3.65 (1.41-4.97)**	**2.35 (1.25-4.43)**
High active	**2.32 (1.27-4.26)**	**2.16 (1.17-3.98)**
Alcohol intake		
None	0.92 (0.72-1.19)	1.10 (0.85-1.42)
Moderate[c]	**1.31 (1.03-1.68)**	**1.35 (1.06-1.73)**
Heavy[c]	Ref (1.00)	Ref (1.00)
Sleep duration		
Short (≤6 hours/ day)	Ref (1.00)	Ref (1.00)
Intermediate (7-8 hours/ day)	1.25 (0.94-1.67)	1.18 (0.89-1.58)
Long (≥9 hours/ day)	1.02 (0.61-1.73)	1.08 (0.64-1.83)
History		
CVD history[d]	-	-
Parental history MI	0.81 (0.66-1.01)	0.84 (0.67-1.04)
Parental history DM	0.80 (0.62-1.03)	0.83 (0.64-1.07)
Psychosocial factors (range: 0-5)		
Mental health	**1.16 (1.01-1.34)**	1.13 (0.98-1.30)
Vitality	1.10 (0.97-1.24)	1.09 (0.96-1.24)
Social role functioning	1.06 (0.95-1.19)	1.05 (0.94-1.18)
Emotional role functioning	1.06 (0.99-1.13)	1.05 (0.98-1.12)

Abbreviations: 95%CI, 95% confidence interval; CVD, cardiovascular disease; DM, diabetes mellitus; MDS, Mediterranean diet score; MI, myocardial infarction; RR, risk ratio. Significant associations (at P<0.05) are printed in bold; [a] Model 1: adjusted for age and gender; [b] Model 2: adjusted for age, gender educational attainment, occupation, civil status, history of cardiovascular disease, and all lifestyle factors. Psychosocial factors were not adjusted for lifestyle factors since lifestyle factors might be an intermediate between the relation with change in risk profile; [c] Moderate alcohol intake: 1-10 gr/day for women and 1-20 gr/day for men; heavy alcohol intake: >10 gr/day for women and >20 gr/ day for men; [d] Model did not converge since no persons with history of CVD attained low risk profile.

Factors associated with attaining low risk profile

Of those having medium/high risk profile at any wave, 97% remained at medium/high risk and only 3% attained a low risk profile by the following wave. After 15 years of follow-up, 95% of those who were medium/high risk at baseline still had a medium/high risk profile.

Among those at medium/high risk at any wave, a higher educational level was associated with a higher likelihood of attaining a low risk profile by the subsequent wave (Table 6.3). Independent of individual educational attainment, having a highly educated partner also increased the likelihood of attaining a low risk profile by 71% (RR: 1.71, 95%CI: 1.24-3.35). Each 10-year increase in age was associated with 42% lower likelihood of attaining low risk profile (RR: 0.58, 95%CI: 0.52-0.65) and women were three times more likely to attain a low risk profile than men (RR: 3.06, 95%CI: 2.38-3.93).

A healthy diet, any amount of physical activity and moderate alcohol consumption were associated with improvements in risk profiles. Each 1-unit increment in the Mediterranean diet score was associated with a 9% higher likelihood of attaining a low risk profile versus remaining at medium/high risk (RR: 1.09, 95%CI: 1.02-1.16) (Table 6.3). Any physical activity --low, intermediate, and high physical activity-- was associated with a more than twofold higher likelihood of attaining a low risk profile compared with being physically inactive ($P<0.05$). Participants who consumed a moderate amount of alcohol had a 35% higher likelihood of attaining low risk profile compared with heavy consumers (RR: 1.35, 95%CI: 1.06-1.73).

Role of individual major CVD risk factors in maintaining or attaining low risk

Participants who were not able to maintain a low risk profile, lost their low risk status mainly due to unfavourable changes in blood pressure levels, followed by changes in TC/HDL cholesterol and BMI (Supplementary Table 6.1). Among those with a medium/high risk status, attainment of a low risk profile was again largely due to improvements in blood pressure levels and to a smaller extent, due to improvements in TC/HDL cholesterol ratio, BMI, and smoking status (Supplementary Table 6.2).

Discussion

For those with an existing low risk profile, the highly educated, women, and younger participants were more likely to maintain a low risk profile; however, lifestyle factors did not seem to affect the likelihood of maintaining a low risk profile. Medium/high risk people who attained a low risk profile were also more likely to be highly educated, female and young, but in addition to that were also more likely to adhere to the Mediterranean diet, were more often physically active and more often had moderate alcohol consumption rather than

heavy alcohol consumption. Changes in blood pressure were the main contributors of both losing and attaining a low risk profile, followed by changes in BMI and TC/HDL cholesterol ratio.

Although numerous studies have demonstrated the benefits of a low risk profile, low risk remains rare.[1-7] The proportion of adults with a low risk profile was small in the present study population, but relatively high compared to the prevalence of low risk observed in most US and European studies, i.e. ranging from 10 to 44% of young adults (age range: 18-39 year)[1,9] to 3-7% of middle-aged adults (age range: 35-79 years).[1-4, 6] A large proportion of adults lost their favourable risk status over time in this study, similar to the CARDIA participants.[9] Moreover, while pharmaceutical and/or lifestyle interventions to prevent CVD are largely directed towards adults with high risk profiles, most CVD cases occur among untreated adults with slightly elevated levels.[25] This underscores the need to identify potential modifiable factors associated with achieving and maintaining low risk profile to facilitate the development of strategies to increase the proportion of adults at low risk and to characterize groups that face higher likelihood of loss of low risk status and exposure to adverse risk profiles who may benefit from earlier/ more intensive interventions.

The observed associations of education, gender and age with maintenance of a low risk profile are consistent with previous studies on individual CVD risk factors. It has been shown that low education, male gender and older age are independent determinants for most major CVD risk factors.[26, 27] Unfavourable changes in blood pressure were the main contributors to loss of low risk status followed by change in cholesterol and BMI. It was therefore unexpected that no lifestyle factors were associated with the maintenance of a low risk profile in this study, since physical activity and diet are important determinants for the individual major CVD risk factors.[28, 29] Although we used repeated measurements, interim changes between measurements in lifestyle factors might have occurred, which may have attenuated the results. In addition, salt intake was not sufficiently measured in our population but might be important since reducing salt intake across the population is an effective way to lower blood pressure.[30]

We did find modifiable determinants associated with attaining a low risk profile among those with medium/high risk profile; adherence to a Mediterranean diet, physical activity and moderate alcohol consumption increased the likelihood of attaining a low risk profile. This is consistent with the established relationship of a Mediterranean diet and alcohol intake with overweight/obesity, hypercholesterolemia, hypertension and diabetes.[28, 31-34] A dose-response relationship between physical activity and the major risk factors has often been observed, i.e. higher physical activity is associated with more favourable risk factor levels.[29] We showed that any physical activity compared with none was similarly associated with a higher likelihood of attaining a low risk profile.

Our finding that only 5% of participants with elevated risk attained a low risk profile after 15 years, stresses the importance of maintaining a low risk profile from young adulthood onwards. Efforts to increase the proportion of adults with a low risk profile should be especially targeted towards those with low educational levels, as this group was most vulnerable to developing unfavourable risk. The current findings also indicate that men are more susceptible to losing low risk status. For those not at low risk, healthy diets, physical activity and moderate alcohol intake can lead to improvements in risk profile. Our results indicate more specifically that even small amounts of physical activity can improve risk profiles.

The strength of this study is that extensive information about risk factors and lifestyle factors was objectively obtained at four points in time over a 15-year period, by the same group of trained staff. Limitations of this study include reliance on self-reported data on lifestyle factors. However, the physical activity and food-frequency questionnaires used have been shown to be reproducible and valid,[11, 35, 36] and self-reported lifestyles were shown to be associated with CVD in the present population.[37-39] Still, recall and misclassification bias due to socially desirable answers may have occurred, possibly resulting in attenuated associations. We focused on attainment and maintenance of an overall low risk profile since this is associated with the lowest risk of CVD.[7] Because of this classification, it was not possible to detect small changes in risk status which would require computation of a continuous risk score. Furthermore, individuals who participate in cohort studies are generally healthier and better educated than non-responders. Participants who were excluded and those who dropped out during follow-up also had slightly less favourable levels of the major risk factors at wave 1 (1987-1991) (data not shown). The underrepresentation of individuals with the worst/unhealthiest levels of the determinants may have mitigated the true difference between the lowest and highest categories, resulting in some underestimation of the observed associations. This potential bias may have been partly addressed by multiple imputation of missing values.

In conclusion, age, gender and educational attainment were the major determinants of attaining and maintaining low risk profile. Participants with lower educational levels and men had lower likelihood of attaining and maintaining low risk status; therefore, these groups may benefit from early, intensive interventions. Lifestyle factors -- diet, moderate alcohol intake, and physical activity -- were associated with a higher likelihood of attaining low risk profile; these should therefore be a fundamental part of CVD prevention programs among adults. The low rate of attaining low risk profile underscores the difficulty of improving overall risk status once adverse risk factors have developed and subsequently the importance of maintaining a low risk status from young adulthood onwards. Finally, more research on modifiable determinants of maintaining a low risk profile is especially needed, to inform the development of effective strategies to promote the achievement and maintenance of low risk profiles.

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Supplementary Methods

Measurement of diet and physical activity

Dietary history was ascertained using a validated semi-quantitative food frequency questionnaire which contained questions on the habitual consumption frequency of 178 food items during the year preceding enrolment. Additional information was obtained on consumption frequency for different subitems, preparations methods and coloured photographs were used to estimate the portion sizes of 28 food items. A healthy diet was operationalized with the 9-scale modified Mediterranean diet score defined by Trichopoulou et al.[1] This score assigned values of 0 to 1 to each nutritional component (i.e. vegetables, fruits, legumes and nuts, grains, fish and seafood, meat products, alcohol, fatty acid ratio, and dairy products) using sex-specific medians. Since alcohol consumption is separately analysed, alcohol intake was not included in the Mediterranean diet score, resulting in a Mediterranean diet score ranging from 0 to 8. Physical activity was categorized using the Cambridge Physical Activity Index (CPAI).[2] The CPAI includes type of work and the amount of leisure-time physical activity. The type of work was classified into four categories; sedentary, standing, physical or heavy manual job. Individuals with no job were classified as having a sedentary job. Leisure-time physical activity consisted of cycling and other physical exercise.[2] Participants were classified as inactive (i.e. sedentary job and no leisure-time physical activity), low active (i.e. sedentary job with <0.5 hour leisure-time physical activity per day or standing job with no leisure-time physical activity), intermediate active (i.e. sedentary job with 0.5-1 hour leisure-time physical activity per day, or standing job with 0.5 hour leisure-time physical activity per day, or physical job with no leisure-time physical activity), and high active (i.e. sedentary job with >1 hour leisure-time physical activity per day, or standing job with >0.5 hour leisure-time physical activity per day, or physical job with at least some leisure-time physical activity or heavy manual job).

Measurement of 'major' CVD risk factors

Body weight and height were measured wearing light indoor clothing with emptied pockets and without shoes. Body weight was measured to the nearest 0.1 kg on calibrated scales and height to the nearest 0.5 cm. BMI was calculated as weight minus 1 kg to adjust for clothing, divided by height squared (kg/m^2). Total cholesterol (TC) and high-density lipoprotein (HDL) cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory, using standardized enzymatic methods. The TC/HDL-ratio was calculated by dividing the TC level by the HDL cholesterol value. Diabetes was defined based on self-reported history and/or non-fasting blood glucose concentration of 11.1 mmol/L or more.[3] All cases were also verified using information from the participant's general practitioner or pharmacist.[4]

Blood pressure

At each examination, diastolic and systolic blood pressure levels were measured twice after 2 minutes of rest and the average of these two measurements was used in the analyses. Participants sat in a chair and a random zero sphygmomanometer (Hawksley and Sons, Lancing, UK) was used in waves one to three. In waves four and five a Speidel Keller meter (Welch Allyn, Skaneateles Falls, NY, USA) was used with participants in a half seated position on a treatment table.

Diastolic and systolic blood pressure measured at wave four were unexpectedly systematically higher compared to blood pressure values in the other waves. As blood pressure increases with aging among adults this age,[5] we expected to observe blood pressure values at wave four to lay between those of waves three and five. In participants with complete follow-up data who did not use anti-hypertensive medication, diastolic blood pressure was on average 78 mmHg in wave three, 83 mmHg in wave four, and 79 mmHg in wave five. A similar pattern was also observed for systolic blood pressure; with systolic blood pressure of 122 mmHg in wave three, 130 mmHg in wave four, and 129 mmHg in wave five. This abnormal pattern was apparent in all 10-year age groups, men and women, during all five research years of a wave, and not related to measurement taken by a specific research employer. We extensively investigated possible causes for this difference such as changes in guidelines for the treatment of hypertension, the change from the random zero sphygmomanometer to the Speidel Keller meter, and the change from sitting position in a chair to a half-sitting position on a treatment table. Calibration reports were also checked. The change in sitting position and measuring device could not explain the difference since the protocol was not changed after wave four, and a decline in blood pressure was apparent from waves four to five. Blood pressure in wave four was also measured with both blood pressure devices among 442 participants. A correction factor was calculated but the abnormal patterns remained.

We decided to statistically adjust blood pressure at wave four since no cause for the abnormal pattern could be identified. To make blood pressures at different waves comparable, blood pressure values were estimated as if the participants were 50 years old using random coefficient analysis. In these models, age was entered as a linear, quadratic, and cubic term. Adults on anti-hypertensive medication or those with missing data at any wave were excluded. Analyses were performed separately for men and women. Blant-Altman plots were constructed to explore whether the abnormal blood pressure values at wave four were similar at different levels of blood pressure. Blant-Altman plots showed that differences in blood pressure between wave four and other waves increased with higher blood pressure values. For example, systolic blood pressure as if the participants were 50 years old was 1 mmHg higher at wave four compared to wave three among adults with an average systolic blood pressure at waves three and four of <110 mmHg (standard deviation:

12 mmHg). On the other hand, systolic blood pressure was 7 mmHg (standard deviation: 16 mmHg) higher among adults with an average systolic blood pressure at waves three and four of ≥ 140 mmHg. The same analyses were performed using the log of diastolic and systolic blood pressure. The results were similar, but showed less variation in the difference in blood pressure across different blood pressure levels. For that reason, the log of diastolic and systolic blood pressure was used in further analyses.

Two correction factors were calculated to adjust diastolic and systolic blood pressure, separately for men and women. The first correction factor took the difference in the abnormality of blood pressure at wave four across different levels of blood pressure into account. A random coefficient analysis was performed with the log diastolic/systolic blood pressure as if the participants were 50 years old as dependent variable and determinants of blood pressure as independent variables i.e. age, quadratic term of age, cubic term of age, total cholesterol, HDL cholesterol, use of cholesterol-lowering medication, educational level, occupational status, body mass index, smoking status, Mediterranean diet score, and physical activity. The log blood pressure was multiplied with the first correction factor before it was entered into the model as dependent variable. The value of this correction factor was changed multiple times until the optimal log-likelihood was observed. The second correction factor was estimated by adding a dummy variable to the model, which indicated whether the blood pressure measurement was taken at wave four or not.

These two correction factors were used to adjust the abnormal diastolic and systolic blood pressure; the first correction factor was multiplied, and the second correction factor and half times the residual were added to the log of the original diastolic and systolic blood pressure, for men and women separately. The exponential of these values were taken to obtain the final adjusted diastolic and systolic blood pressure values, which were used in all analyses.

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Supplementary Table 6.1. Distribution of major cardiovascular disease risk factors at wave 3, 4, and 5 among those who had a low risk profile one wave before.[a]

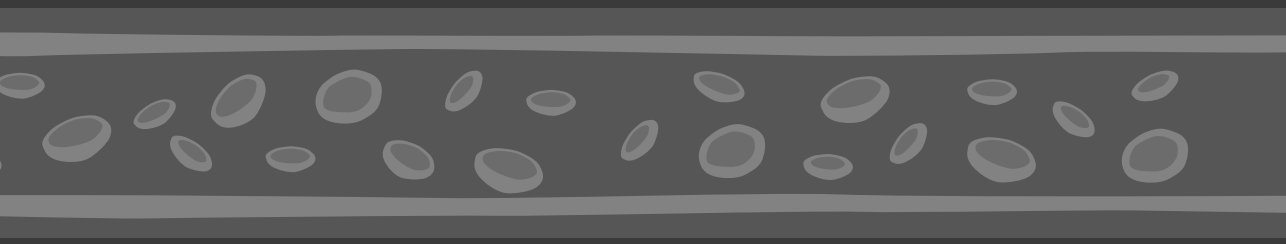
	Wave 3	Wave 4	Wave 5	Average of all waves
Blood pressure				
<80/120 mmHg, untreated	363 (60%)	236 (63%)	188 (54%)	59%
80-89/120-139 mmHg, untreated	204 (34%)	121 (32%)	127 (37%)	34%
≥90/140 mmHg and/or on medication	33 (5%)	21 (6%)	31 (9%)	7%
Anti-hypertensive medication				
Yes	3 (1%)	2 (1%)	4 (1%)	1%
No	597 (99%)	376 (99%)	341 (99%)	99%
Total/HDL cholesterol ratio				
<4.0, untreated	504 (84%)	347 (92%)	293 (85%)	87%
4.0-5.9, untreated	91 (15%)	29 (8%)	52 (15%)	13%
≥6.0 and/or on medication	6 (1%)	3 (1%)	1 (0%)	1%
Cholesterol-lowering medication				
Yes	4 (1%)	3 (1%)	0 (0%)	1%
No	596 (99%)	376 (99%)	345 (100%)	99%
Body mass index				
<25 kg/m^2	479 (80%)	314 (83%)	289 (84%)	82%
25-29.9 kg/m^2	121 (20%)	64 (17%)	56 (16%)	18%
≥30 kg/m^2	1 (0%)	0 (0%)	0 (0%)	0%
Smoking				
Yes	26 (4%)	12 (3%)	9 (3%)	3%
No	575 (96%)	367 (97%)	336 (97%)	97%
Diabetes				
Yes	0 (0%)	0 (0%)	0 (0%)	0%
No	600 (100%)	378 (100%)	345 (100%)	100%
Low risk profile[a]	253 (42%)	187 (49%)	136 (39%)	43%
Medium-low profile[a]	284 (47%)	157 (41%)	170 (49%)	46%
Medium-high profile[a]	61 (10%)	33 (9%)	38 (11%)	10%
High risk profile[a]	2 (0%)	1 (0%)	2 (0%)	0%

[a] Low risk profile was defined as including all of the following: systolic blood pressure < 120 mm Hg, diastolic blood pressure < 80 mm Hg, not taking antihypertensive medication, total cholesterol/HDL-ratio < 4.0, not taking cholesterol-lowering medication, body mass index < 25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk were classified into 3 groups based on 5 risk factors: 1) systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio ≥ 6.0 or taking cholesterol-lowering medication; 3) body mass index ≥ 30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors.

Supplementary Table 6.2. Distribution of major cardiovascular disease risk factors among those who had a medium/high risk profile at wave 2,3, and 4 and attained low risk profile the following wave.[a]

	Wave 2	Wave 3	Wave 4	Average of all waves
Blood pressure				
<80/120 mmHg, untreated	52 (30 %)	56 (32 %)	32 (31 %)	31%
80-89/120-139 mmHg, untreated	111 (65 %)	106 (62 %)	63 (62 %)	63%
≥90/140 mmHg and/or on medication	8 (5 %)	10 (6 %)	7 (7 %)	6%
Anti-hypertensive medication				
Yes	0 (0%)	0 (0%)	0 (0%)	0%
No	171 (100%)	172 (100%)	102 (100%)	100%
Total/HDL cholesterol ratio				
<4.0, untreated	143 (84 %)	130 (76 %)	79 (77 %)	79%
4.0-5.9, untreated	28 (16 %)	38 (22 %)	22 (21 %)	20%
≥6.0 and/or on medication	0 (0 %)	3 (2 %)	1 (1 %)	1%
Cholesterol-lowering medication				
Yes	0 (0%)	1 (1%)	1 (1%)	1%
No	171 (100%)	171 (99%)	101 (99%)	99%
Body mass index				
<25 kg/m^2	140 (82 %)	131 (76 %)	76 (78 %)	79%
25-29.9 kg/m^2	31 (18 %)	40 (23 %)	24 (24 %)	22%
≥30 kg/m^2	0 (0 %)	0 (0 %)	0 (0 %)	0%
Smoking				
Yes	23 (14 %)	30 (17 %)	14 (14 %)	15%
No	148 (86 %)	142 (83 %)	88 (86 %)	86%
Diabetes				
Yes	0 (0 %)	0 (0 %)	0 (0 %)	0%
No	171 (100 %)	172 (100 %)	102 (100 %)	100%
Low risk profile[a]	--	--	--	-
Medium-low profile[a]	140 (82 %)	130 (76 %)	79 (78 %)	79%
Medium-high profile[a]	30 (18 %)	40 (23 %)	23 (22 %)	21%
High risk profile[a]	1 (0 %)	2 (1 %)	0 (0 %)	0%

[a] Low risk profile was defined as including all of the following: systolic blood pressure < 120 mm Hg, diastolic blood pressure < 80 mm Hg, not taking antihypertensive medication, total cholesterol/HDL-ratio < 4.0, not taking cholesterol-lowering medication, body mass index < 25 kg/m^2, not smoking, and no history of diabetes. Persons not low risk were classified into 3 groups based on 5 risk factors: 1) systolic blood pressure ≥ 140 mm Hg, diastolic blood pressure ≥ 90 mm Hg, or taking antihypertensive medication; 2) total cholesterol/HDL-ratio ≥ 6.0 or taking cholesterol-lowering medication; 3) body mass index ≥ 30 kg/m^2; 4) currently smoking and; 5) diabetes. Persons in group 1 (medium-low risk profile) scored high on no risk factors but had a suboptimal score on at least one factor. Persons in group 2 (medium-high risk) scored high on one risk factor. Those in group 3 (high risk profile) scored high on two or more factors.



