Sex differences in causes and consequences of type 2 diabetes

Citation for published version (APA):


Document status and date:
Published: 01/01/2022

DOI:
10.26481/dis.20220908rr

Document Version:
Publisher's PDF, also known as Version of record

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.

Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:
www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:
repository@maastrichtuniversity.nl
providing details and we will investigate your claim.

Download date: 27 Oct. 2023
Chapter 7 Summary and general discussion

Diabetes is associated with an increased risk of cardiovascular disease (CVD) in both sexes(1). Although incidence rates of CVD have been reported to be higher in men than in women, with and without type 2 diabetes, there is compelling evidence that diabetes is a stronger risk factor for cardiovascular complications in women than in men(2-4). The mechanisms underlying sex differences in diabetes-associated CVD are not completely understood(2). This thesis aimed to investigate sex differences in causes and consequences of type 2 diabetes, with a specific focus on providing further insights in biological mechanisms underpinning the observed sex differential in the risk of diabetes-associated CVD. Understanding, and creating awareness of sex differences in the burden of diabetes could, eventually, lead to more personalized diabetes care and diminish the burden in both women and men.

In this final chapter, key findings of the thesis will be summarized and discussed, along with several statistical and methodological aspects. Finally, a general conclusion of this thesis will be provided including recommendations for future research.

Key findings

1. When investigating sex differences and sex-specific effects, it is important to consider the possibility of sex-specific confounding, as possible sex-specific effects of confounders themselves may obscure the evaluation of sex differences (chapters 3 to 6).
2. There was no consistent pattern of sex differences in the associations of either prediabetes or type 2 diabetes or glycemia with early microvascular complications or function (chapter 3).
3. There were sex differences in cardiovascular risk factors associated with prediabetes and type 2 diabetes. Adverse differences in levels of cardiovascular risk factors in prediabetes and type 2 diabetes, as compared to normal glucose metabolism (NGM), were greater in women than in men. Additionally, HbA1c among non-diabetic individuals was more strongly associated with several cardiovascular risk factors in women than in men (chapter 4).
4. There were sex differences in body composition associated with type 2 diabetes. The difference in the amount of subcutaneous adipose tissue, hip circumference and lean mass between type 2 diabetes and NGM was greater in women than in men, whereas the difference in the amount of visceral adipose tissue was greater in men than in women. There was no difference in the percentage of liver fat (chapter 5).
5. There was no consistent pattern of sex differences in the association of prediabetes and type 2 diabetes with cognitive performance, depression and health-related quality of life (chapter 6).

Potential sex differences in causes and consequences of type 2 diabetes

Microvascular complications and function

In chapter 3, we investigated sex differences in the association of prediabetes and type 2 diabetes with microvascular complications and function. We showed that type 2 diabetes, but not prediabetes, was associated with higher odds of microvascular complications and greater adverse differences in microvascular function in men. In women these associations were numerically in the same direction, but generally not statistically significant. There was no consistent pattern of sex differences in the associations of either prediabetes or type 2 diabetes or glycemia with microvascular complications or function. Thus, these results are consistent with the concept that type 2 diabetes does not confer a
greater risk of microvascular complications among women than men. However, the prevalence of advanced-stage complications was too low for evaluation. Therefore, we cannot exclude such sex differences with regard to progression of microvascular complications.

The above results contrast with sex differences in diabetes-associated CVD (i.e., macrovascular complications)(2). It has been hypothesized that women’s cardiovascular risk factors deteriorate to a greater extent in the transition from normoglycemia to type 2 diabetes, possibly due to sex differences in body composition and fat distribution(2). Consequently, women's vasculature may experience a prolonged exposure to metabolic dysfunction prior to diagnosis of type 2 diabetes and this could partly explain women’s excess risk of diabetes-associated CVD(2). Presumably, these sex differences do not, or to a lesser extent, influence microvascular complications. Further study is required to confirm these results and to investigate sex differences with regard to advanced-stage microvascular complications.