Part III

Trajectories of metabolic risk factors and biochemical markers preceding cardiovascular disease and type 2 diabetes

Chapter 7

Trajectories of metabolic risk factors and biochemical markers prior to the onset of cardiovascular disease

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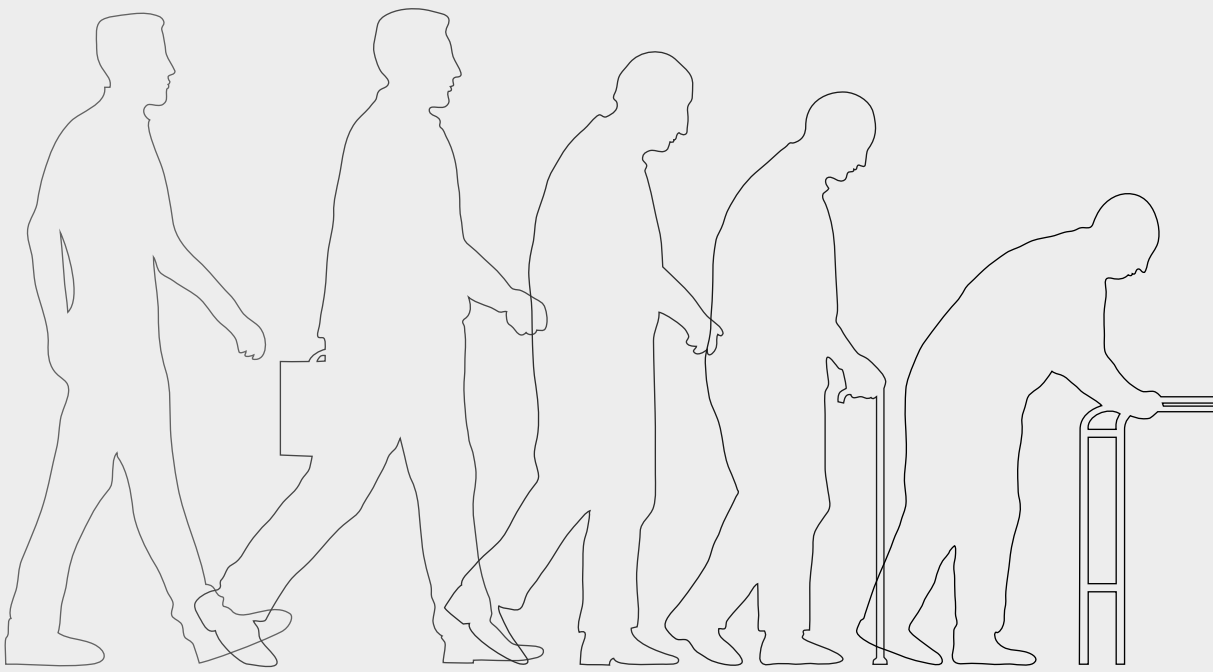


Abstract

Background: Risk factors often develop at young age and are maintained over time, but it is not fully understood how risk factors develop over time preceding cardiovascular disease (CVD). Our objective was to examine how levels and trajectories of metabolic risk factors and biochemical markers prior to diagnosis differ between people who develop CVD and controls over a period of up to 15-20 years.

Methods: A total of 449 incident non-fatal/fatal CVD cases and 1,353 age- and sex-matched controls were identified in a prospective population-based cohort of 7,768 adults between 1993 and 2011. Metabolic risk factors and biochemical markers were measured at five-year intervals prior to diagnosis. Trajectories of risk factors and biochemical markers were analysed using random coefficient analyses.

Results: Participants with CVD had slightly more unfavourable levels for most metabolic risk factors and biochemical markers 15-20 years before diagnosis than controls. Subsequent trajectories until diagnosis were similar in participants with incident CVD and in controls for body mass index, diastolic blood pressure, total cholesterol, HDL cholesterol, random glucose, triglycerides, gamma glutamyltransferase, C-reactive protein and uric acid. Trajectories were more unfavourable in participants with CVD than in controls for systolic blood pressure, waist circumference and estimated glomerular filtration rate ($p \leq 0.05$). For example, among participants with CVD, systolic blood pressure increased on average by 9 mmHg over the 18-year period preceding diagnosis, whereas the increase among controls was 4 mmHg.

Conclusions: Unfavourable levels of metabolic risk factors and biochemical markers are present about 15-20 years before CVD, which indicates that the risk of CVD is already partly determined in young adulthood. This underscores the need for early prevention to reduce the burden of CVD.

Introduction

It is unclear whether cardiovascular disease (CVD) is preceded by gradual accumulation of adverse levels of risk factors starting at young age, by relatively sudden deterioration in risk factors shortly before disease onset, or a combination of both. Although it has been well-established that adverse levels of risk factors often develop early in life and are maintained over time,[1-6] it is not fully understood as to how they progress to CVD. For a better understanding of the natural history of CVD, it is necessary to know how metabolic risk factors and biochemical markers develop during the decades prior to the onset of CVD. The comparison of long-term trajectories of metabolic risk factors and biochemical markers between those who do and those who do not develop CVD will provide insight into the timing and the extent of pathophysiological changes before the occurrence of CVD, which may, therefore, give an indication as to the optimal timing of preventive actions.

Long-term trajectories of metabolic risk factors and biochemical markers preceding CVD have hardly been explored but the few available studies suggest differences between those with and those without CVD long before diagnosis. For example, at the age of 17, males in the Israel Defence Forces who later developed a stenosis of more than 50% in at least one coronary artery had similar BMI levels to the men without coronary stenosis; however, subsequent increases in BMI up to diagnosis at the age of 25-45 years were larger in men who developed the stenosis than among other young men.[7] In the Whitehall II study, British civil servants with CVD had higher levels of C-reactive protein (CRP) 14 years prior to diagnosis of fatal CVD than those individuals without CVD.[8] This difference remained similar until the occurrence of CVD. Insight into trajectories of other important CVD risk factors such as blood pressure,[9] lipids,[10, 11] liver fat accumulation[12] and kidney function[13] may lead to a better understanding of the long-term physiological changes preceding CVD. Therefore, we examined how levels and trajectories of several metabolic risk factors and biochemical markers prior to diagnosis among initially healthy men and women differed between those who later developed CVD and those who did not, over a period of up to 15-20 years.

Method

Population

The Doetinchem Cohort Study is an ongoing population-based longitudinal study of men and women aged 20-59 years at the start of the study from Doetinchem, a town in the eastern part of the Netherlands. Men and women were invited to undergo a clinical examination from 1987-1991 (wave 1: N=7,768, participation rate: 62%), 1993-1997 (wave 2: N=6,117), 1998-2002 (wave 3: N=4,918), 2003-2007 (wave 4: N=4,520) and 2008-2012 (wave 5: N=4,018).

Response rates were 75% or higher in waves 2-5. Details are described elsewhere.[14] We excluded 2,250 participants from the current analyses based on the following exclusion criteria: participation in only one wave (N=1,378); missing follow up information on CVD or no informed consent for linkage with Statistics Netherlands or the Dutch Hospital Discharge Registry (n=416); prevalent CVD at baseline or wave 2 based on hospital discharge data and self-reporting (N=184); missing data on biochemical markers in all waves due to absence of informed consent to use blood samples for future research (N=90); and non-participation in the wave prior to diagnosis of CVD (N=182). This led to a population of 2,517 men and 3,001 women. Pregnant women were excluded for the wave in which they were pregnant. All participants gave written informed consent for each wave and the study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Measurements

Weight, height, waist circumference, diastolic and systolic blood pressure were measured and blood samples were taken according to standard protocols.[14] Total cholesterol and HDL cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum, with standardised enzymatic methods. In 2013-2014, standardised enzymatic methods were used to retrospectively determine triglycerides, alanine aminotransferase (ALT), gamma-glutamyltransferase (GGT), high sensitivity CRP, uric acid, cystatin C and creatinine of waves 2-5 for the whole population using blood plasma that had been stored in freezers. Details of all measurements are described in the Supplementary Methods. The examination of all available samples from consecutive waves in one assay run reduced the chance of measurement error to an absolute minimum.[15] Estimated glomerular filtration rate (eGFR) was estimated with the Chronic Disease Epidemiology Collaboration (CKD-EPI) equation using a combination of cystatin C and creatinine.[16] Data on educational attainment, smoking status and use of anti-hypertensive and cholesterol-lowering medication were obtained by questionnaire.

Cardiovascular disease

Non-fatal and fatal cardiovascular events that occurred after the second examination wave were ascertained until January 1, 2011. Cause of death was ascertained through linkage with Statistics Netherlands, and morbidity data were obtained through probabilistic linkage with the Dutch Hospital Discharge Registry.[17] We defined fatal CVD cases (where CVD was the primary or secondary cause of death) and non-fatal CVD cases according to ICD-9 codes 410–414, 415.1, 427.5, 428, 430–438, 440-442, 443.9, 444, 798.1, 798.2, 798.9[18] and corresponding ICD-10 codes.[19]

Selection of controls

For each incident CVD case (N=449), three controls were randomly selected from the same study wave and matched on age (±3 years) and sex using incidence density sampling, the preferred method for a nested case-control design and recently proposed for retrospective, longitudinal analysis.[20, 21] This led to a study population of 1,804 participants. We matched to control as much as possible for differences in metabolic risk factors and biochemical markers between those with and those without CVD caused by differences in age and length of follow-up.

Data analysis

From the date of diagnosis, participants were followed back in time for 5-24 years (Table 7.1), i.e. participants diagnosed between waves 2-3, 3-4, 4-5 and after wave 5 could be followed back in time over 6-11, 11-16, 16-21 or 21-24 years respectively. BMI, blood pressure, total cholesterol and HDL cholesterol were followed back in time for a maximum of 24 years, and other metabolic risk factors and biochemical markers were followed back in time for a maximum of 18 years, as those factors were not measured during the first examination wave.

Trajectories were constructed by random coefficient analyses adjusted for sex, age (linear, plus quadratic and cubic age-terms if it statistically significantly improved model fit), examination wave and time prior to event as a linear function for each metabolic risk factor and biochemical marker (dependent variable) separately. For participants with incident CVD, the exact time of each examination prior to diagnosis was calculated by subtracting the examination date from the date of diagnosis of CVD. Matched controls were assigned the same follow-up time as their respective cases. Age was centred at 60 years, which was approximately the mean age at wave 5, and examination wave was centred at wave 4 to fit trajectories for a hypothetical population of 60 year olds in 2002-2007 (T_0). We centred the examination wave at wave 4 and not wave 5 for optimal power since there were only few incident CVD cases after wave 5 (N=38). Trajectories of diastolic and systolic blood pressure were also adjusted for anti-hypertensive medications, and trajectories of total cholesterol, HDL cholesterol and triglycerides were adjusted for cholesterol-lowering medications. We log-transformed triglycerides, ALT, GGT and CRP and reported back-transformed geometric means since these biochemical markers did not have a normal distribution.

Table 7.1. Number of incident CVD cases at each wave and the corresponding follow-up time of metabolic risk factors and biochemical markers.

Moment of diagnosis	Number of incident CVD cases	Number of years prior to diagnosis that measurements of BMI, DBP, SBP, TC and HDLc were available	Number of years prior to diagnosis that measurements of glucose, WC, TG, ALT, GGT, CRP, UA, eGFR were available	Age at diagnosis Mean ± standard deviation
Between waves 2-3	120	6-11	0-5	55.4 ± 8.2
Between waves 3-4	128	11-16	5-10	59.3 ± 8.6
Between waves 4-5	163	16-21	10-15	64.0 ± 9.1
After wave 5	38	21-24	15-18	63.5 ± 8.8

Abbreviations: BMI, body mass index; CVD, cardiovascular disease; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; HDLc, high-density lipoprotein cholesterol; WC, waist circumference; TG, triglycerides; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; CRP, C-reactive protein; UA, uric acid; eGFR, estimated glomerular filtration rate; Note: For example, cases diagnosed after wave 5 had measurements of BMI, DBP, SBP, TC and HDLc for up to 21-24 years and measurements of other risk factors and biochemical markers for up to 15-18 years prior to diagnosis. Cases diagnosed between waves 3-4 had measurements of BMI, DBP, SBP, TC and HDLc for up to 11-16 years and measurements of other risk factors and biochemical markers for up to 5-10 years prior to diagnosis.

Non-linearity was investigated by fitting first-order fractional polynomials of time.[22] The following transformations of time before event were fitted: linear, quadratic, cubic, square root, logarithmic, and the inverse of linear, quadratic and square root. A non-linear function of time replaced the linear function of time when the model fit was statistically significantly better based on the likelihood ratio test ($P<0.05$). A second polynomial was included in the model when inclusion further improved the model fit.

We tested differences between individuals with CVD and controls in 1) levels of metabolic risk factors and biochemical markers 18 years before CVD, 2) subsequent trajectories until diagnosis and 3) levels at diagnosis. Participants with incident CVD and controls were contrasted using the contrast statement in SAS to test differences in levels of metabolic risk factors and biochemical markers at diagnosis and 18 years prior to diagnosis, the maximum follow-up time of most metabolic risk factors and biochemical markers. Differences in trajectories of metabolic risk factors and biochemical markers between participants with incident CVD and controls were statistically tested ($P< 0.10$) using an interaction term or interaction terms for the interaction(s) between CVD status and the time function(s) (i.e. time before event). A three-way interaction between age, CVD status and the time function(s) tested whether differences in trajectories between individuals with CVD and controls differed significantly by age ($P<0.10$)

We had limited power to stratify the primary analyses by specific CVD endpoint (coronary heart disease, stroke). To get an indication of potential differences in trajectories between subtypes of CVD, we modelled trajectories of BMI, diastolic and systolic blood

pressure, total cholesterol and HDL cholesterol separately for coronary heart disease and stroke as a sensitivity analysis. These were chosen because they are major risk factors of CVD, and the full follow-up period was available for these risk factors. Trajectories were additionally adjusted for BMI and centred at 25 kg/m^2 in sensitivity analyses to investigate whether differences in trajectories between participants with incident CVD and controls could be explained by BMI, a key driver of the other metabolic risk factors and biochemical markers.[23-26] All analyses were performed using SAS 9.3 software.

Results

In the total study population, 449 participants developed CVD (290 men; 159 women): 281 coronary heart disease events, 117 strokes and 51 other cardiovascular events occurred. For participants with incident CVD and controls, blood pressure, total cholesterol, HDL cholesterol and BMI (that were measured from the first wave onwards) were followed back in time for an average of 2.9 waves and 14.9 years (range: 6.0-23.8), while the other risk factors and biochemical markers (that were measured from the second wave onwards) were followed back for an average of 2.2 waves and 11.1 years (range: 5.0-17.8). Participants diagnosed with CVD later during follow-up were older (Table 7.1). At baseline, the average age was 45.4 (range: 20.1-59.8). Participants with incident CVD were more likely to have had a lower level of education, to have been a smoker and to have been on anti-hypertensive medication than controls (Table 7.2).

Table 7.2. Baseline characteristics of participants who developed cardiovascular disease and those who did not.

	Cardiovascular disease N=449	**No cardiovascular disease N=1,347**
Demographics		
Age (years), mean (SD)	45.4 (8.7)	45.5 (8.7)
Women (%)	159 (35%)	477 (35%)
Low educational level (%)[a]	304 (68%)	804 (60%)
Smoking status		
Currently smoking (%)	198 (44%)	372 (28%)
Ex-smoker (%)	123 (27%)	475 (35%)
Medication		
Anti-hypertensive medication (%)	29 (6%)	56 (4%)
Cholesterol-lowering medication (%)	0 (0%)	1 (0%)

[a] Intermediate secondary education or less.

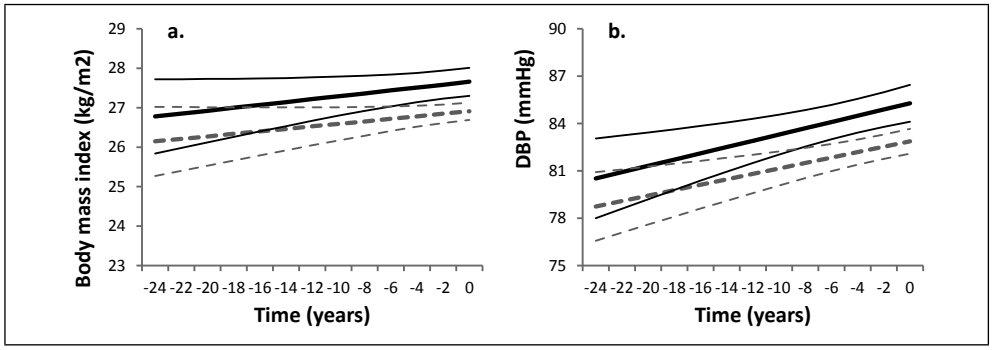
Differences in initial levels of metabolic risk factors and biochemical markers between participants with CVD and controls

With the exception of systolic blood pressure, waist circumference, ALT and eGFR, participants with incident CVD had, on average, slightly more unfavourable levels of metabolic risk factors and biochemical markers than controls at 18 years before diagnosis in age-adjusted analyses (Figure 7.1 and Table 7.3). This difference was only statistically significantly for HDL cholesterol, triglycerides and random glucose (P<0.05).

Table 7.3. Age-adjusted mean levels of metabolic risk factors 18 years prior to diagnosis of cardiovascular disease and at diagnosis, separately for cases and controls.

	Mean level at T_{-18} [a]			Mean level at diagnosis [a]		
	Cases	**Controls**	**P-value for difference**	**Cases**	**Controls**	**P-value for difference**
Body mass index (kg/m^2)	27.0	26.3	NS	27.7	26.9	<0.01
Diastolic blood pressure (mmHg)	82	80	NS	85	83	<0.01
Systolic blood pressure (mmHg)	132	131	NS	141	135	<0.01
Total cholesterol (mmol/L)	6.1	5.7	NS	6.0	5.9	<0.10
HDL cholesterol (mmol/L)	1.35	1.43	<0.05	1.30	1.42	<0.01
Triglycerides (mmol/L)	1.5	1.4	<0.01	1.5	1.4	<0.01
Glucose (mmol/L)	5.8	5.5	<0.01	5.6	5.3	<0.05
Waist circumference (cm)	95	95	NS	99	97	<0.01
ALT (U/L)	18	17	NS	19	18	NS
GGT (U/L)	22	20	<0.01	28	24	<0.01
C-reactive protein (mg/L)	1.3	0.9	NS	1.7	1.5	NS
Uric acid (mmol/L)	0.30	0.29	NS	0.32	0.30	<0.01
EGFR (ml/min/1.73 m^2)	93	93	NS	87	92	<0.01

Abbreviations: ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate; NS: not significant (P≥0.05); [a] Mean levels were estimated using random coefficient analyses.



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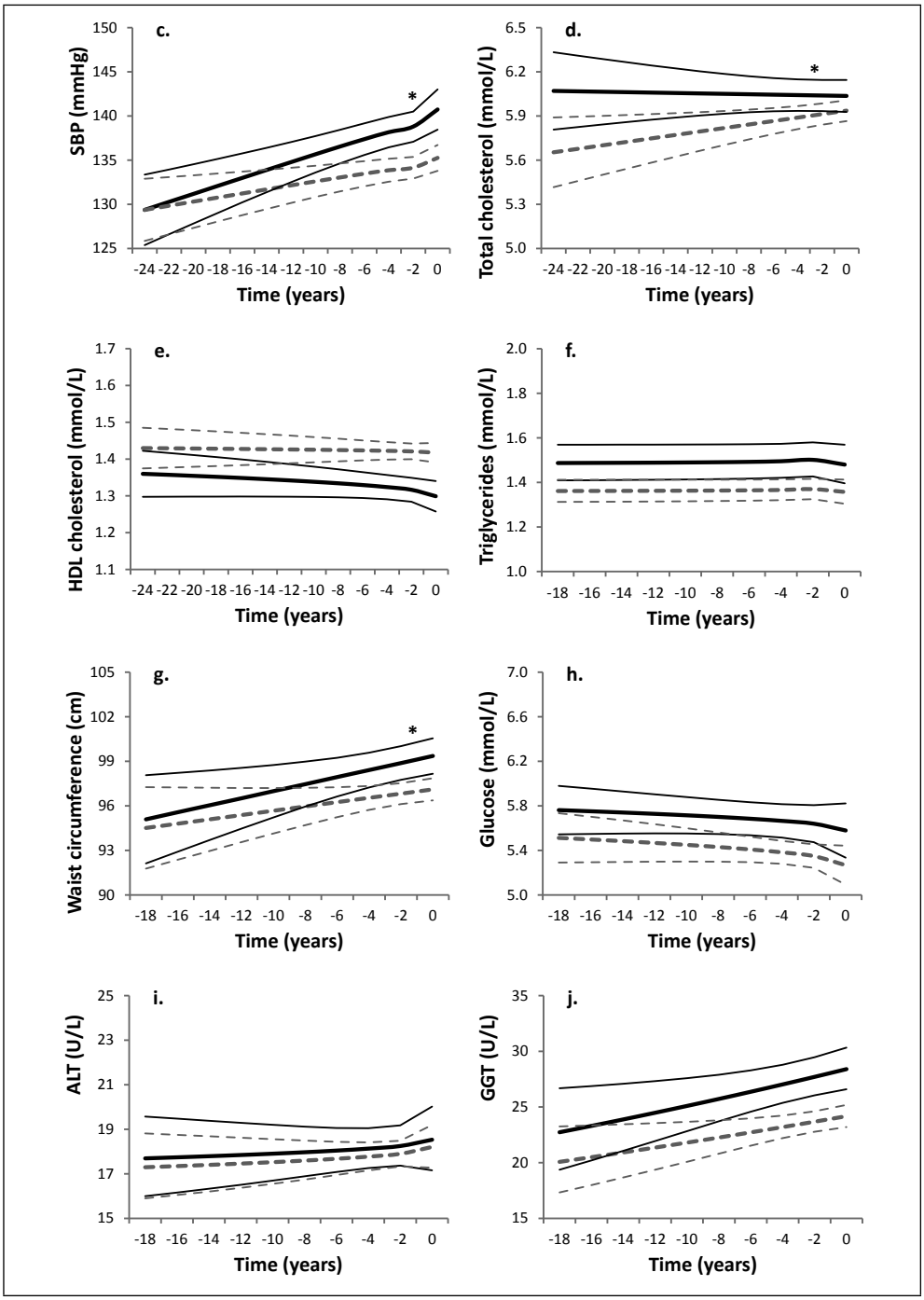

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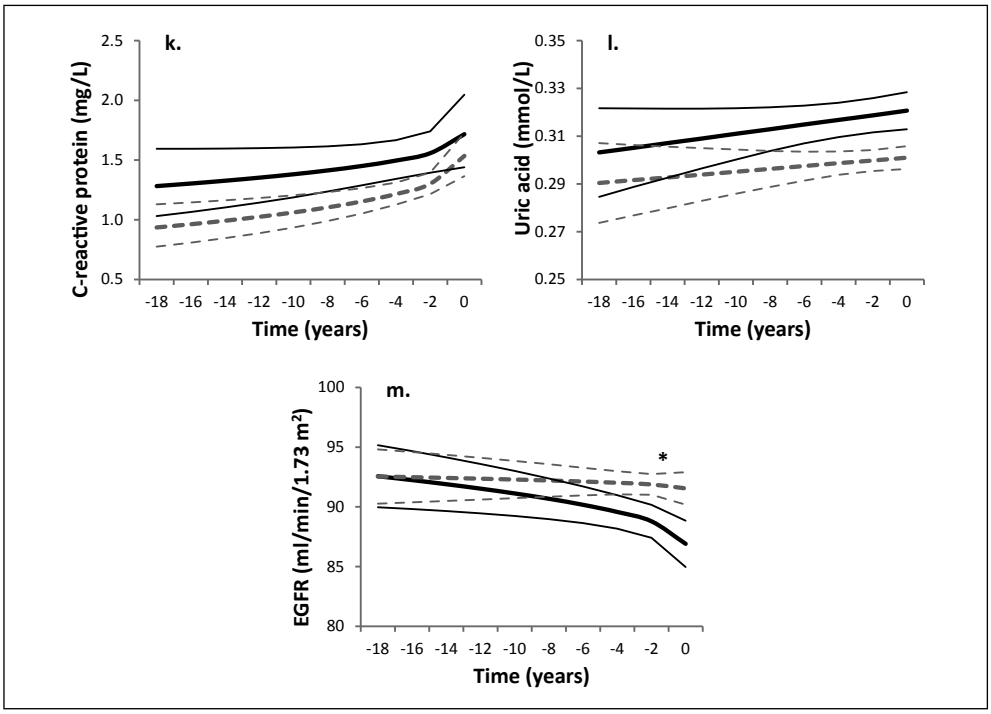


Figure 7.1. Trajectories of body mass index (a), DBP (b), SBP (c), total cholesterol (d), HDL cholesterol (e), triglycerides (f), waist circumference (g), random glucose (h), ALT (i), GGT (j), C-reactive protein (k), uric acid (l), and eGFR (m) of those participants with incident cardiovascular disease (solid black lines) and controls (dashed grey lines) for a hypothetical population of 60 year olds at diagnosis.
Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate. The thin black lines represent the 95% confidence intervals of mean levels of metabolic risk factors and biochemical markers for individuals with CVD. The thin dashed grey lines represent the 95% confidence intervals of mean levels of metabolic risk factors and biochemical markers for controls. Geometric means are shown for triglycerides, alanine aminotransferase, gamma glutamyltransferase and C-reactive protein. An asterisk (*) indicates a statistically significant difference in trajectory between cases and controls (P<0.10).

Differences in trajectories of metabolic risk factors and biochemical markers between participants with CVD and controls

The differences observed between participants with incident CVD and controls 15-20 years before diagnosis remained stable over time for most metabolic risk factors and biochemical markers (P≥0.10) (Figure 7.1 and Table 7.4). During the 18 years preceding diagnosis, in both participants with incident CVD and controls, increasing levels were seen for mean BMI (2%), diastolic blood pressure (4%), random glucose (3-4%), ALT (5%), GGT (20-25%), CRP (34-64%) and uric acid (4-6%); decreasing levels for HDL cholesterol (-3 to -4%); and stable

levels for triglycerides. In individuals with CVD, this resulted in statistically significantly more unfavourable levels of BMI, diastolic blood pressure, HDL cholesterol, triglycerides, random glucose, GGT and uric acid at diagnosis compared to controls (P<0.01).

Levels of ALT, CRP and total cholesterol were not statistically significantly different in participants with CVD and controls 15-20 years before diagnosis and at the end of follow-up/at diagnosis (P≥0.05). Trajectories of ALT and CRP were similar in participants with CVD and controls (P≥0.10), while the trajectory of total cholesterol was statistically significantly less unfavourable in individuals with incident CVD than controls (P<0.05).

More unfavourable trajectories were observed in participants with CVD than in controls for systolic blood pressure (P<0.01), waist circumference (P=0.05) and eGFR (P<0.01) (Figure 7.1 and Table 7.4). The similar levels of these risk factors in participants with CVD and controls 15-20 years before CVD slowly turned into more unfavourable levels in those with CVD at diagnosis (P<0.01). In men and women with incident CVD the mean systolic blood pressure increased from 132 to 141 mmHg (7%) in the 18 years preceding diagnosis, while systolic blood pressure increased from 131 to 135 mmHg (3%) in controls. During the same period, waist circumference increased from 95 to 99 cm (4%) and eGFR decreased from 93 to 87 ml/min/1.73m^2 (-6%) in participants with incident CVD, while changes were 2-6 times smaller in controls.

There were no statistically significant differences by age in the comparison of trajectories of metabolic risk factors and biochemical markers between people with CVD and controls (P≥0.10 for three-way interactions), with the exception of HDL cholesterol (P=0.07 for three-interaction) and ALT (P=0.02 for three-way interaction).

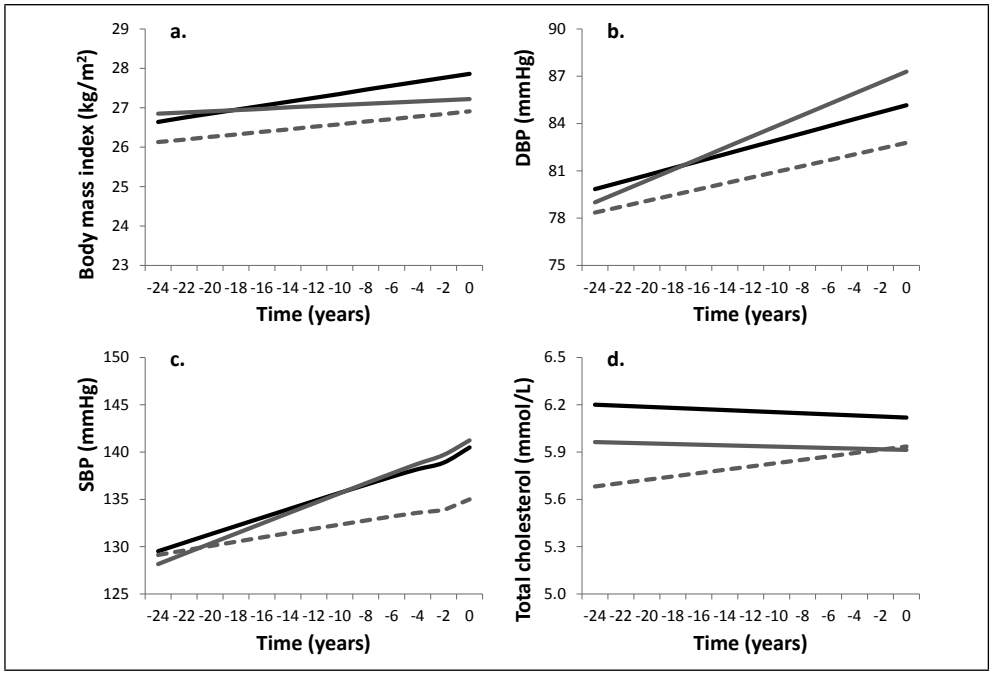
Sensitivity analyses

Participants with coronary heart disease and stroke had no statistically significantly different levels or trajectories of BMI, diastolic and blood pressure, total cholesterol and HDL cholesterol during the 15-20 years prior to diagnosis (Figure 7.2). Other sensitivity analyses showed that adjustment for BMI only slightly attenuated differences in metabolic risk factors and biochemical markers between participants with incident CVD and controls (Supplementary Figure 7.1).

Table 7.4. Percentage change in metabolic risk factors and biochemical markers during the 18 years prior to diagnosis of cardiovascular disease until diagnosis, separately for cases and controls.

	Percentage change between T_{-18} and diagnosis		
	Cases	**Controls**	**P-value for difference** [a]
Body mass index (kg/m^2)	2%	2%	NS
Diastolic blood pressure (mmHg)	4%	4%	NS
Systolic blood pressure (mmHg)	7%	3%	<0.01
Total cholesterol (mmol/L)	0%	4%	<0.05
HDL cholesterol (mmol/L)	-3%	-1%	NS
Triglycerides (mmol/L)	0%	0%	NS
Glucose (mmol/L)	-3%	-4%	NS
Waist circumference (cm)	4%	2%	0.05
ALT (U/L)	5%	5%	NS
GGT (U/L)	25%	20%	NS
C-reactive protein (mg/L)	34%	64%	NS
Uric acid (mmol/L)	6%	4%	NS
EGFR (ml/min/1.73 m^2)	-6%	-1%	<0.01

Abbreviations: ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate; NS: not significant (P≥0.10); [a] Difference in trajectory was statistically tested with interaction term(s) between time prior to diagnosis and cardiovascular disease status.



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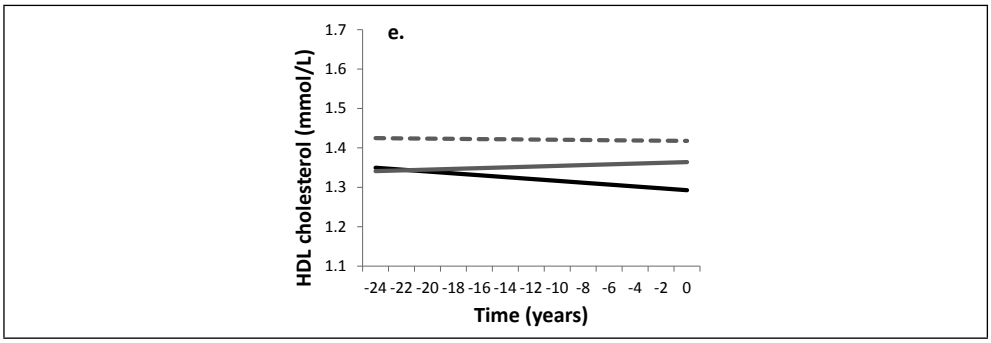

Figure 7.2. Trajectories of body mass index (a), DBP (b), SBP (c), total cholesterol (d) and HDL cholesterol (e) of those participants with incident coronary heart disease (solid black lines), incident stroke (solid grey lines) and controls (dashed grey lines) for a hypothetical population of 60 year olds at diagnosis. Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure.

Discussion

Long before (up to 15-20 years prior to) the diagnosis of CVD, people with incident CVD had slightly more unfavourable metabolic risk factor and biochemical marker levels than controls. Subsequent trajectories in BMI, diastolic blood pressure, HDL cholesterol, random glucose, triglycerides, GGT, ALT, CRP and uric acid were similar in people with incident CVD and controls until diagnosis. For systolic blood pressure, waist circumference and eGFR, similar levels were seen at 15-20 years before diagnosis, but more unfavourable subsequent trajectories were observed for participants with incident CVD compared to controls.

Differences in all metabolic risk factors and biochemical markers were small in our population but are in line with results from the INTERHEART study that also revealed small differences in mean levels of BMI (0.3 kg/m^2) and HDL cholesterol (0.03 mmol/L) between individuals with myocardial infarction and controls.[27, 28] Our finding of a difference in CRP of 0.2-0.4 mg/L over an 18 year period is also in accordance with findings from the Whitehall II study that showed a constant difference in mean CRP of 0.3-0.4 mg/L between people with incident fatal CVD and controls during the 14 years prior to diagnosis.[8] Extending the findings of the Whitehall II study to other metabolic risk factors, we showed that differences in most other metabolic risk factors and biochemical markers were also present long before diagnosis and, in general, subsequent trajectories were similar between persons with CVD and controls up to the occurrence of CVD. These differences were not explained by differences in BMI, and, in general, did not differ by age at diagnosis. These findings underscore the fact that CVD is caused by a long-term multifactorial disease process in which adverse effects of elevated levels of multiple risk factors are already present at young age

and slowly accumulate over time, rather than being the result of rapidly increasing levels of risk factors in the years preceding diagnosis.

We observed a larger increase in total cholesterol in controls than in participants with CVD. This may be the result of a larger increase in the use of statins in individuals with CVD than controls, which resulted in a 50% higher use of cholesterol-lowering medication in participants with CVD in the present study population (data not shown). Perhaps adjusting the analyses for cholesterol-lowering medication did not fully account for this increase in statin use.

During the 15-20 years preceding CVD, the course of only two of the metabolic risk factors and one of the biochemical markers was different in individuals with incident CVD than in controls: increases in systolic blood pressure, waist circumference and eGFR were larger in participants with incident CVD than in controls. This suggests that unfavourable changes in these three risk factors in the 15-20 years prior to diagnosis might be important in the development of CVD. Independently from BMI, waist circumference is associated with systolic blood pressure and GFR,[29, 30] which suggests that the unfavourable trajectory in waist circumference partially drives the unfavourable trajectories of blood pressure and eGFR. Blood pressure and GFR are also highly related, and each may adversely affect the other.[31, 32] The trajectory of eGFR may, therefore, resemble the trajectory of systolic blood pressure, and vice versa. In addition, unfavourable changes in either GFR or systolic blood pressure may also exacerbate the effect of the other. Although the unfavourable trajectories of systolic blood pressure, waist circumference and eGFR in people with incident CVD are biological plausible, the possibility of chance findings cannot be ruled out.

The more unfavourable trajectory in waist circumference but not in BMI in participants with incident CVD in comparison to controls suggests that unfavourable changes in intra-abdominal adiposity may be more important in the development of CVD than changes in general adiposity. The INTERHEART study also observed a stronger relationship for waist circumference and myocardial infarction than with BMI.[33] This is compatible with the fact that adipose tissue produces and secretes many bioactive molecules such as leptin, adiponectin, angiotensinogen, and inflammatory molecules. These adipokines also interact with other tissues and cells in the body that are linked with CVD.[34] In addition, the reduction of waist circumference and not of BMI in people with incident CVD also suggests a decline in muscle mass and an increase in fat tissue. This is in line with our secondary analyses, in which we found that, in people with CVD, the decline in eGFR based on cystatin C (-7.3 ml/min/1.73m^2) was larger than the decline in eGFR based on creatinine (-3.0 ml/min/1.73m2) during the 18 years prior to diagnosis (data not shown). Creatinine might remain higher due to a decline in muscle mass, while cystatin C better demonstrates the true decline in kidney function.[35, 36]

The presence of unfavourable risk factor levels 15-20 years or more prior to CVD, the fact that CVD event rates increase progressively after the age of 45 years,[37-39] and, of course, given that we want to prevent or postpone as many of those cases as possible underscore the need for CVD prevention in young adulthood. Small elevations in metabolic risk factors and biochemical markers are often not considered clinically relevant at a young age, but our findings indicate that small elevations in most metabolic risk factors and biochemical markers remain the same – they do not disappear – during the 15-20 years preceding CVD. These findings suggests that unfavourable levels are already harmful at a young age, and they emphasize the importance of maintaining favourable levels of general adiposity, blood pressure, lipids, markers of liver fat, chronic inflammation and kidney function from young adulthood onwards by way of a healthy lifestyle, including a healthy diet and physical activity. Preventing deterioration of systolic blood pressure, abdominal adiposity and kidney function in young adulthood and middle age might be especially warranted since these factors deteriorated more rapidly in people with CVD than controls until diagnosis. In addition, when extrapolating our findings to CVD risk prediction, the relatively small differences in mean levels of metabolic risk factors and biochemical markers between people with CVD and controls underscores the difficulty in differentiating between those who will and those who will not develop CVD long before diagnosis, which might explain the modest performance of CVD prediction models.[40] The similar long-term course of the majority of risk factors in people with CVD and controls also suggests that it is unlikely that multiple measurements of risk factors can improve the performance of such CVD prediction models.

Our approach using the modelling of long-term trajectories preceding CVD yields important insights into physiological changes preceding CVD. The strengths of this study are that the same group of trained study personnel objectively measured various metabolic risk factors and biochemical markers in a population-based cohort over a long period. Our study has also certain limitations, in that relatively few participants had measurements of metabolic risk factors over the full follow-up period due to the limited incidence of CVD after wave 5 (N=38), which led to relatively large 95% confidence intervals at 20 years or more before diagnosis. Although all types of CVD share common risk factors, the strength of risk factors differs by endpoint. For example, hypertension is more strongly associated with stroke than with coronary heart disease.[41, 42] In sensitivity analyses, we observed only small statistically insignificant differences in levels and trajectories between people with stroke and coronary heart disease. Combining all subtypes of CVD has, therefore, probably led to a small underestimation of the differences in metabolic risk factors and biochemical markers between cases and controls. Furthermore, the non-responders and the excluded participants had slightly more unfavourable levels of the metabolic risk factors and biochemical markers than the study participants. This may have led to an underestimation of the proportion

of adults with incident CVD and, as a consequence may have slightly underestimated differences in levels of metabolic risk factors and biochemical markers between people with incident CVD and controls.

In conclusion, the present study provides novel findings regarding the more unfavourable levels, but similar course, of most metabolic risk factors and biochemical markers in people with incident CVD compared to controls during the 15-20 years preceding CVD. In contrast, the course of systolic blood pressure, waist circumference and kidney function was more unfavourable in people with CVD than in controls, leading to increasing differences during the 15-20 years preceding diagnosis. These findings seem to indicate that the risk of CVD is already partly determined in young adulthood; thereby stressing the need for primary prevention measures targeted at all risk factors, such as encouraging physical activity and a healthy diet in individuals starting from childhood/young adulthood onwards.