**Cardiovascular and metabolic risk factors**

To further understand the biological processes underlying women’s observed higher relative risk of diabetes-associated CVD, we determined, in chapter 4, sex differences in the associations of prediabetes and type 2 diabetes (reference category: NGM) and of HbA1c (continuously, among individuals without type 2 diabetes) with cardiovascular risk factors. We demonstrated that adverse differences in cardiovascular risk factors (i.e., systolic blood pressure, HDL cholesterol, total-to-HDL cholesterol ratio, triglycerides and inflammation markers) between individuals with prediabetes and NGM are already more pronounced in women than in men. These sex differences were similar to, but smaller than, the sex differences observed in type 2 diabetes. In addition, HbA1c in individuals without type 2 diabetes is more strongly associated with several cardiovascular risk factors (i.e., systolic and diastolic blood pressure, HDL and LDL cholesterol) in women than in men. Taken together, these observations are consistent with the concept that these sex differences reflect a continuous process that already emerges in early hyperglycemia. Since no consistent pattern of sex differences was observed in the associations with lifestyle risk factors, biological factors are more likely to underlie the higher relative risk of diabetes-associated CVD in women.

Previous studies have observed that BMI and waist circumference differ more between women with and without diabetes than between men with and without diabetes(5, 6), which is in line with our findings. When newly diagnosed with type 2 diabetes, the BMI of women, as compared to men, has shown to be almost 2 kg/m² higher despite similar levels of HbA1c(7, 8). These sex differences suggest a link with a differential pattern of fat storage and insulin resistance between women and men(9, 10). It has been hypothesized that women need to attain higher levels of BMI and deteriorate related risk factors to a greater extent than men to develop insulin resistance and eventually diabetes(2, 11). Although not statistically significant, we found a tendency towards more adverse differences in levels of BMI and insulin sensitivity in women than in men associated with prediabetes, and with HbA1c in those without type 2 diabetes. Furthermore, after adjustment for BMI, all significantly different sex differences in cardiovascular risk factors associated with prediabetes and type 2 diabetes were attenuated. Thus, even small sex differences in BMI may induce a greater deterioration of related risk factors before the onset of type 2 diabetes in women than men. With regard to clinical implications, tailoring cardiovascular risk management for women could benefit from the insight that women possibly already have a greater deterioration of cardiovascular risk factors before the onset of type 2 diabetes than men. However, further research into biological factors, specifically into fat distribution and metabolism, is needed to unravel the underlying mechanisms of sex differences in the association between type 2 diabetes and vascular function.

As previously mentioned it has been hypothesized that sex differences in body composition may influence the development of type 2 diabetes and play a role in women's observed higher relative
risk of diabetes-associated CVD(2). However, the interplay between sex, body composition, type 2 diabetes and CVD is complicated. In chapter 5, we investigated sex differences in body composition associated with prediabetes and type 2 diabetes. We showed that both women and men with prediabetes and type 2 diabetes, as compared to their NGM counterparts, had more fat and lean mass. Furthermore, this study indicates that there are sex differences in body composition associated with type 2 diabetes. The differences in the amount of abdominal subcutaneous adipose tissue (SAT), hip circumference and lean mass between type 2 diabetes and NGM were greater in women than in men, and the difference in visceral adipose tissue (VAT) was greater in men than in women. There was no difference in the percentage of liver fat. So, when developing type 2 diabetes, women possibly have a smaller increase in VAT, but a higher increase in SAT than men. Due to the harmful effects of deep SAT(12), this might have a similar adverse effect on liver fat deposition and the development of type 2 diabetes. Besides that, VAT may also have sex-specific effects on diabetes development. VAT seem to have a stronger association with insulin resistance in women than in men(13). Thus, although women possibly have a smaller increase in VAT, this does not necessarily indicate that it is less detrimental for diabetes development. The sex-specific role of SAT and VAT in diabetes development is unclear(13, 14) and requires further investigation.