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Supplementary Methods

Description of measurements

Body weight was measured to the nearest 0.1 kg on calibrated scales and 1 kg was subtracted to adjust for clothing; height was measured to the nearest 0.5 cm. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured twice to the nearest 0.5 cm, at the level midway between the lowest rib and the iliac crest at the end of expiration, with participants in standing position. The mean of two measurements was used for analysis. Diastolic and systolic blood pressure levels were measured twice after 2 minutes of rest and the average of these two measurements was used in the analyses. Participants were measured in sitting position with a random zero sphygmomanometer (Hawksley and Sons, Lancing, UK) in waves one through three. In waves four and five a Speidel Keller meter (Welch Allyn, Skaneateles Falls, NY, USA) was used. Mean diastolic and systolic blood pressure levels measured during wave four were unexpectedly higher compared to the blood pressure levels in the previous and following waves. No clear cause could be identified, therefore, we statistically corrected blood pressure values of wave 4, as described extensively elsewhere (Supplementary Methods Chapter 6).[1] Total cholesterol and HDL cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam, using standardised enzymatic methods. In 2013-2014, standardised enzymatic methods (Roche/Hitachi Modular P analyser, Mannheim, Germany) were used to retrospectively determine additional biochemical markers from waves 2-5 in non-fasting plasma samples that had been stored at -20 degree Celsius until June 1995 and at -80 degree Celsius from July 1995 onwards. Gamma-glutamyltransferase (GGT), uric acid, triglycerides (GPO-PAP assay) and alanine aminotransferase (ALT) (kinetic UV assay) were measured with a colorimetric method. ALT measurements were excluded until June 1995 (N=2,495) because prior to that blood plasma was stored at -20 degree Celsius, a temperature at which ALT has poor stability.[2] ALT (N≤2), and GGT (N≤29) values greater than three times the upper normal reference were recoded as missing for that wave since this may indicate liver problems.[3] High sensitivity CRP was measured with the principle of particle-enhanced immunological agglutination (Tina-quant CRP) and cystatin C measurement was based on a particle enhanced-turbidimetric immunoassay using reagents from Gentian (Gentian, Moss, Norway). Creatinine was measured with a Creatinine Plus assay (IDMS traceable). CRP values above 10 mg/L were recoded as missing for that wave because this may have indicated an acute-phase response to infection, for example, or physical injury rather than chronic subclinical inflammation (N≤80).[4]

Supplementary References

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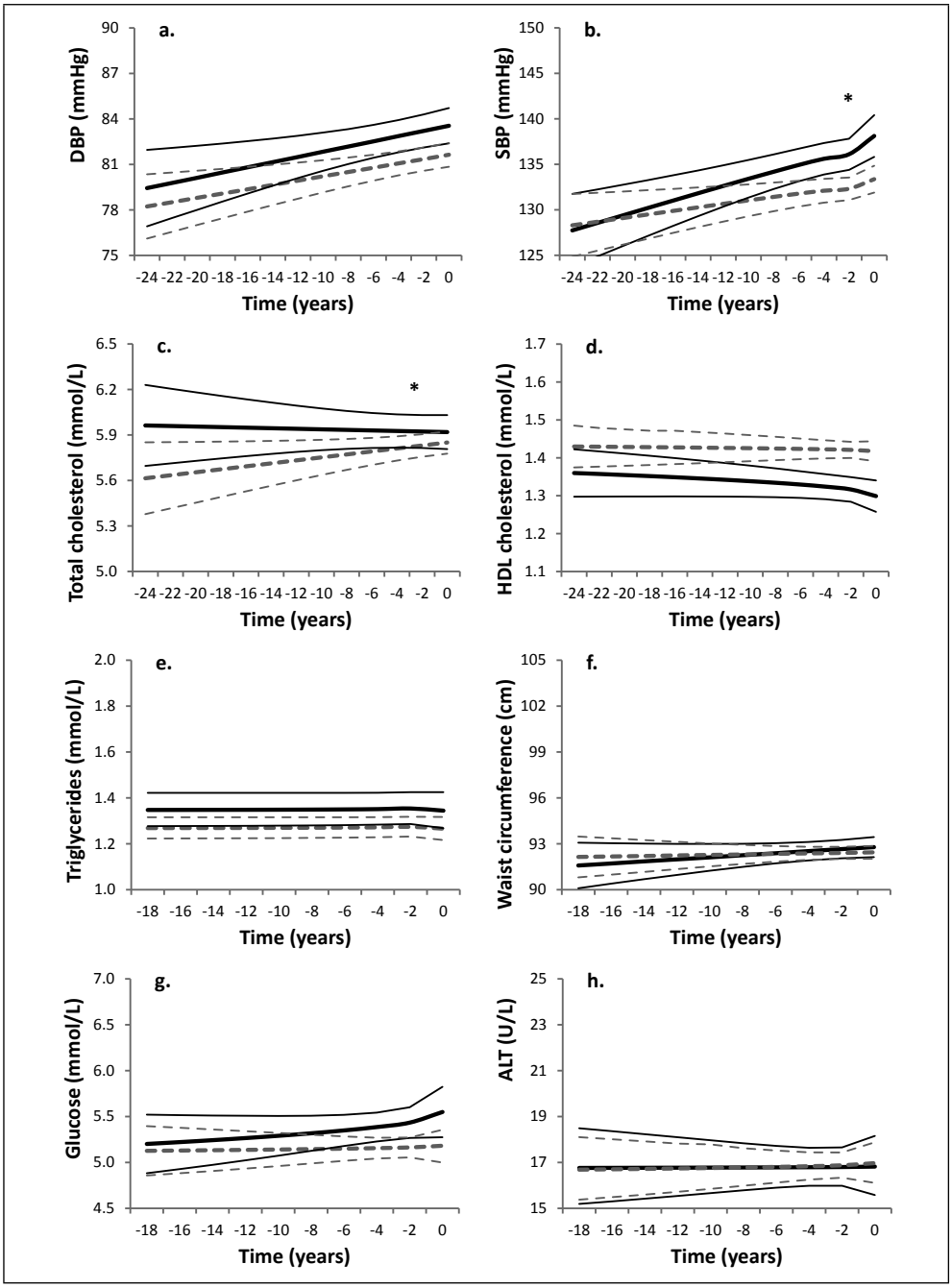

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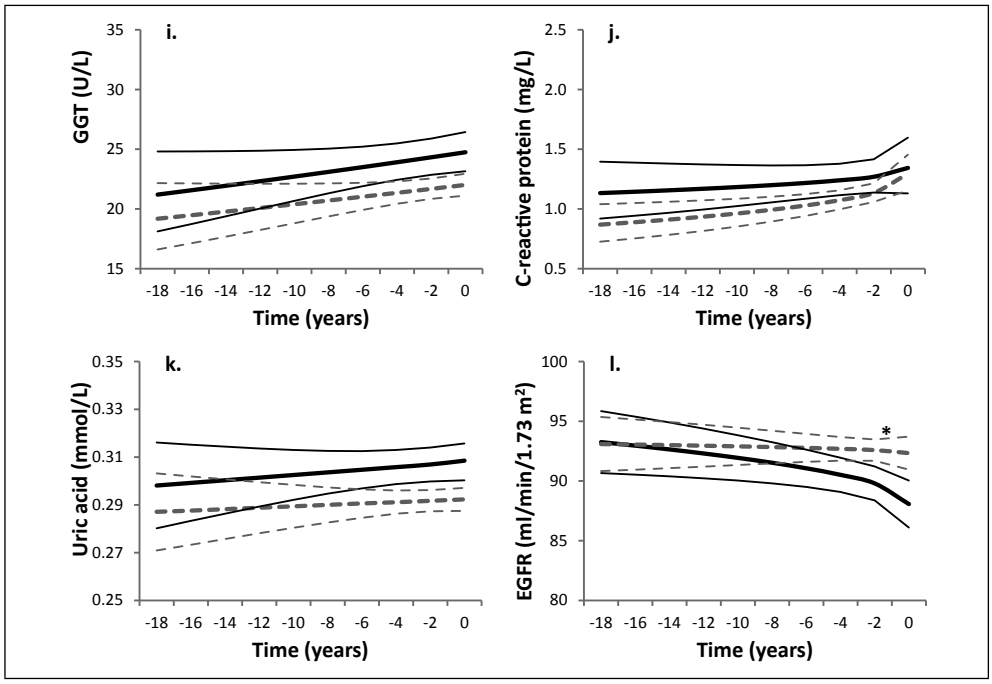

Supplementary Figure 7.1. Results adjusted for body mass index. Trajectories of DBP (a), SBP(b), total cholesterol (c), HDL cholesterol (d), triglycerides (e), waist circumference (f), random glucose (g), ALT (h), GGT (i), C-reactive protein (j), uric acid (k), and eGFR (l) of those participants with incident cardiovascular disease (solid black lines) and controls (dashed grey lines) for a hypothetical population of 60 year olds at diagnosis.
Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate. The thin black lines represent the 95% confidence intervals of mean levels of metabolic risk factors and biochemical markers for people with CVD. The thin dashed grey lines represent the 95% confidence intervals of mean levels of metabolic risk factors and biochemical markers for controls. Geometric means are shown for triglycerides, alanine aminotransferase, gamma glutamyltransferase and C-reactive protein. An asterisk (*) indicates a statistically significant difference in trajectory between cases and controls ($P<0.10$).

Chapter 8

Trajectories of metabolic risk factors and biochemical markers prior to the onset of type 2 diabetes

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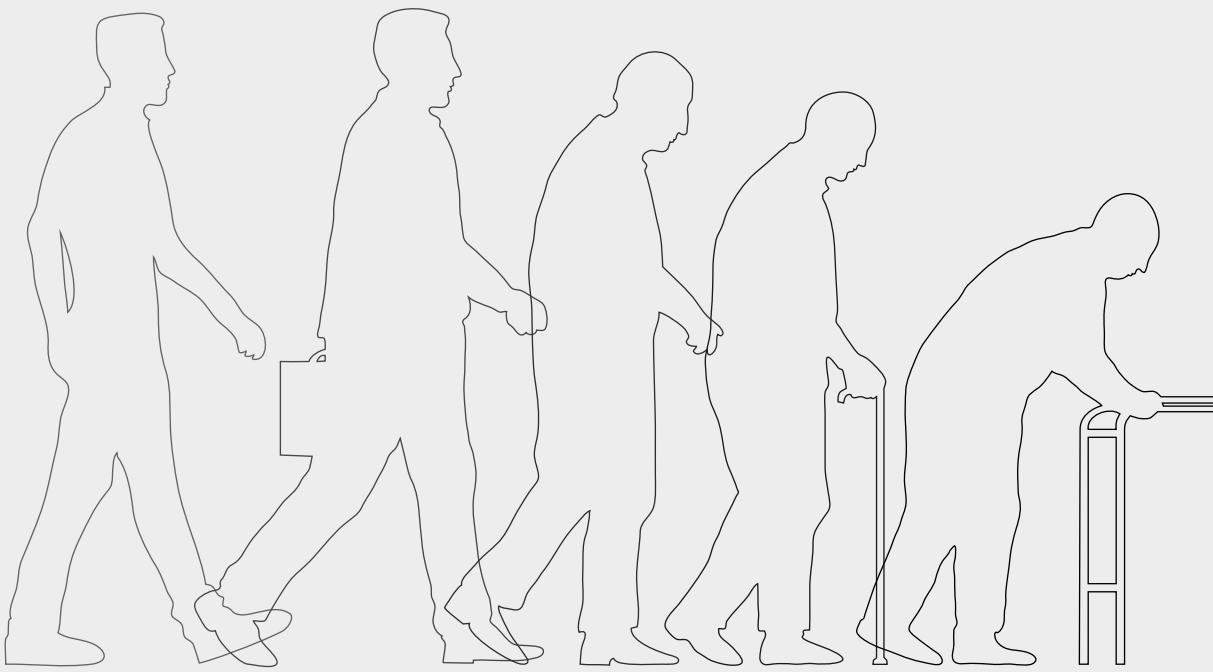


Abstract

Objective: To examine how trajectories of metabolic risk factors and biochemical markers prior to diagnosis differ between persons who develop type 2 diabetes and controls over a 15-20-year period.

Methods: A total of 355 incident type 2 diabetes cases (285 self-reported and 70 with random glucose≥11.1 mmol/L) and 2,130 age-matched and sex-matched controls were identified in a prospective population-based cohort between 1987 and 2012. Risk factors and biochemical markers were measured at five-year intervals. Trajectories preceding case ascertainment were analysed using generalised estimating equations.

Results: Participants with type 2 diabetes had higher levels of most metabolic risk factors and biochemical markers 15-20 years before case ascertainment than controls. Subsequent trajectories were more unfavourable in participants with incident type 2 diabetes for body mass index (BMI), HDL cholesterol and glucose ($P<0.01$), and to a lesser extent for diastolic and systolic blood pressure, waist circumference, triglycerides, alanine aminotransferase, gamma glutamyltransferase, C-reactive protein, uric acid and estimated glomerular filtration rate compared with controls. Among persons with incident type 2 diabetes, BMI increased by 5-8% over a 15-year period whereas the increase among controls was 0-2%. The observed differences in trajectories of metabolic risk factors and biochemical markers were largely explained by unfavourable changes in BMI. Results were similar for men and women.

Conclusions: Participants with type 2 diabetes had more unfavourable levels already 15-20 years before diagnosis and worse subsequent trajectories in metabolic risk factors and biochemical markers than controls. Our results highlight the need in particular for maintenance of a healthy weight from young adulthood onwards for diabetes prevention.

Introduction

Long-term changes in metabolic risk factors before the onset of type 2 diabetes are not well characterised. The comparison of long-term trajectories of risk factors between those who do and those who do not develop type 2 diabetes will reveal the changes before the onset of type 2 diabetes. This may help to identify at which time point these factors start deteriorating before overt disease. Such insight into the timing and extent of pathophysiological changes before symptoms become manifest may provide indications as to the optimal timing of preventive actions. Relevant factors associated with type 2 diabetes, include glucose levels,[1] β-cell function,[2] insulin resistance,[2] body mass index (BMI),[3, 4] waist circumference,[3] blood pressure,[4] lipids,[4] liver fat markers,[5, 6] markers of chronic inflammation[7] and kidney function.[8]

Several studies have described gradual changes in β-cell function, insulin resistance, fasting glucose and two-hour post-load glucose many years before diagnosis of type 2 diabetes with steeper unfavourable changes three to five years before diagnosis.[9-13] Only a few studies, mainly among men, have examined progressive changes of other risk factors, but so far findings have been inconsistent. The Whitehall II study showed that adults who developed type 2 diabetes had more unfavourable trajectories of systolic blood pressure and high-density lipoprotein (HDL) cholesterol but similar trajectories of BMI and C-reactive protein (CRP) compared with adults without diabetes, over a period of approximately 14 years.[14, 15] In contrast, a small study of 177 men observed larger changes in BMI but no differences in blood pressure, HDL cholesterol and liver fat markers in men who developed impaired fasting glucose compared with men who did not, over a nine-year period.[16] A short-term study (i.e. over 1.5 years) observed differences in changes of alanine aminotransferase (ALT) and triglycerides but not in blood pressure, total cholesterol and HDL cholesterol between high-risk men with incident type 2 diabetes and controls.[11]

A longer follow-up period in a population-based study and inclusion of other metabolic risk factors and biochemical markers is needed for more insight in the physiological changes preceding the onset of type 2 diabetes. There is also a need to investigate differences between men and women since previous studies reported several sex-related differences in the associations of risk factors such as systolic blood pressure, HDL cholesterol and uric acid with type 2 diabetes.[17, 18] Therefore, we examined whether trajectories of metabolic risk factors and biochemical markers among initially healthy men and women differed for those who developed type 2 diabetes and those who did not over a period of up to 15-20 years.

Methods

Population

The Doetinchem Cohort Study is an ongoing population-based longitudinal study of men and women aged 20-59 at the start of the study from Doetinchem, a town in the eastern part of the Netherlands. Men and women were invited to undergo a clinical examination in 1987-1991 (wave 1, N=7,768, participation rate: 62%), 1993-1997 (wave two, N=6,117), 1998-2002 (wave 3, N=4,918), 2003-2007 (wave 4, N=4,520) and 2008-2012 (wave 5, N=4,018). Response rates were 75% or higher in waves 2-5. Details of the study are described elsewhere.[19] We excluded 1,551 participants from the current analyses based on the following exclusion criteria: participation in only one wave (N=1,378); prevalent type 2 diabetes at baseline (N=48); missing diabetes status in all waves (N=3); and missing data on biochemical markers in all waves due to absence of informed consent to use blood samples for future research (N=122). This led to a population of 2,913 men and 3,304 women. Pregnant women were excluded for the wave in which they were pregnant. All participants gave written informed consent in each wave and the study was approved by the Medical Ethics Committee of the University Medical Center Utrecht.

Measurements

Weight, height, waist circumference, diastolic and systolic blood pressure measurements, and blood samples were taken according to standard protocols.[19] Total cholesterol and HDL cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum, using standardised enzymatic methods. In 2013-2014, standardised enzymatic methods were used to retrospectively determine triglycerides, ALT, gamma glutamyltransferase (GGT), high sensitivity CRP, uric acid, cystatin C and creatinine levels of waves 2-5 for the whole population using blood plasma that had been stored in freezers. Details of all measurements are described in the Supplementary Methods. GGT (N≤29) and ALT (N≤2) values greater than three times the upper normal reference were recoded as missing for that wave since this may indicate liver problems [20]. CRP values above 10 mg/L were recoded as missing for that wave because this may indicate an acute-phase response to infection for example or physical injury rather than chronic subclinical inflammation (N≤80).[21] Estimated glomerular filtration rate (eGFR) was calculated using the combination of cystatin C and creatinine.[22] Data on educational attainment, smoking status and use of anti-hypertensive and cholesterol-lowering medication were obtained by questionnaire.

Type 2 diabetes

Type 2 diabetes was ascertained by self-report (i.e. response of yes or no to the question: "Do you have diabetes?"). Of the self-reported cases up to 31 December 2007, 80% were checked with the general practitioner or pharmacist registries (N=201):[23] 176 of these 201 self-reported cases were confirmed as having type 2 diabetes. All self-reported incident diabetes cases that were not checked (N=109) were considered to have type 2 diabetes since the youngest participant in 2007 was 37 years old. In addition, 70 participants were ascertained as having incident type 2 diabetes by a measurement of random glucose of ≥11.1 mmol/L in the physical examination for our study. This gives a total of 355 participants with incident type 2 diabetes.

Selection of controls

For each incident type 2 diabetes case (N=355), three controls were randomly selected from the same study wave and matched on age (±2 years) and sex using incidence density sampling, the preferred method for a nested case-control design and recently proposed for retrospective, longitudinal analyses.[24,25] This led to a study population of 2,485 participants. We matched to control as much as possible for differences in metabolic risk factors and biochemical markers between those with and those without CVD caused by differences in age and differences in length of follow-up.

Data analysis

The time of case ascertainment was the first examination wave in which participants reported that they had type 2 diabetes and/or were found to have a random glucose ≥11.1 mmol/L. The same wave was used for their matched controls. Participants were followed back in time for 6-21 years, depending on the wave in which they were ascertained as being a type 2 diabetes case or control (Figure 8.1), i.e. participants ascertained in wave 2, 3, 4 or 5 could be followed back in time over 6, 11, 16 or 21 years respectively. BMI, blood pressure, total cholesterol and HDL cholesterol were followed back in time for a maximum of 21 years, referred to as 20 years for easier reading. Other metabolic risk factors were followed back in time for a maximum of 15 years since those factors were not measured in the first examination wave.

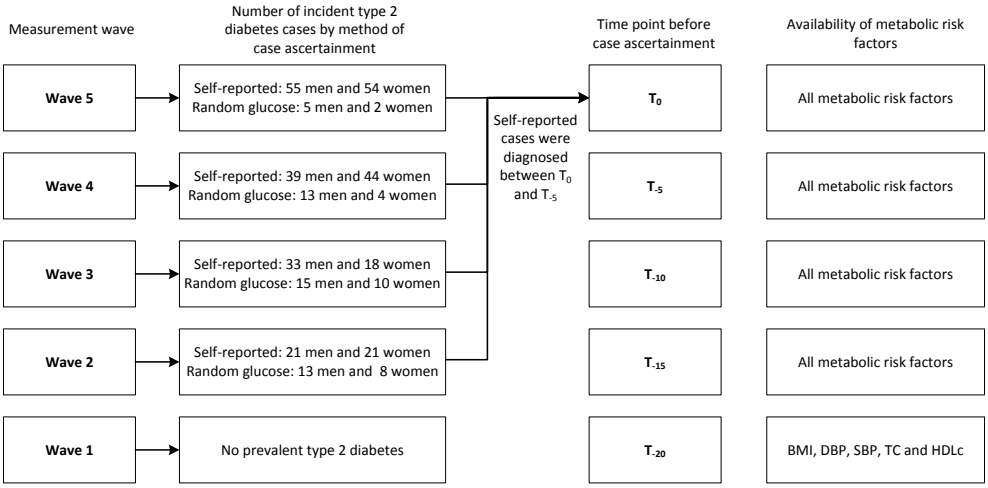


Figure 8.1. Flow chart of incident type 2 diabetes cases at each wave leading to the study population at case ascertainment (T_0).
Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; HDLc, high-density lipoprotein cholesterol; Note: for example, cases ascertained at wave 5 (T_0) had measurements of BMI, DBP, SBP, TC and HDLc for up to 20 years and measurements of other risk factors up to 15 years prior to diagnosis. Cases ascertained at wave 3 (T_0) had measurements of BMI, DBP, SBP, TC and HDLc for up to 10 years and measurements of other risk factors up to 5 years prior to case ascertainment.

Trajectories preceding case ascertainment were constructed by estimating the means at four or five points in time using linear generalised estimating equation models with an autoregressive correlation structure, separately for each metabolic risk factor and biochemical marker (dependent variable). This method takes the correlations between repeated measurements on the same participant into account. Analyses were stratified by sex and the model included linear, quadratic and cubic terms of age, examination wave, time as a categorical variable (i.e. examination wave) and diabetes status. Age was centred at 60 years, which was approximately the mean age at wave 5, and examination wave was centred at wave 5 to fit trajectories for a hypothetical population of 60 year olds in 2008-2012 (T_0). Trajectories of diastolic and systolic blood pressure were also adjusted for anti-hypertensive medications, and trajectories of total cholesterol, HDL cholesterol and triglycerides were adjusted for cholesterol-lowering medications. We log-transformed triglycerides, ALT, GGT and CRP and reported back-transformed geometric means since these biochemical markers did not have a normal distribution.

For participants with a self-reported diagnosis, the date of diagnosis was somewhere between the first wave in which they reported that they had diabetes (case ascertainment) and the previous wave. Treatment after diagnosis (that took place in between two successive waves of our study) may have changed the trajectories of participants with a self-

reported diagnosis, and would be reflected in the trajectory over the last five years before case ascertainment. Therefore, the trajectory over the last five years was not taken into account when testing differences between those with type 2 diabetes and controls in the total trajectories of metabolic risk factors and biochemical markers. The time from 15/20 years prior to case ascertainment up to five years prior to case ascertainment was used to statistically test differences. This was done using an interaction term for the interaction between diabetes status and time (dummy relating $T_{-15/-20}$ to T_{-5}), assuming a linear pattern over that period. Differences in trajectories of risk factors and biochemical markers between those subjects with type 2 diabetes and the controls during the last five years prior to case ascertainment were also tested using an interaction term for the interaction between the dependent variable diabetes status and time (dummy relating T_{-5} to T_0). A P-value<0.10 was considered statistically significant for interactions. The analyses were also stratified by method of case ascertainment (i.e. self-report and random glucose ≥11.1 mmol/L) to further investigate the potential effects of medical treatment after the diagnosis of type 2 diabetes among the self-reported cases during the five years preceding case ascertainment. This stratification was done for BMI, systolic blood pressure, total cholesterol and glucose since medical treatment after the diagnosis of type 2 diabetes is mostly directed at these risk factors. The stratification was restricted to male subjects since too few women were diagnosed on the basis of glucose levels (N=28). To investigate whether differences in trajectories between participants with type 2 diabetes and controls could be explained by BMI, trajectories were additionally adjusted for BMI and centred at 25 kg/m^2 in sensitivity analyses. All analyses were performed using SAS 9.3 software (SAS Institute, Cary, North Caroline, USA).

Results

In total, 194 men and 161 women developed type 2 diabetes. In those participants with type 2 diabetes and their matched controls, blood pressure, total cholesterol, HDL cholesterol and BMI were followed back in time for an average of 14.0 years while the other risk factors and biochemical markers were followed back for an average of 10.6 years. At case ascertainment (T_0), the average age was 60.5 (range: 34-80) for men and 61.2 (range: 33-80) for women. Participants with incident type 2 diabetes were more likely to have a low level of educational attainment and to be on anti-hypertensive and cholesterol-lowering medication (Table 8.1).

Trajectories

At 20 years prior to case ascertainment, those participants with type 2 diabetes had higher levels of BMI, and, although not statistically significantly so, more unfavourable levels of

diastolic and systolic blood pressure, total cholesterol and HDL cholesterol than controls (Figure 8.2A-E). At 15 years prior to case ascertainment, levels of other metabolic risk factors and biochemical markers were similar (glucose and eGFR) or higher (waist circumference, triglycerides, ALT, GGT, CRP and uric acid) among those subjects with incident type 2 diabetes compared with the matched controls (Figure 8.2F-M).

Table 8.1. Population characteristics of those participants with incident T2D and controls at case ascertainment (T_0), stratified by sex.

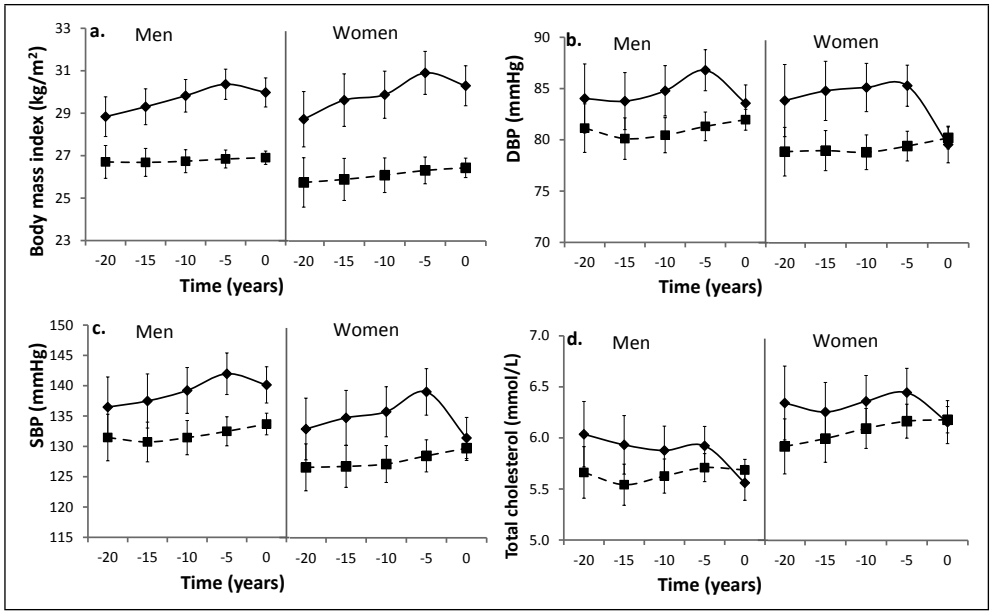
	Men		**Women**	
	T2D N=194	**No T2D** N=1,164	**T2D** N=161	**No T2D** N=966
Age (years)	60.4 ±8.8	60.5 ± 8.9	61.2 ±8.6	61.2 ±8.7
Low education	104 (54%)	531 (46%)	119 (74%)	630 (65%)
Smoking status				
Current smoker	38 (20%)	217 (19%)	61 (39%)	183 (19%)
Ex-smoker	118 (61%)	651 (56%)	35 (22%)	369 (38%)
Medication				
Anti-hypertensive	78 (40%)	201 (17%)	86 (54%)	212 (22%)
Cholesterol-lowering	68 (35%)	139 (12%)	64 (40%)	98 (10%)
Risk factors				
BMI (kg/m^2)	29.6 ±4.3	26.6 ±3.2	30.3 ±5.6	26.5 ±4.4
DBP (mm Hg)	84 ±11	83 ±10	80 ±9	81 ±10
SBP (mm Hg)	142 ±18	135 ±17	135 ±19	133 ±19
TC (mmol/L)	5.2 ±1.2	5.6 ±1.0	5.5 ±1.1	5.9 ±1.1
HDLc (mmol/L)	1.09 ±0.31	1.24 ±0.33	1.33 ±0.39	1.56 ±0.39
Random glucose (mmol/L)	9.5 ±3.7	5.4 ±1.1	8.0 ±4.3	5.2 ±0.9
WC (cm)	109 ±11	100 ±9	103 ±13	92 ±11
TG (mmol/L)	1.9 [1.3-2.7]	1.5 [1.1-2.1]	1.8 [1.4-2.4]	1.3 [1.0-1.7]
ALT (U/L)	22 [17-32]	18 [14-23]	19 [15-23]	15 [12-19]
GGT (U/L)	36 [25-60]	26 [20-39]	25 [17-37]	17 [13-25]
CRP (mg/L),	2.1 [1.1-3.9]	1.2 [0.6-2.5]	2.2 [1.1-4.6]	1.3 [0.6-2.5]
UA (mmol/L)	0.34 ±0.08	0.34 ±0.07	0.30 ±0.07	0.27 ±0.07
eGFR (ml/min/1.73 m^2)	92 ±18	89 ±16	87 ±20	87 ±15

Values are mean ±standard deviation, number and (percentage) or median and [interquartile range]. Abbreviations: T2D, type 2 diabetes; BMI, body mass index; WC, waist circumference; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; HDLc, high-density lipoprotein cholesterol; TG, triglycerides; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; CRP, C-reactive protein; UA, uric acid; eGFR, estimated glomerular filtration rate.

As regards the development in metabolic risk factors and biochemical markers during the 15-20 years prior to case ascertainment, those subjects with incident type 2 diabetes had in particular larger unfavourable changes over time (i.e. unfavourable trajectories) in BMI, HDL cholesterol and random glucose (P-value for interaction<0.01) (Figure 8.2, Tables 8.2-8.3). For example, BMI increased among men and women with type 2 diabetes by 5-8%

(2 kg/m^2) between T_{-20} and T_{-5}, while BMI increased among controls by 0-2%. Trajectories of diastolic and systolic blood pressure, waist circumference, triglycerides, ALT, GGT and CRP were also more unfavourable in those participants with type 2 diabetes than in the controls, although the difference was not statistically significant for triglycerides, ALT, GGT and CRP. For example, GGT increased by 13% among men with type 2 diabetes and by 5% among controls between T_{-15} and T_{-5}. During the last five years before case ascertainment, levels of metabolic risk factors and biochemical markers remained stable or decreased, except for glucose in both sexes and CRP in men.

Trajectories of uric acid and eGFR were more unfavourable (i.e. increasing for uric acid and declining eGFR) for women with incident type 2 diabetes than controls up to five years prior to case ascertainment (P-value for interaction<0.05), whereas there was no significant difference among men (P-value for interaction≥0.10). Trajectories of total cholesterol were not significantly different between those participants with incident type 2 diabetes and controls (P-value for interaction≥0.10) (Figure 8.2, Tables 8.2-8.3).



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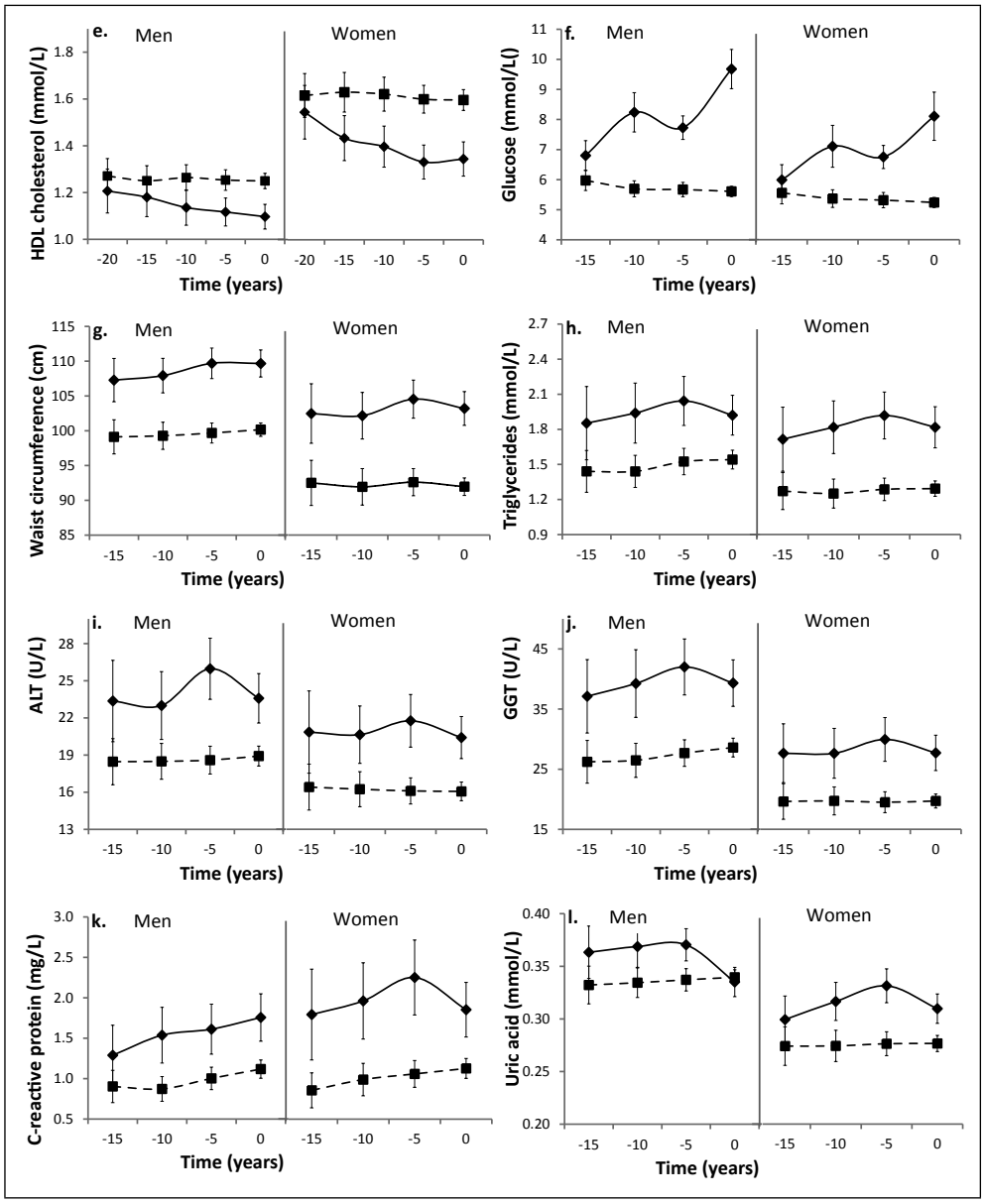

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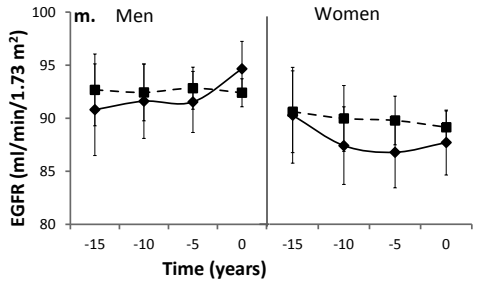

Figure 8.2. Trajectories of body mass index (a), DBP (b), SBP (c), total cholesterol (d), HDL cholesterol (e), random glucose (f), waist circumference (g), triglycerides (h), ALT (i), GGT (j), C-reactive protein (k), uric acid (l), and eGFR (m) of those with incident type 2 diabetes (solid lines) and controls (dashed lines) for men and women who were hypothetically 60 years old at the time of case ascertainment. Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate. Geometric means are shown for triglycerides, ALT, GGT and C-reactive protein.

Differences in trajectories between cases ascertained by self-report and by random glucose

Trajectories of BMI, systolic blood pressure, total cholesterol and random glucose appeared to be similar for participants ascertained by self-report and those ascertained by elevated random glucose up to five years prior to case ascertainment (Figure 8.3). In contrast, during the last five years prior to case ascertainment, levels of BMI, systolic blood pressure and total cholesterol decreased among participants with self-reported type 2 diabetes but not among participants diagnosed by elevated random glucose based on our study examination.

Adjustment for BMI

Adjustment for BMI strongly attenuated differences in trajectories between participants with incident type 2 diabetes and controls for all metabolic risk factors and biochemical markers except for random glucose (Supplementary Figure 8.1). After adjustment for BMI, differences in trajectories between subjects with type 2 diabetes and controls remained statistically significant only for random glucose in men and women, and HDL cholesterol, uric acid and eGFR in women (P-value for interaction<0.10).

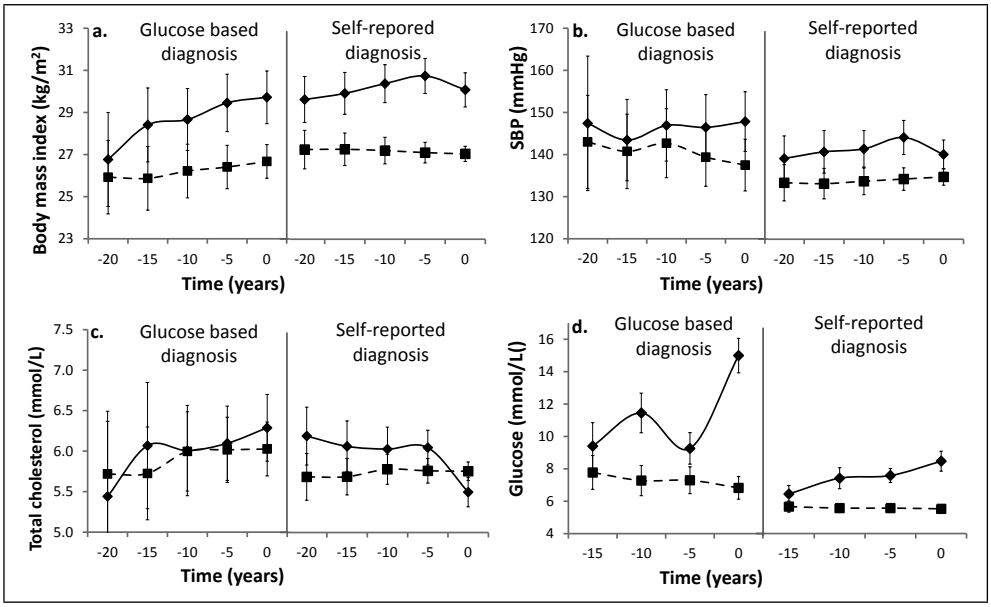

Figure 8.3. Trajectories of body mass index (a), SBP (b), total cholesterol (c) and random glucose (d) of those with incident type 2 diabetes (solid lines) and controls (dashed lines) for men who were hypothetically 60 years old at the time of case ascertainment, stratified by diagnosis based on glucose ≥11.1 mmol/L and self-reported diabetes.
Abbreviation: SBP, systolic blood pressure. Note: time before diagnosis ranged from -17.5 to 2.5 among the self-reported cases since participants were diagnosed somewhere between case ascertainment (year 0) and the previous wave.

Discussion

Men and women with incident type 2 diabetes had slightly more unfavourable levels of metabolic risk factors and biochemical markers than matched controls 15-20 years prior to diagnosis. Subsequent trajectories were also more unfavourable in participants with type 2 diabetes than controls for BMI, HDL cholesterol, glucose and to a lesser extent for diastolic and systolic blood pressure, waist circumference, triglycerides, markers of liver fat and chronic inflammation, uric acid and kidney function. The patterns were similar for men and women. Differences in trajectories between participants with type 2 diabetes and controls were much smaller after adjustment for BMI, with the exception of random glucose.

We observed that in our study, levels of metabolic risk factors and biochemical markers were often more favourable at case ascertainment than five years prior to ascertainment. Since the diagnosis of type 2 diabetes occurred at an unknown time point during the five years preceding case ascertainment, medical treatment and lifestyle changes will have often already started before the wave in which a respondent reported a diagnosis

of type 2 diabetes. This implies that medical treatment and lifestyle intervention after the diagnosis of type 2 diabetes had a large favourable impact on levels of almost all metabolic risk factors and biochemical markers. This is supported by analyses stratified by method of case ascertainment, which showed that the drop in BMI, systolic blood pressure and total cholesterol was only apparent in those participants with a self-reported diagnosis (diagnosed in the years before ascertainment), and not in those cases ascertained by elevated random glucose levels during the examination for our study.

Extending earlier findings that unfavourable changes in ALT and triglycerides precede the diagnosis of type 2 diabetes in men over a 1.5-year period,[11] we showed that differences in ALT, triglycerides and additionally GGT between men and women with incident type 2 diabetes and controls already exist 10 to 15 years before the onset of type 2 diabetes. These differences continue to increase until diagnosis, although no longer at a statistically significantly more unfavourable rate in persons with type 2 diabetes than in the controls. The increase in ALT and GGT, indicating hepatic fat accumulation, leads to higher concentrations of very low density lipoprotein (VLDL) particles in the circulation, which may lead to hypertriglyceridemia and lower HDL cholesterol.[26] This is consistent with our observed unfavourable trajectories in triglycerides and HDL cholesterol that occurred concurrently with unfavourable trajectories in ALT and GGT among those participants with incident type 2 diabetes.

The present work also extends previous work on the relation between uric acid and type 2 diabetes[27] by showing that unfavourable changes in uric acid precede the diagnosis of type 2 diabetes in women but not in men over a period of more than 10 years. This is in line with results from a meta-analysis that showed that each ml/dl increase in uric acid increased the risk of type 2 diabetes by 28% among women but only by 9% among men.[27] This indicates that uric acid may be a more important factor for the development of type 2 diabetes in women than men. The observed sex difference could be related to hormonal differences. For example, uric acid levels in women have been shown to increase due to menopause-related changes in their metabolism[28] and due to hormone replacement therapy.[29] Sex differences might also reflect differences in other metabolic risk factors related to uric acid, such as drug use and dietary patterns. Furthermore, although it is still uncertain whether elevated uric acid is causally related to type 2 diabetes,[30] possible mechanisms include increased oxidative stress, low-grade inflammation and endothelial dysfunction, which are all related to the development of type 2 diabetes.[31-33]

Table 8.2. Percentage change in metabolic risk factors and biochemical markers over time for men with incident type 2 diabetes and controls.

	$T_{-15/20}$ to T_{-5} [a]		T_{-5} to T_0	
	T2D	**No T2D**	**T2D**	**No T2D**
Body mass index	5%	0%***	-1%	0%***
Diastolic blood pressure	3%	0%*	-4%	1%***
Systolic blood pressure	4%	1%*	-1%	1%**
Total cholesterol	-2%	1%	-6%	0%***
HDL cholesterol	-7%	-1%***	-2%	0%
Random glucose	14%	-5%***	25%	-1%***
Waist circumference	2%	1%**	0%	0%
Triglycerides	10%	6%	-6%	1%
Alanine aminotransferase	11%	1%	-9%	2%**
Gamma glutamyltransferase	13%	5%	-6%	3%**
C-reactive protein	25%	11%	9%	11%
Uric acid	2%	1%	-10%	1%***
Estimated glomerular filtration rate	1%	0%	3%	0%***

Asterisks (*) indicate a difference between those with incident type 2 diabetes and controls: * P-value<0.10, **P-value< 0.05, ***P-value<0.01; [a] T: time, indicating the number of years before ascertainment of type 2 diabetes or the same point in time for matched controls.

Table 8.3. Percentage change in metabolic risk factors and biochemical markers over time for women with incident type 2 diabetes and controls.

	$T_{-15/20}$ to T_{-5} [a]		T_{-5} to T_0	
	T2D	**No T2D**	**T2D**	**No T2D**
Body mass index	8%	2%***	-2%	0%***
Diastolic blood pressure	2%	1%	-7%	1%***
Systolic blood pressure	5%	1%*	-5%	1%***
Total cholesterol	2%	4%	-4%	0%***
HDL cholesterol	-14%	-1%***	1%	0%
Random glucose	13%	-4%***	20%	-2%***
Waist circumference	2%	0%*	-1%	-1%
Triglycerides	12%	1%	-5%	0%
Alanine aminotransferase	4%	-2%	-6%	0%
Gamma glutamyltransferase	8%	-1%	-7%	1%*
C-reactive protein	26%	24%	-18%	6%**
Uric acid	11%	1%***	-6%	0%***
Estimated glomerular filtration rate	-4%	-1%**	1%	-1%

Asterisks (*) indicate a difference between those with incident type 2 diabetes and controls: * P-value<0.10, **P-value< 0.05, ***P-value<0.01; [a] T: time, indicating the number of years before ascertainment of type 2 diabetes or the same point in time for matched controls.