Investigating whether sex differences in body composition can explain sex differences in the risk of diabetes-associated CVD was beyond the scope of this thesis. However, our results of chapters 4 and 5 may give some further insights. Taken together, our results indicate that women have a greater deterioration of cardiovascular risk factors, even before the onset of type 2 diabetes, than men. Additionally, our results imply that there is a sex-specific effect of body composition on the development of type 2 diabetes. Besides the sex differences in SAT and VAT, we showed that the difference in total fat mass between type 2 diabetes and NGM was numerically greater, but not statistically significant, in women than in men. Interestingly, we did observe a statistically significant greater difference in total and peripheral lean mass between type 2 diabetes and NGM in women than in men (chapter 5). This might contribute to the women’s observed greater difference in BMI between type 2 diabetes and NGM (chapter 4). As discussed in more detail in chapter 2, previous research has shown that women are more likely to store fat subcutaneously and on their lower extremities, whereas men are more likely to store fat in the abdominal region(15). It has been hypothesized that sex differences in the preferred location of fat storage may provide women with more cardiometabolic reserves(9). As a consequence, men need to gain less weight and progress more quickly to insulin resistance and diabetes, whereas women need to put on more weight and other cardiovascular risk factors may need to deteriorate further before reaching the amount of abdominal and ectopic fat required to develop type 2 diabetes(9, 16). This hypothesis is supported by the observation that women have, on average, a 2-year longer pre-diabetic phase compared to men(17). It can be postulated that besides sex differences in ‘good’ peripheral vs. ‘bad’ abdominal fat, there are also sex differences in the pathophysiology of abdominal fat itself (i.e., sex-specific associations of superficial and deep SAT and VAT with liver fat deposition and diabetes development). Possibly, this may also be related to sex differences in cardiovascular risk factors associated with diabetes. In addition, weight gain is not necessarily only an increase in fat mass. Increased adiposity has been suggested to act as a chronic overload stimulus on the muscles, increasing muscle size and strength(18). Additionally, in people with increased adiposity and type 2 diabetes, lean mass may be less functional because of skeletal muscle lipid infiltration(19). In turn, lean mass has to increase to compensate malfunction. Women’s observed greater difference in lean mass between type 2 diabetes and NGM, as compared to men, possibly also plays an important role with regard to CVD risk, as it has been suggested that more lean mass is not always beneficial(19). It has been shown that individuals with both high fat mass and high lean mass have the most unfavorable cardiometabolic risk profile(19). Taken together, the exact underlying mechanisms of women’s observed higher relative risk of diabetes-associated CVD
are not clear, and further study is required to investigate sex differences in the pathophysiology. It is important to take both fat and lean mass into account when investigating the interplay between sex, body composition, diabetes and CVD (risk).

Mental health aspects and quality of life
Next to sex differences in diabetes-associated CVD, a meta-analysis has shown that diabetes is also a stronger risk factor for vascular dementia in women than in men(20). In chapter 6, we aimed to investigate whether there were sex differences in other consequences of diabetes. We investigated these sex differences with regard to cognitive performance, depression and quality of life. As vascular function is associated with both cognitive performance and depression(21), we hypothesized that diabetes would be a stronger risk factor for worse cognitive performance and depression in women than in men. However, type 2 diabetes was associated with worse cognitive performance and depression, but, like with microvascular complications, there were no sex differences in these associations. Our population is generally relatively healthy, most of the participants with cognitive decline probably have early stages of cognitive decline, and the number of participants with depression is relatively low. Therefore, we cannot exclude sex differences in the associations of (pre)diabetes with later stages of cognitive decline and depression and further research is required. In addition, type 2 diabetes was associated with poorer quality of life, but we did not find sex differences in these associations independent of lifestyle factors. However, it must be noted that sex-differences in the associations with the physical component of quality of life were statistically significant to the disadvantage of women if lifestyle risk factors were not taken into account. This suggests that the sex-specific effect of diabetes on physical quality of life is in part driven by these lifestyle factors. Explanations for potential sex differences in physical quality of life may be related to the excess risk of comorbidities, such as CVD(3, 4), and more adverse cardiometabolic profile(22) associated with type 2 diabetes in women. Also, gender roles and gender differences in health consciousness and health perception may affect management and control of diabetes and related health problems(23, 24). As research on sex differences in diabetes-associated quality of life is sparse, further research is required to investigate whether type 2 diabetes is a stronger risk factor for poorer physical quality of life in women than in men and to investigate possible explanations.

Sex hormones, diabetes and CVD
Women and men are fundamentally different with regard to the expression of sex hormones(25), and hormones are involved in virtually all physiologic processes across the life span(25). Although beyond the scope of this thesis, women’s higher relative risk of diabetes-associated CVD could be partly due to hormonal differences. Sex hormones are, next to the reproductive system, involved in cardiovascular health, obesity, glucose metabolism and inflammation. It has been shown that these hormones exert different effects in women and men(26, 27). It is well recognized that women develop CVD, on average, 10 years later than men. This observation have led to the hypothesis that estrogen protect women against atherosclerotic complications(28). This hypothesis is supported by the observation that women with premature or early menopause are at increased risk of CVD, compared to those with normal or late menopause(29). However, it has also been observed that higher levels of oestradiol were not associated with a decreased risk of myocardial infarction in women. The presumed cardioprotective effects of oestradiol may be largely confounded by age and further confounded by other cardiovascular risk factors(30). In both women and men, the amount of body fat and its distribution are related to the state of sex hormones. In women, menopause is often associated with a shift from gynoid (pear-shaped) fat distribution to android (apple-shaped) fat distribution, which is mostly seen in men(26). In premenopausal women, most estrogen production occurs in the ovaries. In postmenopausal women, most estrogen production occurs via conversion of testosterone to estradiol in white fat. Similarly to postmenopausal women, most estrogen production in men occurs
via conversion of testosterone to estradiol in white fat(26). Testosterone has been reported to have an ambivalent role in women and men. Low levels of testosterone in men have been associated with impaired glucose homeostasis and type 2 diabetes, whereas women with higher levels of testosterone are at increased risk of developing type 2 diabetes and have worse cardiovascular risk profiles(26). Studying the sex-specific impact of sex hormones is complex, especially because of the cyclic fluctuations in hormone levels among women. In The Maastricht Study, data with regard to sex hormones are not readily available. The impact of sex hormones on the observed sex differential in risk of diabetes-associated CVD is uncertain and requires further study.

Health care aspects
In this thesis, we mainly investigated sex differences in biological aspects. However, in addition to these biological aspects, disparities in uptake and provision of healthcare may in part explain sex differences in the risk of diabetes-associated CVD (chapter 2). Potential sex differences may occur throughout the pathway - starting with healthy women and men being exposed to certain risk factors, at some point being diagnosed with diabetes, and eventually develop complications – and may include, i.e., diagnostic delay, inadequate risk factor screening, disparities in adequate interventions, and non-adherence. Future studies are needed to improve the understanding of sex disparities in the uptake and provision of healthcare as possible underlying mechanisms of the observed sex differential in the risk of diabetes-associated CVD.

Statistical considerations

Sex versus sex-by-(pre)diabetes as determinant
In this thesis, we mainly investigated the interaction of sex-by-(pre)diabetes with regard to several outcomes. The majority of studies investigating sex differences with regard to complications of type 2 diabetes, investigated these differences within populations with type 2 diabetes (for example: (31-33)). These studies did not include a reference group of people without type 2 diabetes. As such, it is unknown whether type 2 diabetes, next to CVD, also is a stronger risk factor for the development of other complications in women than in men. As the development of type 2 diabetes is a gradual process of increasing glucose intolerance, it is pertinent to include comparisons with individuals at different stages of the glucose intolerance spectrum, so to understand where in the spectrum any sex difference might emerge and where appropriate interventions may be initiated. Furthermore, we have experienced that the different approaches to investigate sex differences and their interpretations are difficult to understand and explain. Therefore, in chapter 3 we have illustrated the differences between sex as determinant and sex-by-diabetes as determinant and pointed out that it is important to distinguish between these two approaches. Figure 1 in chapter 3, repeated below, showed that it is possible that, for a certain outcome, sex is not a determinant in type 2 diabetes, while there is a sex-by-diabetes interaction (Figure 1, part C). Turned around, sex could be a determinant in type 2 , while there is no sex-by-diabetes interaction (Figure 1, part D). Of course, it is also possible that sex is a determinant in type 2 diabetes and there is also a sex-by-diabetes interaction (Figure 1, part B), or there are no sex differences at all (both as determinant as the interaction; Figure 1, part A).
Figure 1: Simulation data to illustrate differences (A, B, C, D – described below) between sex as a determinant and sex-by-T2D interaction as a determinant for microvascular disease. Precise prevalences will vary in real data.