In line with our findings, data from the Whitehall II study and the Framingham Heart Study showed that subjects with type 2 diabetes had higher mean BMI levels than controls at 18 and 20 years prior to diagnosis respectively.[14, 34] However, we also observed more unfavourable trajectories of BMI among those participants who developed type 2 diabetes, while the Whitehall II study found no difference in the trajectories of BMI.[14] Our findings indicate that, independent of whether the subject is overweight, gaining weight is important in the development of type 2 diabetes. Contrasting findings might be the result of matching on age, sex and examination wave in our study, leading to a stricter adjustment for age and a smaller difference in selective dropout between those participants with type 2 diabetes and controls. This may have led to trajectories of controls that were more favourable in our study than in the Whitehall II study, and thereby to larger differences in trajectories between those subjects with type 2 diabetes and controls.

In general, the incidence of type 2 diabetes is relatively low before the age of 45 and increases exponentially thereafter, with approximately 90% of the incident type 2 diabetes cases being diagnosed after the age of 45.[35-37] Since the present study showed that differences in trajectories of metabolic risk factors and biochemical markers between those with incident type 2 diabetes and controls start to develop more than 15-20 years before diagnosis, this indicates that measures to prevent type 2 diabetes are already warranted before the age of 25 and onwards. Our results showed particularly unfavourable changes in adiposity, HDL cholesterol and random glucose, and to a lesser extent in blood pressure, triglycerides, markers of liver fat accumulation and chronic inflammation, uric acid and kidney function. Obesity is a major risk factor for dyslipidaemia, hypertension, liver fat accumulation, chronic inflammation and kidney dysfunction,[38-41] and BMI largely explained unfavourable trajectories in those metabolic risk factors and biochemical markers among participants with type 2 diabetes. Thus, our findings highlight the need for lifestyle interventions to promote the maintenance of a healthy weight from young adulthood onwards to reduce the burden of type 2 diabetes. Our results further suggest that it may be of interest to investigate whether repeated measurements of risk factors can improve risk prediction of type 2 diabetes.

One of the strengths of the present study is that the same group of trained study personnel objectively measured various metabolic risk factors and biochemical markers in a population-based cohort at four or five points in time over a long follow-up period. We were able to describe long-term trajectories for men and women separately, and match each participant with incident type 2 diabetes with three controls on age and sex. The limitations of the present study include the limited number of participants with type 2 diabetes with a follow-up period of 15 or 20 years, leading to relatively large 95% confidence intervals for 15 and 20 years prior to case ascertainment. We identified participants with type 2 diabetes based on self-report or random glucose levels in blood plasma. Most of these self-

reported cases were confirmed by the general practitioner or pharmacist and a validation study indicated a high level of accuracy of self-reported diagnosis in our population.[23] Nevertheless, misclassification may have occurred, including cases not detected by random glucose levels, which could have led to underestimation of the differences in trajectories between those participants with incident type 2 diabetes and controls. Furthermore, individuals who participate in cohort studies are generally healthier and better educated than non-responders, and participants who were excluded and those who dropped out during follow-up also had slightly less favourable levels of the investigated risk factors at baseline. This has most likely led to underestimation of the number of participants with type 2 diabetes and differences in trajectories between people with type 2 diabetes and controls.

Our results showed that metabolic risk factors and biochemical markers were more unfavourable in people with type 2 diabetes than controls 15-20 years or more before diagnosis of type 2 diabetes and that BMI, HDL cholesterol, random glucose and to a lesser extent diastolic and systolic blood pressure, waist circumference, triglycerides, liver fat and inflammatory markers, uric acid and kidney function gradually deteriorate further up to diagnosis. Unfavourable changes in these metabolic risk factors and biochemical markers occurred at the same time and showed a similar pattern in men and women. Differences in trajectories between subjects with incident type 2 diabetes and controls seemed to be explained largely by unfavourable changes in BMI among participants with type 2 diabetes, stressing the importance of maintaining a healthy weight. These findings underscore the need for primary prevention that starts more than 15 years before the diagnosis of type 2 diabetes, i.e. from young adulthood onwards.

Acknowledgement

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Supplementary Methods

Measurements

At each examination, body weight was measured to the nearest 0.1 kg on calibrated scales and 1 kg was subtracted to adjust for clothing and height was measured to the nearest 0.5 cm. BMI was calculated as weight divided by height squared (kg/m^2). Waist circumference was measured twice to the nearest 0.5 cm, at the level midway between the lowest rib and the iliac crest at the end of expiration, with participants in standing position. The mean of two measures was used for analysis. Diastolic and systolic blood pressure levels were measured twice after 2 minutes of rest and the average of these two measurements was used in the analyses. Participants were measured in sitting position with a random zero sphygmomanometer (Hawksley and Sons, Lancing, UK) in waves one to three. In waves four and five a Speidel Keller meter (Welch Allyn, Skaneateles Falls, NY, USA) was used. Mean diastolic and systolic blood pressure levels measured at wave four were unexpectedly higher compared to the blood pressure levels in the previous and following waves. No clear cause could be identified, therefore, we statistically corrected blood pressure values of wave 4, as described extensively elsewhere (Supplementary Methods Chapter 6).[1] Total cholesterol and HDL cholesterol were measured until 1998 in non-fasting EDTA-plasma and from 1998 onwards in serum at the Lipid Reference Laboratory of the University Hospital Dijkzigt in Rotterdam, using standardised enzymatic methods. In 2013-2014, standardised enzymatic methods (Roche/Hitachi Modular P analyzer, Mannheim, Germany) were used to retrospectively determine additional metabolic risk factors from waves 2-5 in non-fasting plasma samples that had been stored at -20 degree Celsius until June 1995 and at -80 degree Celsius from July 1995 onwards. Gamma-glutamyltransferase (GGT), uric acid, triglycerides (GPO-PAP assay) and alanine aminotransferase (ALT) (kinetic UV assay) were measured with a colorimetric method. ALT measurements until June 1995 were recoded as missing (N=2,495) because during those years blood plasma was stored at -20 degree Celsius, a temperature at which ALT has poor stability.[2] High sensitivity CRP was measured with the principle of particle-enhanced immunological agglutination (Tina-quant CRP) and cystatin C measurement was based on a particle enhanced-turbidimetric immunoassay using reagents from Gentian (Gentian, Moss, Norway). Creatinine was measured with a Creatinine Plus assay (IDMS traceable).

Supplementary references

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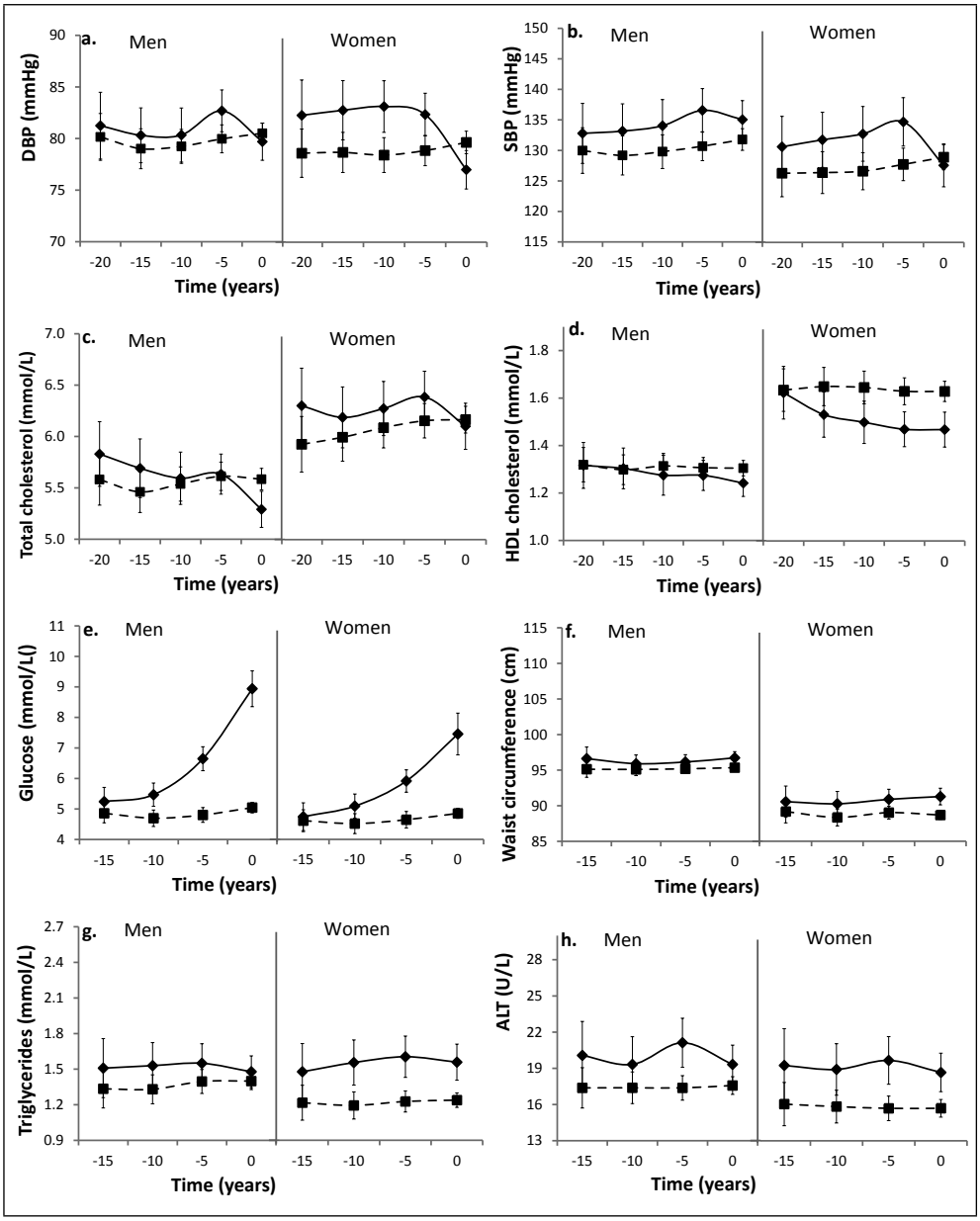

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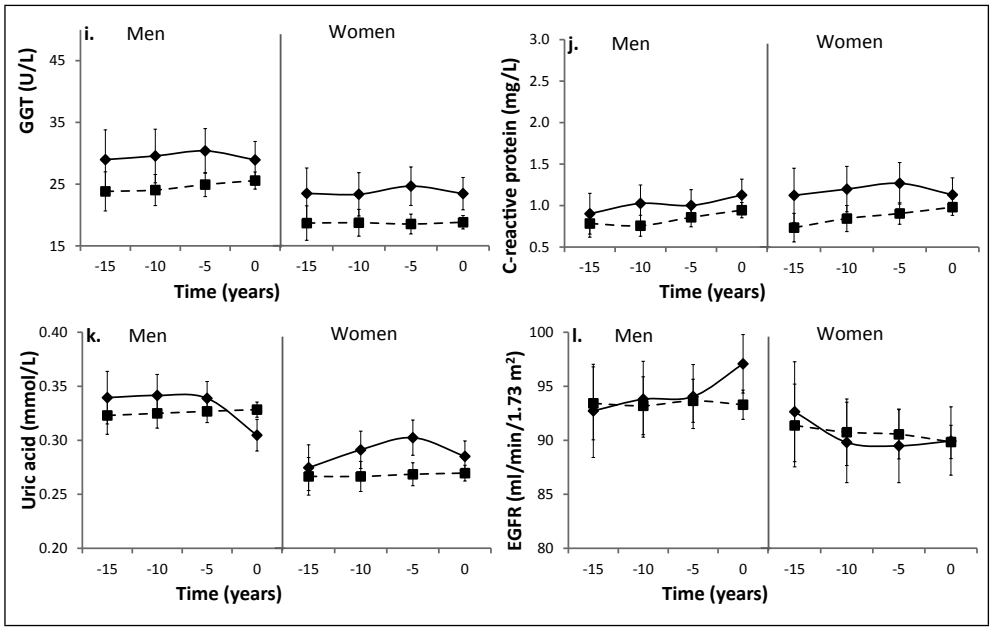
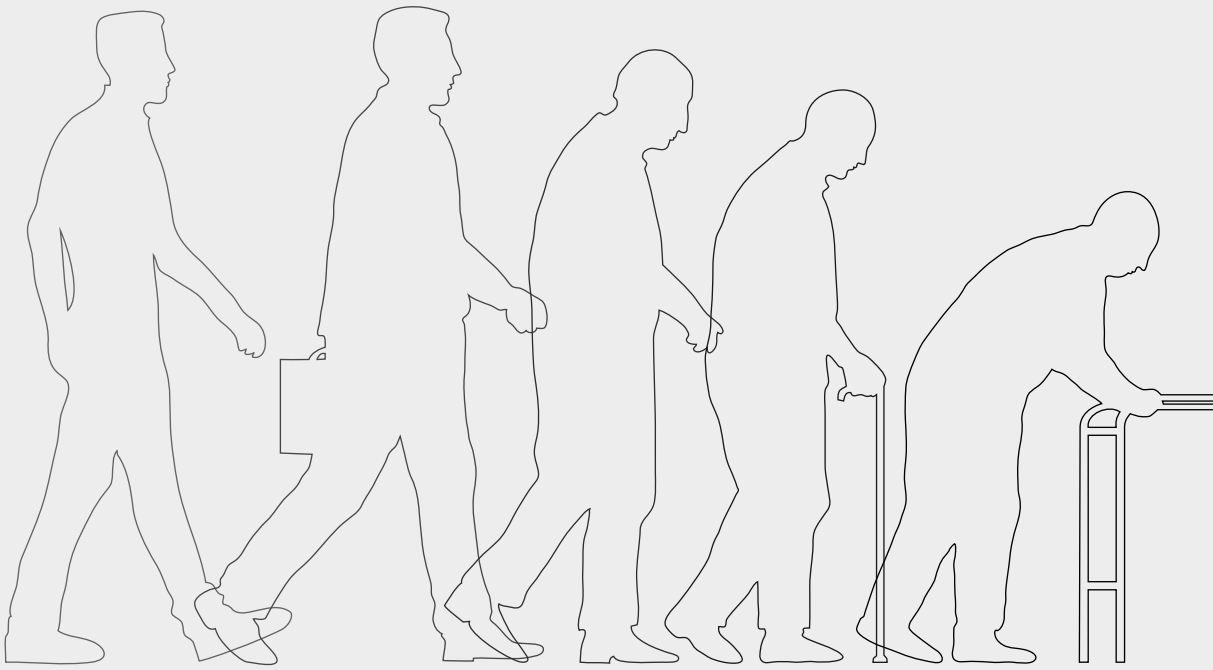


Supplementary Figure 8.1. Results adjusted for body mass index. Trajectories of DBP (a), SBP (b), total cholesterol (c), HDL cholesterol (d), random glucose (e), waist circumference (f), triglycerides (g), ALT (h), GGT (i), C-reactive protein (j), uric acid (k), and eGFR (l) of those with incident type 2 diabetes (solid lines) and controls (dashed lines) for men and women who were hypothetically 60 years at the time of case ascertainment.
Abbreviations: DBP, diastolic blood pressure; SBP, systolic blood pressure; ALT, alanine aminotransferase; GGT, gamma glutamyltransferase; eGFR, estimated glomerular filtration rate. Geometric means are shown for triglycerides, alanine aminotransferase, gamma glutamyltransferase and C-reactive protein.

Chapter 9

General discussion



In this thesis, medium-term and long-term changes in lifestyle and metabolic risk factors were studied in relation to the risk of cardiovascular diseases (CVD), using data from the longitudinal population-based Doetinchem Cohort Study. Also, changes across generations were analysed. Age-specific levels and prevalences of metabolic risk factors and biochemical markers in four generations were described in **part I**; associations of medium-term lifestyle profiles and long-term metabolic risk profiles with risk of CVD were presented in **part II**; and trajectories of metabolic risk factors and biochemical markers preceding CVD and type 2 diabetes were described in **part III**. In this chapter, the main findings are reviewed and implications for public health and future research are discussed.

Part I - Metabolic risk factors across generations

Main findings

- At any given age, younger generations had a higher prevalence of overweight and obesity than older generations. These unfavourable generation shifts in overweight and obesity were observed across all generations of men and the youngest generations of women (**chapter 2**).

- Unfavourable generation shifts were also observed for the prevalence of hypertension, but not for the prevalence of hypercholesterolemia, low HDL cholesterol and levels of markers of oxidative stress and inflammation (**chapter 2** and **3**).

- Levels of obesity-related biochemical markers increased with age in most generations of men and women. This was most pronounced in individuals whose body mass index increased with age (about half of the population) (**chapter 3**).

Interpretation

Obesity and the number of cases of type 2 diabetes and cardiovascular disease in the Netherlands

The findings of **chapter 2** indicate a higher age-specific prevalence of obesity in each successive generation of men and in the youngest generation of women (i.e. 20-29 years). This implies that the increasing prevalence of obesity in the general adult population over the last decades resulted from an increasing trend in age-specific prevalence of obesity in each younger generation of men and in the youngest generation of women. With obesity developing at a younger age, an increase can be expected in lifelong exposure to obesity in current and future generations compared to previous generations. In all adult generations,

the increase in obesity prevalence with ageing was associated with concomitant unfavourable changes in cardio-metabolic markers (**chapter 3**). These findings suggest that current and future generations are at a higher risk of chronic diseases such as type 2 diabetes and CVD.

The number of people diagnosed with type 2 diabetes has more than doubled in the period from 2000 to 2013.[1-3] This enormous increase in the number of type 2 diabetes cases was the result of growth and ageing of the population, the intensified and improved detection of type 2 diabetes by general practitioners and the increase in the prevalence of obesity as a risk factor for diabetes.[1-3] A recent modelling study showed that when the current prevalence of overweight and obesity would remain stable over time, the number of type 2 diabetes cases in the Netherlands is projected to increase from 0.9 million in 2013 to 1.1 million in 2035,[3] solely as a result of growth and ageing of the population. Another modelling study projected the increase in the number of type 2 diabetes cases under different scenarios. With the obesity prevalence increasing at the present rate, about 80.000 extra type 2 diabetes cases will occur over a 20-year period compared to a scenario where the prevalence of obesity remains stable.[4] Thus, both past and future trends in the prevalence of obesity have a major effect on the number of type 2 diabetes cases.

With respect to CVD, the age-standardised mortality rate of CVD has been decreasing since the 1970s, and is expected to decrease further.[5, 6] A recent modelling study explored the contribution of changes in risk factor levels to the decline in age-standardised mortality from coronary heart disease in the Netherlands during the period from 1997 to 2007.[7] This study concluded that in addition to the observed decline in coronary heart disease, 10% more cases could have been prevented or postponed if body mass index and type 2 diabetes would not have increased. The unfavourable generation shifts in obesity will continue to have an unfavourable impact on the number of CVD cases in the coming years. Although the decrease in age-standardised mortality rate of CVD is expected to continue,[5, 6] the modelling study indicates that the rate of decrease in CVD may slow down as a result of the unfavourable trends in obesity. In conclusion, the unfavourable trends in the prevalence of obesity contributed to the large increase in the number of type 2 diabetes cases over the last decades. The effect of unfavourable trends in the prevalence of obesity on the number of CVD cases is less clear, as age-standardised mortality rates have been declining over the last decades. Yet, a large number of CVD cases were attributable to the increase in the prevalence of obesity.

Concomitant changes in other cardiovascular risk factors and the effect on cardiovascular disease

The future trends in type 2 diabetes and CVD are not only determined by trends in overweight and obesity but also by trends in other risk factors such as smoking. With data from the Doetinchem Cohort Study, it has previously been shown that the prevalence of

smoking was lower in each younger generation of men and in the youngest generation of women.[8] The generations with an unfavourable shift in obesity prevalence simultaneously showed a favourable shift in smoking prevalence. Also, the use of preventive medication for CVD such as cholesterol-lowering drugs has increased over the last decades and favourable generation shifts were observed for total and HDL cholesterol (**chapter 2**).[9] Overall, favourable generation shifts in several cardiovascular factors may partly counteract the unfavourable shifts in overweight and obesity. Thus, it remains to be seen how the generation shifts in all cardiovascular risk factors combined influences future trends in CVD.

Part II - Cardiovascular risk factor profiles and risk of cardiovascular disease

Main findings

- Only 16% of the current young and middle-aged generations maintained a healthy lifestyle profile (i.e. adhering to at least four of five healthy lifestyle factors) over a period of five years (**chapter 4**).

- Young and middle-aged generations who maintained a healthy lifestyle profile over a period of five years had a 2.5 times lower risk of CVD during a subsequent period of follow-up of 8-15 years than adults who maintained an unhealthy lifestyle profile (**chapter 4**).

- Deterioration in lifestyle profile in a period of five years resulted in a 35% higher risk of CVD for each healthy lifestyle factor lost during subsequent follow-up (8-15 years), while improvement in lifestyle profile did not significantly lower CVD risk (**chapter 4**).

- Only 7% of the current young and middle-aged generations maintained a low metabolic risk profile (i.e. ideal levels of the 'major' metabolic risk factors, non-smoking and no diabetes) over a period of 11 years (**chapter 5**).

- Young and middle-aged generations with a long-term low metabolic risk profile had a 7 times lower risk of CVD during the subsequent 5-10 years follow-up than adults who maintained a high metabolic risk profile (**chapter 5**).

- Improvement in metabolic risk profile in a period of 11 years resulted in a twofold lower risk of CVD the following 5-10 years and deterioration in metabolic risk profile in a twofold higher risk of CVD (**chapter 5**).

- Adherence to a Mediterranean diet, physical activity, and moderate alcohol consumption increased the likelihood of attaining a low metabolic risk profile (**chapter 6**).

Interpretation

Improvement in lifestyle profile and metabolic risk profile

The findings in this thesis suggest that for the larger part of adults it is difficult to maintain a healthy lifestyle profile, defined as adhering to a healthy diet, being physically active, not smoking, drinking alcohol in moderation and not being obese, during adulthood (**chapter 4**). This is even more so for the components of the low metabolic risk profile (favourable levels of blood pressure, cholesterol and no diabetes) (**chapter 5** and **6**), where, besides modifiable (often lifestyle) factors, also non-modifiable factors such as genetic susceptibility play a role.[10] Intervention studies in mainly 'high-risk' populations have shown that improvements in lifestyle factors may lower blood pressure, cholesterol and the risk of type 2 diabetes.[11-15] It remains, however, unclear from these studies whether the low metabolic risk profile (low levels of all 'major' CVD risk factors simultaneously) can be attained and maintained by adopting a healthy lifestyle profile in young and middle-aged adults. **Chapter 6** indicates that adherence to a healthy diet, being physically active and moderate alcohol intake increases the likelihood of attaining a low metabolic risk profile in young and middle-aged adults. In line with those findings, young adults who maintained a healthy lifestyle profile over a period of 20 years were more likely to have a low metabolic risk profile at the end of that period than others.[16] Thus, improvements in lifestyle profile are likely to lead to a higher proportion of adults with low levels of all 'major' CVD risk factors.

Public health impact

To estimate which fraction of the CVD burden can be attributed to suboptimal lifestyle profiles and suboptimal metabolic risk profiles, the population attributable risk was estimated (see footnote Table 9.1 for method). When estimates were based on a single, baseline, measurement, 25% of the CVD cases were attributable to suboptimal lifestyle profiles (Table 9.1). When taking into account the lifestyle profiles over a five-year period, a substantially greater proportion of 40% of the CVD cases could be attributed to suboptimal lifestyle profiles, implying that approximately 165.000 non-fatal/fatal CVD cases yearly in the Netherlands can be attributed to the low number of people who maintain a healthy lifestyle in the long term. Attributable fractions were considerably greater for metabolic risk profiles than for lifestyle profiles. That is, when estimates of the prevalence of a low metabolic risk profile were based on one baseline measurement at a single point in time, approximately 200.000 (49%) of the CVD cases were attributable to suboptimal metabolic risk profiles. When metabolic risk profiles over an 11-year period were taken into account,

approximately 355.000 (86%) non-fatal/fatal CVD cases yearly in the Netherlands could be attributed to the fact that most adults do not maintain a low metabolic risk profile in the long term. In conclusion, the low prevalence of a healthy lifestyle profile and low metabolic risk profile have a major public health impact, and maintaining these favourable profiles in the long term is of great importance.

Table 9.1. Public health impact of (medium-term) healthy lifestyle profile and (long-term) low metabolic risk profile on non-fatal/fatal cardiovascular disease.

	Prevalence (%)	Population attributable risk (%) [a]	Number of cardiovascular disease cases attributable to suboptimal lifestyle profiles and metabolic risk profiles in the Netherlands [b]
Healthy lifestyle profile, single point in time	29%	25%	100,000
Maintaining a healthy lifestyle profile over a five-year period	16%	40%	165,000
Low metabolic risk profile, single point in time	12%	49%	200,000
Maintaining a low metabolic risk profile over an 11-year period	7%	86%	355,000

[a] The population attributable risk (PAR) was calculated using the prevalence of all (medium-term) lifestyle profiles and (long-term) metabolic risk profiles (i.e. exposures), and their hazard ratios (HRs) of cardiovascular disease, with (medium-term) healthy lifestyle profile and (long-term) low metabolic risk profile as reference. The following formula was used: PAR = prevalence exposed (HR-1) / [1 + prevalence exposed (HR-1)]. For each lifestyle profile and metabolic risk profile the PAR was calculated, leading to two PARs based on the baseline lifestyle profiles, six PARs based on the medium-term lifestyle profiles, three PARs based on the baseline metabolic risk profiles and nine PARs based on the long-term metabolic risk profiles. To obtain a combined PAR, the separate PARs of each of the four profiles were combined as follows: 1-[(PAR1-1)(PAR2-1)(PAR3-1)….]. [b] The combined PARs were used to calculate the number of non-fatal/fatal cardiovascular disease cases that could theoretically be attributed to suboptimal lifestyle profiles and suboptimal metabolic risk profiles, considering that 413.772 incident non-fatal/fatal cardiovascular disease events occurred in the Netherlands in 2012,[17] numbers were rounded to the nearest 5,000.

Changes in lifestyle profile and metabolic risk profile and risk of cardiovascular disease

Changes with age in lifestyle profile and metabolic risk profile have a large effect on the risk CVD. In **chapter 4**, it was shown that each healthy lifestyle factor lost over a period of five years was associated with on average a 35% higher risk of CVD in subsequent years. Improvement in lifestyle did, however, not lower risk, implying that the benefits of healthy lifestyles are easier lost than gained. Long-term poor lifestyle habits at younger ages may already have resulted in considerable damage in early adulthood or middle age, and improvement in lifestyle in a five-year period (implying an improved lifestyle over a period of at most five years but probably less), might be insufficient to lower the risk of CVD. This illustrates the importance of maintaining a healthy lifestyle profile throughout the life course.

Improvement in metabolic risk profile – not due to treatment effects – in an 11-year period was associated with risk reduction: improvement resulted in an approximate twofold lower risk of CVD (**chapter 5**). The observation that metabolic risk profiles were more strongly associated with risk of CVD than the lifestyle profile, could be explained by the fact that metabolic risk factors are more proximal to development of CVD. Also, the association may be less subject to misclassification than the lifestyle factors since these can be measured more accurately. Maintaining a low metabolic risk profile from a young age onwards is obviously to be preferred, but this finding shows that it is "never too late" for improvement in metabolic risk profile to lower the risk of CVD.

Part III - Trajectories of metabolic risk factors and biochemical markers preceding cardiovascular disease and type 2 diabetes

Main findings

- Already about 15-20 years before diagnosis, individuals with incident CVD and type 2 diabetes had more unfavourable levels of most metabolic risk factors and biochemical markers than controls (**chapter 7** and **8**).

- Yet, apart from these initial levels, for most metabolic risk factors and biochemical markers the trajectories did not show an increased rate of change in individuals who developed CVD compared to controls during the 15-20 years preceding disease occurrence (**chapter 7**); levels of metabolic risk factors and biochemical markers deteriorated more rapidly in individuals who developed type 2 diabetes than in controls (**chapter 8**).

Interpretation

Trajectories of metabolic risk factors and biochemical markers before disease onset: differences between cardiovascular disease and type 2 diabetes

Although CVD and type 2 diabetes share a number of risk factors, the trajectories of these risk factors preceding diagnosis differ. For CVD small differences in metabolic risk factors and biochemical markers between cases and controls were already present 15-20 years before diagnosis (**chapter 7**). In general, these differences remained constant during these subsequent 15-20 years. This implies that adults who develop CVD follow 'normal' age-related trajectories of metabolic risk factors and biochemical markers during the 15-20 years before disease onset with a more unfavourable level of risk factors but similar trajectory as adults who do not develop CVD. In contrast, for type 2 diabetes, levels of metabolic risk factors and biochemical markers deteriorated more rapidly in cases than in controls over

the 15-20 years before diagnosis (**chapter 8**). This more rapid deterioration of levels of metabolic risk factors and biochemical markers was likely largely due to the more rapid increase in body mass index in individuals with type 2 diabetes than in controls. This is consistent with previous work that has shown that obesity plays an important part in the development of insulin resistance and beta-cell dysfunction, which eventually progresses to type 2 diabetes.[18-21] Moreover, increasing BMI is associated with several of the other risk factors, such as hypertension and hypercholesterolemia.[22, 23] Overall, these findings strongly suggest that the process leading to type 2 diabetes is mainly driven by increasing adiposity during adulthood, whereas the process leading to CVD is driven by the adverse effects of multiple risk factors starting earlier in life. If this is the case, the prevention of CVD and that of type 2 diabetes would require different strategies to be optimally effective. In particular, the window for starting an intervention should probably be focussing on different stages of life, and prevention should be targeted at different risk factors.

The finding that unfavourable levels of metabolic risk factors and biochemical markers are already present long before the onset of symptomatic CVD (**chapter 7**) reinforces earlier evidence that the process leading to CVD often begins early in life. This may be due to an inherited biological predisposition to unfavourable levels of metabolic risk factors and/or deterioration of metabolic risk factors in utero/childhood/adolescence due to adverse environmental factors. Previous studies also showed that already in children and adolescents, suboptimal levels of metabolic risk factors were often present, and were associated with atherosclerosis.[24-26] Metabolic risk factors in childhood and adolescence have also been shown to track into adulthood.[27-30] Thus, unfavourable levels of metabolic risk factors in childhood may persist over time and can eventually lead to CVD.

Small differences in mean levels of risk factors and cardiovascular disease

The differences in metabolic risk factors and biochemical markers between individuals with and without CVD seem relatively small (e.g. body mass index differed by 0.8 kg/m^2) (**chapter 7**). This is in line with the results of a paper from the Framingham Heart study in the early 1970s, that reported minor difference in the distribution of total cholesterol between individuals with incident coronary heart disease and controls.[31] The INTERHEART study, including data from 52 countries, observed even smaller differences than observed in this thesis: a difference in body mass index of only 0.3 kg/m^2 and of HDL cholesterol of only 0.03 mmol/L between individuals with myocardial infarction and controls.[32, 33]

Yet, when looking at the extreme ends of the risk factor distribution, i.e. those with the highest levels versus those with the lowest levels, large differences in CVD risk exist (**part II**). When comparing individuals with low levels of all 'major' CVD risk factors (low metabolic risk profile) with individuals with high levels of all 'major' CVD risk factor (high metabolic risk profile) even larger differences in CVD risk exist. Thus, the relatively small mean differences

in the risk factor distribution between those with and without CVD may still cause a high CVD risk when a number of such risk factors are present in the same individual. Moreover, an unfavourable shift in the mean level of a risk factor in the whole population leads to an increased risk of CVD across the entire risk factor distribution. It does not only lead to a higher number of CVD cases among the group with high risk factor levels but also among the group with low and intermediate risk factor levels.[34-36] In addition, there is the cumulative effect over time: the mean levels of many risk factors were higher in CVD cases than in controls over a long period (at least 15 years). Thus, the relatively minor differences in mean risk factor levels between those with and without CVD, may at first sight seem irrelevant, but are actually important differences in CVD risk. These differences become fully visible when considering the population as a whole, and taking into account interaction and additive effects of multiple risk factors as well as the accumulation of adverse effects of risk factors over time.

Implications for public health

Monitoring metabolic risk factors

Information about the metabolic risk factor burden in the general population becomes outdated rapidly, as new generations carry different metabolic risk profiles, and these change the burden of the population as a whole (**part I**). In addition, metabolic risk factors often deteriorate with age, which increases the overall burden of CVD in an ageing population (**part I-III**). These findings emphasise the need for monitoring of metabolic risk factors in the future in order to be able to estimate the future disease burden and to set priorities for interventions. Special focus on obesity-related diseases and, in particular, type 2 diabetes is needed, since these will be major public health problems when today's young and middle-aged generations reach old age.

Prevention at an environmental and population level

In this thesis, unfavourable changes with age in lifestyle factors, metabolic risk factors and biochemical markers were shown in all four generations (**part I-III**). The findings of **part II** of this thesis indicate that the combination of healthy lifestyle factors and favourable metabolic risk factor levels had a large effect on reduction of the risk of CVD, especially when favourable levels are maintained over time. This emphasises the importance of primary prevention that influences the distribution of risk factors in the whole population.[37, 38] Population-oriented approaches to primary prevention, such as removal of trans fatty acids from products and taxation of sugar sweetened beverages, have the potential of shifting risk factor levels of the whole population in a favourable direction, including those at high risk of CVD, intermediate

risk and low risk. This implies that risk reduction is accomplished in a large proportion of the population, and, therefore, it leads to a significant risk reduction on the population level.

The unfavourable changes in metabolic risk factors and specifically in obesity were most likely the result of behavioural and environmental changes over time in the population.[39, 40] Major environmental drivers of unfavourable changes might be, for example, the increased supply of easily available cheap, energy-dense foods; increased marketing; urbanisation; motorisation and computerisation.[39, 40] This stresses the need for a renewed and reinvigorated emphasis on population-based prevention that promotes health and changes the environment in such a way that the healthy choice becomes the preferred choice. The ultimate goal should be to make it possible for the whole population to maintain a healthy lifestyle profile throughout the whole life course.

The presence of unfavourable levels of metabolic risk factors and biochemical markers long before the onset of CVD and type 2 diabetes shows that prevention should start at least 15-20 years earlier than symptomatic disease is expected to become manifest. This means starting prevention in young adulthood or even before, given that CVD and type 2 diabetes event rates increase progressively after the age of 45, and, of course, given that we want to prevent or postpone as many cases as possible.[17, 41-45] The urgency of such an approach is emphasised by the findings that a low metabolic risk profile was so rare in our study (**part II**). Other studies have also shown that the proportion of children with healthy lifestyle factors and favourable metabolic risk factor levels is higher than in adults.[46, 47] This implies that favourable lifestyle profiles and metabolic risk profiles are often lost during, or shortly after adolescence. The findings of this thesis reinforce earlier evidence that prevention should start early in life.

Several initiatives to improve lifestyle profiles and metabolic risk profiles of the population are currently taking place. An important initiative is the focus on cardiovascular health of the American Heart Association that started in 2010 with the launch of its 2020 goals.[48] The American Heart Association defined cardiovascular health as ideal based on four behavioural factors ('ideal health behaviours': non-smoking, healthy diet, low body mass index and physical activity at goal levels) and three metabolic risk factors ('ideal health factors': total cholesterol, blood pressure and fasting glucose all below threshold), comparable to the definitions of a healthy lifestyle profile and low metabolic risk profile in this thesis. Cardiovascular health is not just the absence of high risk factor levels. Rather it means having all lifestyle factors and metabolic risk factors at ideal levels, i.e. better than suboptimal, without needing drug treatment to achieve such levels. The focus on cardiovascular health was also adopted by other cardiovascular societies such as the European Society of Cardiology and the European Association for Cardiovascular Prevention and Rehabilitation. In a recent joint statement by these American and European associations, a policy was proposed aimed at creating a paradigm shift from a focus on sickness and

disease towards wellness and prevention to achieve the adoption of a healthy lifestyle profile in the whole population.[49] To achieve this goal new strategic directions in research, clinical, public health policy and advocacy programs are now being implemented that focus on increasing the proportion of people with ideal lifestyle behaviours and metabolic risk factor levels.[49, 50]

Prevention by health care providers

Health care providers are increasingly focussing on preserving healthy lifestyles and favourable metabolic risk factor levels of their patients, which may help them lead a healthy CVD-free life as long as possible. A culture of health in which everyone is stimulated by his or her care provider to achieve a healthy lifestyle is desirable: where lifestyle profile or metabolic risk profile is suboptimal, it must be improved; and where ideal, it must be preserved. Promoting a long-term healthy lifestyle should be a key task of health care providers but the most effective way to do so, is not clear yet. For example, a simple single counselling session is, for example, ineffective in changing lifestyle behaviours.[51] Future research should elucidate what interventions are effective and should inform training programs for clinicians and other health workers that improve lifestyle profiles of their patients regardless of the presence of symptomatic disease.

Research on the development of preventive measures to improve cardiovascular risk factor profiles

At a population level, interventions that change lifestyle behaviour have been found to be effective,[52, 53] but interventions with long-term positive effects are scarce.[53, 54] Behaviour is multifactorially determined and is difficult to change.[55] Complex interventions with many interactive and dynamic components that incorporate the whole environment and community seem most promising to establish and sustain healthy lifestyle behaviours.[53, 54, 56, 57] However, knowledge in this area is still limited,[53, 54, 57] especially regarding interventions that improve total metabolic risk profiles. More insight into the effects of lifestyle and environmental factors on long-term metabolic risk profiles might be helpful in developing effective interventions that improve cardiovascular health of the population, and consequently reduce the burden of CVD.

Overall conclusion

The prevalence of overweight and obesity was higher in younger generations and this leads to an increased lifelong exposure to obesity in the population. Obesity will therefore be one of the most important determinants of the future burden of type 2 diabetes and to

a lesser extent of CVD. Levels of metabolic risk factors and biochemical markers started deteriorating more than 15-20 years before disease onset, emphasising the importance of early prevention already in young adulthood or even childhood and onwards. This insight was reinforced by the finding that the benefits of a healthy lifestyle profile were easier lost than gained, and by the finding that a low metabolic risk profile was already lost in the great majority of people reaching adulthood. During adulthood, few people were able to maintain a healthy lifestyle profile (1 in 7 over five years) and a low metabolic risk profile (1 in 14 over 11 years). There is an urgent need to increase the currently low proportion of adults who maintain these favourable profiles because 40% and 86% of all CVD cases were attributable to suboptimal lifestyle profiles and suboptimal metabolic risk profiles respectively. Thus, population-based CVD prevention strategies are required that focus on the maintenance of healthy lifestyles and favourable metabolic risk factors levels from young age onwards to minimize the burden of CVD.

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Chapter 10

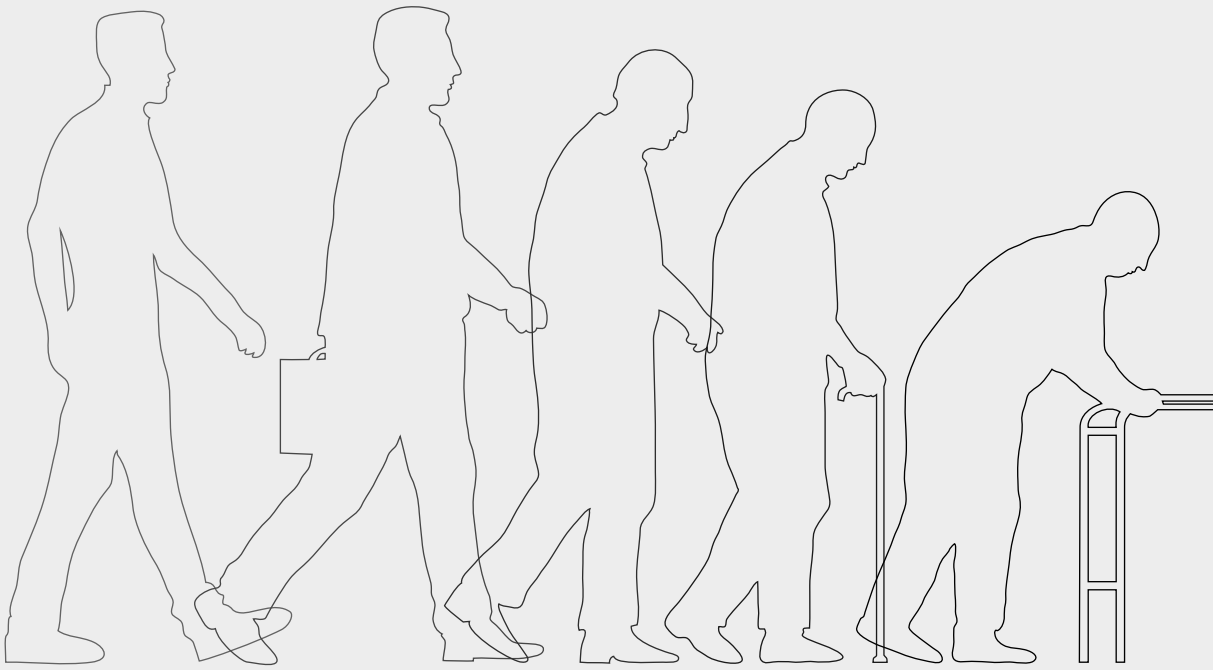
Summary

Samenvatting

Dankwoord

Curriculum vitae

List of publications



Summary

Cardiovascular disease (CVD) usually manifests itself at middle age or beyond, but it is the result of an ongoing disease process. Unhealthy lifestyles and metabolic risk factors accumulate with ageing and interact, eventually leading to CVD. This stresses the need for insight into the changes in lifestyle and metabolic risk factors that occur throughout the life course, and their impact on CVD. Two 'dimensions' of change are studied and described in this thesis. First, **Part I** describes changes across generations, that is, higher or lower age-specific levels of risk factors in successive generations. Second, **Part II** and **III** describe changes with age in lifestyle, metabolic risk factors and biochemical markers within individuals, and the association with CVD. **Chapter 1** gives the background and objectives of this thesis.

Data from the Doetinchem Cohort Study was used in all analyses described in this thesis. The Doetinchem Cohort Study is an ongoing prospective population-based cohort study that started with almost 7,800 men and women aged 20-59 years. Extensive information about demographics, lifestyle, metabolic risk factors and biochemical markers was obtained from 1987-1991 onwards, with measurements every five years over a 21-year period. Data about non-fatal and fatal CVD events was obtained through linkage with registries.

Part I – Metabolic risk factors across adult generations

Chapter 2 shows that the prevalence of overweight, obesity, and hypertension increased with age within all generations, but, in general, younger generations had a higher age-specific prevalence of these risk factors than 10-year older generations. Unfavourable generation shifts were most pronounced for overweight/obesity: in men, an unfavourable shift was observed between every generation, while in women only between the two most recently born generations. These findings imply that the prevalence of obesity and the lifelong exposure to obesity is increasing in younger generations. Consequently, more elderly of the future will develop overweight-related diseases such as type 2 diabetes, resulting in an increased risk of CVD. This evidence strengthens the need of stimulating a healthy weight, both in general practice and by preventive interventions. Interventions should at least target young adult generations.

Chapter 3 describes the extent to which the age-related increases in body mass index across generations was reflected in age-specific levels of markers of oxidative stress and chronic inflammation. In all generations, individuals with a stable body mass index had no or slightly increasing levels of gamma glutamyltransferase, uric acid and C-reactive protein during follow-up, while individuals with increasing body mass index had increases that were 2-4 times larger. These findings reinforce the importance of maintaining a stable weight to improve population levels of markers of oxidative stress and chronic inflammation, and consequently lower risk of related chronic diseases such as type 2 diabetes and CVD.

Part II – Cardiovascular risk factor profiles and risk of cardiovascular disease

Chapter 4 shows the associations of maintenance of, and improvement or deterioration in lifestyle profiles over a five-year period with risk of CVD and all-cause mortality. Maintaining a healthy lifestyle profile (i.e. at least four of the following five healthy lifestyle factors: non-smoking, a healthy diet, adequate physical activity, moderate alcohol consumption and a healthy body mass index) was associated with the lowest risk of CVD, i.e. 2.5 times lower risks compared to maintaining only 0-1 healthy lifestyle factor. It was notable that only a small proportion of adults had a healthy lifestyle profile (29%) and an even smaller proportion was able to maintain this healthy lifestyle profile over five years (16%). Independent of the lifestyle profile at young adulthood/ middle age, each individual healthy lifestyle factor 'lost' over a five-year period was associated with a one-third higher risk of CVD and all-cause mortality, whereas improvement in lifestyle over the same period did not significantly reduce those risks in this cohort. Thus, in young adulthood and middle age, the benefits of a healthy lifestyle are easily lost by deterioration in lifestyle.

Chapter 5 describes associations between the maintenance of, and the changes in metabolic risk profiles over an 11-year period with risk of CVD. Only 12% of the participants had a low metabolic risk profile (i.e. ideal levels of blood pressure, cholesterol and body mass index, non-smoking and no diabetes) at baseline, and only 7% maintained it over 11 years. Participants who maintained a low metabolic risk profile over 11 years had a 7 times lower risk of CVD than participants who maintained a high metabolic risk profile over 11 years, whereas those with a low metabolic risk profile at baseline whose profile deteriorated over time had only a 3 times lower risk of CVD. The results further suggest that improvement in metabolic risk profiles is associated with up to two-fold lower risk of CVD, and deterioration in metabolic risk profiles with about a two-fold higher risk. These findings underscore the importance of achieving and maintaining a low metabolic risk profile from young adulthood onwards and demonstrate the full benefit of a low metabolic risk profile.

For the development of effective preventive strategies to increase the proportion of adults with a low metabolic risk profile, it is necessary to identify factors associated with achieving and maintaining a low metabolic risk profile. **Chapter 6** describes the associations of demographic, lifestyle, psychological factors and family history of CVD with attainment and maintenance of a low metabolic risk profile. Age, gender and educational level were the major determinants of attaining and maintaining a low metabolic risk profile. Since people with lower educational levels and men were less likely to attain and maintain a low metabolic risk profile, these groups may benefit from early, intensive interventions. Lifestyle factors – healthy diet, physical activity and moderate alcohol intake – were associated with a higher likelihood of attaining a low metabolic risk profile; these lifestyle factors should therefore be a fundamental part of CVD prevention programs among adults.

Part III – Trajectories of metabolic risk factors and biochemical markers preceding cardiovascular disease and type 2 diabetes

Chapter 7 describes differences in levels and trajectories of metabolic risk factors and biochemical markers prior to diagnosis between individuals with CVD and controls. Adults with incident CVD had slightly more unfavourable levels of most metabolic risk factors and biochemical markers than controls, already 15-20 years prior to their diagnosis: a difference that remained stable up to diagnosis. The exceptions were systolic blood pressure, waist circumference and estimated kidney function: levels of these risk factors deteriorated more rapidly in people with incident CVD than in controls during the 15-20 years prior to diagnosis. These findings indicate that the risk of CVD is already partly determined in young adulthood. This stresses the need for primary prevention measures targeted at all risk factors, such as encouraging physical activity and a healthy diet in individuals starting from childhood and young adulthood onwards.

Chapter 8 shows differences in levels and trajectories of metabolic risk factors and biochemical markers prior to diagnosis between individuals with type 2 diabetes and controls. Metabolic risk factors and biochemical markers were slightly more unfavourable in adults who developed type 2 diabetes 15-20 years prior to diagnosis, compared to controls. Levels of most metabolic risk factors and biochemical markers deteriorated more rapidly in cases than controls up to diagnosis. The differences in the trajectories were largely explained by differences in body mass index, stressing the importance of maintaining a healthy weight. These findings show the need for primary prevention that starts more than 15-20 years before symptomatic disease is expected to become manifest, i.e. from young adulthood onwards.

General discussion

Finally, **chapter 9** discusses the main findings of this thesis, their implications and suggestions for future research. The effects of both favourable and unfavourable changes in young and middle-aged generations on the number of type 2 diabetes and CVD cases are discussed. The effects of unfavourable lifestyles and levels of metabolic risk factors over the life course on CVD are addressed, and the public health impact of medium-term lifestyle profiles and long-term metabolic risk profiles is reviewed. About 40% and 86% of all CVD cases were attributable to suboptimal lifestyle profiles and metabolic risk profiles respectively. Furthermore, this chapter discusses differences between CVD and type 2 diabetes in trajectories of metabolic risk factors and biochemical markers prior to disease onset, and the consequences for prevention.

In conclusion, the prevalence of overweight and obesity and the lifelong exposure to overweight and obesity is increasing in younger generations, and will therefore be one of the most important determinants of the future burden of type 2 diabetes and to a lesser extent of CVD. The findings of this thesis also underscore the need to increase the currently low proportion of adults who maintain a healthy lifestyle and favourable levels of metabolic risk factors over the life course to minimalize the burden of CVD. The best way to achieve this is by population-oriented approaches to primary prevention from young age onwards that lead to favourable behavioural and environmental changes across the whole population.

Samenvatting

Hart- en vaatziekten manifesteren zich over het algemeen op middelbare leeftijd of later, maar zijn het resultaat van een langdurig onderliggend ziekteproces. De effecten van een ongezonde leefstijl en metabole risicofactoren accumuleren met het ouder worden, en kunnen uiteindelijk tot hart- en vaatziekten leiden. Daarom is het van belang inzicht te hebben in veranderingen in leefstijl en metabole risicofactoren die gedurende de levensloop optreden, en de effecten hiervan op hart- en vaatziekten. Twee dimensies van veranderingen zijn bestudeerd en beschreven in dit proefschrift. **Deel I** beschrijft veranderingen over generaties, ofwel, hogere en lagere leeftijdsspecifieke niveaus van risicofactoren in opeenvolgende generaties. **Deel II** en **III** beschrijft veranderingen in individuen met de leeftijd in leefstijl, metabole risicofactoren en biochemische markers en de relatie met hart- en vaatziekten. **Hoofdstuk 1** geeft de achtergrond en doelen van dit proefschrift.

In alle analyses die beschreven zijn in dit proefschrift zijn gegevens van de Doetinchem Cohort Studie gebruikt. Dit is een langlopende prospectieve cohort studie begonnen in de periode 1987-1991 onder bijna 7.800 mannen en vrouwen van 20-59 jaar. Uitgebreide informatie over demografie, leefstijl, metabole risicofactoren en biochemische markers is elke vijf jaar verzameld over een periode van 21 jaar. Gegevens over niet-fatale en fatale hart- en vaatziekten zijn verkregen door koppelingen met registraties.

Deel I – Metabole risicofactoren in verschillende generaties

Hoofstuk 2 laat zien dat de prevalentie van overgewicht, obesitas en hypertensie toenam met de leeftijd in alle generaties, maar dat elke jongere generatie hogere leeftijdsspecifieke prevalenties van deze risicofactoren had dan 10 jaar oudere generaties. Ongunstige generatieverschillen waren het duidelijkst voor overgewicht en obesitas: bij mannen werden deze ongunstige generatieverschillen geobserveerd tussen alle generaties, terwijl dit bij vrouwen alleen het geval was voor de twee jongste generaties. Deze bevindingen suggereren dat de prevalentie van obesitas en de levenslange blootstelling aan obesitas zal toenemen. Hierdoor zullen in de toekomst meer ouderen overgewicht-gerelateerde ziekten zoals type 2 diabetes ontwikkelen, wat resulteert in een hoger risico op hart- en vaatziekten. Dit onderzoek onderschrijft het belang van het stimuleren van een gezond gewicht, zowel in de huisartsenpraktijk als door preventieve interventies. Interventies dienen in ieder geval gericht te worden op jongvolwassen generaties.

Hoofdstuk 3 beschrijft in hoeverre de leeftijdsgerelateerde toename in body mass index (BMI) over generaties weerspiegeld werd in leeftijdsspecifieke niveaus van markers van oxidatieve stress en chronische ontsteking. In alle generaties hadden respondenten met een stabiele BMI geen of slechts een kleine stijging in niveaus van gamma-glutamyltransferase, urinezuur en C-reactief proteïne gedurende follow-up, terwijl de respondenten met een

toegenomen BMI een 2-4 keer grotere stijging hadden in deze markers. Deze bevindingen benadrukken het belang van het behouden van een gezond gewicht om de niveaus van markers van oxidatieve stress en chronische ontsteking in de algemene bevolking te verbeteren, en daarmee het risico op chronische ziekten zoals type 2 diabetes en hart- en vaatziekten te verkleinen.

Deel II – Cardiovasculaire risicoprofielen en het risico op hart- en vaatziekten

Hoofdstuk 4 beschrijft de associaties van het behouden van en verbetering of verslechtering in leefstijlprofielen over een periode van vijf jaar met het risico op hart- en vaatziekten en sterfte. Het behouden van een gezonde leefstijl (minimaal vier van de volgende vijf leefstijlfactoren: niet roken, gezond voedingspatroon, adequate lichamelijke activiteit, gematigde alcohol inname en een gezonde BMI) was geassocieerd met het laagste risico op hart- en vaatziekten: een 2,5 keer lager risico vergeleken met het behouden van 0-1 gezonde leefstijlfactoren. Het was opmerkelijk dat slechts een klein gedeelte van de volwassenen een gezonde leefstijl had (29%) en dat een nog kleiner gedeelte een gezonde leefstijl behield gedurende een periode van vijf jaar (16%). Onafhankelijk van het leefstijlprofiel gedurende jongvolwassenheid en middelbare leeftijd was elke individuele gezonde leefstijlfactor die 'verloren' ging over een periode van vijf jaar geassocieerd met een één derde hoger risico op hart- en vaatziekten en sterfte, terwijl verbetering in leefstijl over dezelfde periode niet significant het risico verlaagde in dit cohort. Dus bij jongvolwassenen en personen van middelbare leeftijd lijken de voordelen van een gezonde leefstijl snel verloren te gaan door verslechtering van de leefstijl.

Hoofstuk 5 beschrijft de associaties tussen het behouden van en veranderingen in metabole risicoprofielen over een periode van 11 jaar en het risico op hart- en vaatziekten. Slechts 12% van de deelnemers had een gunstig metabool risicoprofiel (optimale niveaus van bloeddruk, cholesterol en BMI, niet roken en geen diabetes) op baseline en slechts 7% behield dit gedurende de 11-jaarsperiode. Deelnemers die een gunstig metabool risicoprofiel behielden over een periode van 11 jaar hadden een 7 keer lager risico op hart- en vaatziekten dan deelnemers die een ongunstig metabool risicoprofiel behielden gedurende de 11-jaarsperiode. Degene die een gunstig metabool risicoprofiel hadden op baseline, maar verslechterden gedurende de 11-jaarsperiode hadden slechts een 3 keer lager risico op hart- en vaatziekten. De resultaten suggereren ook dat verbetering in metabool risicoprofiel is geassocieerd met een ongeveer 2 keer lager risico op hart- en vaatziekten en verslechtering in metabool risicoprofiel met een ongeveer 2 keer hoger risico. Deze bevindingen onderstrepen het belang van het bereiken en behouden van een gunstig metabool risicoprofiel vanaf jongvolwassenheid en kwantificeren de enorme verlaging van het risico bij een volledig gunstig metabool risicoprofiel.

Voor de ontwikkeling van effectieve preventieve strategieën om het aandeel van volwassenen met een gunstig metabool risicoprofiel te vergroten is het noodzakelijk om factoren te identificeren die geassocieerd zijn met het bereiken en behouden van een gunstig metabool risicoprofiel. **Hoofstuk 6** beschrijft de associaties van demografische, leefstijl, psychosociale factoren en familiegeschiedenis van hart- en vaatziekten met het bereiken en behouden van een gunstig metabool risicoprofiel. Leeftijd, geslacht en opleidingsniveau waren de belangrijkste determinanten van het bereiken en behouden van een gunstig metabool risicoprofiel. Personen met een laag opleidingsniveau en mannen hadden minder kans op het bereiken en behouden van een gunstig metabool risicoprofiel, dus deze groepen hebben mogelijk veel voordeel bij vroege en intensieve interventies. Leefstijlfactoren (gezond voedingspatroon, lichamelijke activiteit en gematigde alcoholinname) waren geassocieerd met een hogere kans op het bereiken van een gunstig metabool risicoprofiel; deze leefstijlfactoren zijn daarom een essentieel onderdeel van cardiovasculaire preventieprogramma's.

Deel III – Trajecten van metabole risicofactoren en biochemische markers voorafgaand aan hart- en vaatziekten en type 2 diabetes

Hoofstuk 7 beschrijft de verschillen in niveaus en trajecten van metabole risicofactoren en biochemische markers, voorafgaand aan diagnose, tussen individuen met hart- en vaatziekten en controlepersonen. Volwassenen die hart- en vaatziekten ontwikkelden hadden al 15-20 jaar voor diagnose iets ongunstigere niveaus van de meeste metabole risicofactoren en biochemische markers dan controlepersonen; dit verschil bleef stabiel tot diagnose. Systolische bloeddruk, middelomtrek en geschatte nierfunctie waren uitzonderingen: de niveaus van deze risicofactoren verslechterden sneller in personen die hart- en vaatziekten ontwikkelden dan in controlepersonen gedurende de 15-20 jaar voorafgaand aan diagnose. Deze bevindingen geven aan dat het risico op hart- en vaatziekten al gedeeltelijk is bepaald in jongvolwassenheid. Dit benadrukt de noodzaak voor primaire preventieve maatregelen die gericht zijn op alle risicofactoren, zoals het aanmoedigen van lichamelijke activiteit en een gezond voedingspatroon vanaf de kindertijd/jongvolwassenheid.

Hoofdstuk 8 beschrijft de verschillen in niveaus en trajecten van metabole risicofactoren en biochemische markers voorafgaand aan diagnose tussen individuen met type 2 diabetes en controlepersonen. Metabole risicofactoren en biochemische markers waren 15-20 jaar voor diagnose iets ongunstiger in volwassenen die type 2 diabetes ontwikkelden dan in controlepersonen. Niveaus van de meeste metabole risicofactoren en biochemische markers verslechterden tot diagnose sneller in personen die type 2 diabetes ontwikkelden dan in controlepersonen. De verschillen in trajecten werden grotendeels verklaard door verschillen in BMI, wat de noodzaak van het behouden van een gezond gewicht benadrukt. Deze bevindingen laten het belang van primaire preventie zien die moet starten 15-20 jaar voor de symptomen van de ziekte zich manifesteren, dus vanaf jongvolwassenheid.

Algemene discussie

Tot slot bediscussieert **hoofdstuk 9** de belangrijkste bevindingen van dit proefschrift en implicaties en suggesties voor toekomstig onderzoek. De effecten van zowel gunstige als ongunstige veranderingen van generaties op de incidentie van type 2 diabetes en hart- en vaatziekten worden bediscussieerd. De effecten van een ongunstige leefstijl en niveaus van metabole risicofactoren gedurende de levensloop op hart- en vaatziekten zijn bestudeerd en de gevolgen voor de volksgezondheid zijn beschreven. Ongeveer 40% en 86% van alle hart- en vaatziekten waren toe te schrijven aan respectievelijk suboptimale leefstijlprofielen en metabole risicoprofielen. Verder worden de verschillen in trajecten van metabole risicofactoren en biochemische markers voorafgaand aan het optreden van hart- en vaatziekten en type 2 diabetes besproken, en de consequenties voor preventieve interventies.

Concluderend, de prevalentie van overgewicht en obesitas en de levenslange blootstelling aan overgewicht en obesitas is aan het toenemen. Daarom zullen overgewicht en obesitas één van de belangrijkste determinanten van de toekomstige ziektelast van type 2 diabetes en in mindere mate van hart- en vaatziekten zijn. De bevindingen van dit proefschrift benadrukken ook het belang om onder volwassenen de prevalentie van een langdurig gezonde leefstijl en een gunstig metabool risicoprofiel toe te laten nemen om daarmee de ziektelast van hart- en vaatziekten te minimaliseren. Dit kan het beste gedaan worden door middel van preventieve strategieën op populatieniveau die gericht zijn op het bewerkstelligen van gunstige veranderingen in de omgeving en in het gedrag van de algemene bevolking.

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Curriculum vitae

Gerben Hulsegge was born on December 7th 1986 in Bennekom, the Netherlands. In 2004, he completed secondary school at the CSG Het Streek in Ede. He received his Bachelor's degree in physical therapy at the HU University of Applied Sciences Utrecht in 2008. Subsequently, he worked as a physical therapist at the St. Antonius Hospital Utrecht and Nieuwegein for three years. During his study physical therapy, Gerben developed a high interest in research on the prevention of chronic diseases, and therefore combined his work as a physical therapist with the premaster program Health Sciences followed by the two-year research master program Lifestyle and Chronic Disorders at the VU University Amsterdam from which he graduated cum laude in 2011. His master thesis was performed at the George Institute for Global Health in Sydney, Australia; where he investigated fundamental movement skills, physical fitness and physical activity among Australian children with juvenile idiopathic arthritis. After he graduated in 2011, he started his PhD project leading to this thesis at the Dutch National Institute for Public Health and the Environment, in collaboration with the Julius Center for Health Sciences and Primary Care of the University Medical Center Utrecht. During his PhD project, Gerben followed several courses from the program of the Postgraduate Master of Clinical Epidemiology at Utrecht University. In 2013, he also attended the 45th Ten Day Teaching Seminar in Cardiovascular Disease Epidemiology and Prevention in Incheon, South Korea, from the International Society of Cardiovascular Disease Epidemiology and Prevention with PhD students from over 20 different countries. In 2014, he was nominated for the 'young Investigator Award - Prevention & Epidemiology and Sports Cardiology' at the annual meeting of the European Society of Cardiology and Preventive Cardiology (EuroPRevent) in Amsterdam for his research paper on lifestyle changes and cardiovascular diseases (chapter 4 of this thesis). Currently, Gerben has a postdoc position at the VU University Medical Center and studies the metabolic health effects of shift work and the role of physical activity and diet.

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Presentations

Hulsegge G, Picavet HS, Blokstra A, Nooyens AC, Spijkerman AM, van der Schouw YT, Smit HA, Verschuren WMM. Today's adult generations are less healthy than their predecessors: generation shifts in metabolic risk factors- the Doetinchem Cohort Study. Jun 2012, annual meeting of the Netherlands Epidemiology Society (WEON), Rotterdam, the Netherlands (oral presentation)

Hulsegge G, Smit HA, van der Schouw YT, Daviglus ML, Verschuren WMM. Quantifying the benefits of achieving or maintaining long-term low risk profile for cardiovascular disease-the Doetinchem Cohort Study. March 2013, AHA Conference on Cardiovascular Disease Epidemiology and Prevention - Nutrition, Physical Activity and Metabolism, New Orleans, United States (moderated poster presentation)

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Hulsegge G, Smit HA, van der Schouw YT, Daviglus ML, Verschuren WMM. Determinants of attaining and maintaining a low cardiovascular risk profile. May 2014, annual meeting of the European Society of Cardiology and Preventive Cardiology (EuroPRevent), Amsterdam, the Netherlands (moderated poster presentation, awarded with poster price)

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Hulsegge G, Spijkerman AMW, van der Schouw YT, Bakker SJL, Gansevoort RT, Smit HA, Verschuren WMM. Trajectories of traditional risk factors and biomarkers before the onset of type 2 diabetes. Nov 2014, Annual Dutch Diabetes Research Meeting, Oosterbeek, the Netherlands (oral presentation)

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