Black circles represent women and white squares represent men. NGM: normal glucose metabolism; T2D: type 2 diabetes. Microvascular disease: # investigation of sex as a determinant; + women with T2D vs. women with NGM; ‡ men with T2D vs. men with NGM; * investigation of sex-by-T2D interaction as a determinant. Statistically significantly differences (# and/or * p<0.05) are typed in bold.

A: Sex is a determinant in neither NGM nor T2D; the sex-by-T2D interaction is not a determinant
B: Sex is a determinant in T2D but not in NGM; hence the sex-by-T2D interaction is a determinant
C: Sex is a determinant in NGM but not in T2D; hence (!) the sex-by-T2D interaction is a determinant
D: Sex is a determinant in both NGM and T2D; the sex-by-T2D interaction is not a determinant

Sex-specific effects of potential confounders
In all our statistical analyses, we adjusted for potential confounders (chapters 3 to 6). For each potential confounder included, an interaction term (sex by confounder) was also incorporated in the same models. In doing so, adjustments made in the interaction models were allowed to have a sex-specific effect. In the thesis of Dr. Marit de Jong, in chapter 3, an illustrative example of statistical approaches to assess sex differences is included(34). They also illustrated that sex differences can be biased if sex specific confounding is not taken into account. The majority of studies investigating sex differences analyze sex differences by using a single interaction term with sex and/or by stratifying by sex, for example(13). In this study, sex differences in the associations of waist circumference, VAT and SAT with insulin resistance and insulin secretion were investigated by using single interaction terms with sex (sex by waist, sex by VAT and sex by SAT), and results were stratified by sex. They did not include interaction terms of sex by confounders in the models. By not using full interaction models, including interaction terms of sex by confounders, the impact of sex-specific confounding remains unknown and biased conclusions may be reached. Illustratively, we here noted that a sex difference was reported, while results of the stratified analyses were comparable between men and women. By not including interaction terms of sex by confounders, the adjustments made in the single interaction model were not allowed to have sex-specific effects, in contrast to those in the stratified analyses, which may possibly explain the observed discrepancy. Taken together, we would like to emphasize
that it is important to properly account for the possibility of sex-specific confounding and we recommend to use full interaction models when studying sex differences and sex-specific effects. This method has also been described by Professor Mark Woodward in a tutorial on how to design, analyze and describe sex differences in cardiovascular associations(35).

Analyzing and interpretation of data with regard to sex differences
We were interested in sex-specific effects of body composition in the development of type 2 diabetes. Therefore, we investigated, in chapter 4, sex differences in body composition associated with type 2 diabetes. Although it may seem counterintuitive, we specifically chose to analyze the data with prediabetes and type 2 diabetes as determinants and measures of body composition as outcome, and not the other way around, because results of analyses with body composition measures as determinants are difficult to interpret. For example, men have more VAT than women, and therefore 1 cm² greater VAT is a relatively smaller increase for men than for women. Hence, the results, e.g. the odds of having (pre)diabetes compared to NGM per 1 cm² greater VAT, is difficult to compare between women and men. Similarly, men also have a higher standard deviation of VAT than women, because of their greater amount of VAT, so comparing standard deviations between men and women would give results which are also difficult to interpret. In chapter 4, results are expressed as linear regression coefficients (95% CI), which indicate mean differences (βs) in measures of body composition (g or cm²) according to (pre)diabetes (reference category: NGM). These results are presented for men and women separately and can be seen as a snapshot which indicates the amount of fat and lean mass in (pre)diabetes vs NGM. Taken together, it is important to take into account these difficulties while analyzing or interpreting data with regard to sex differences.

Mathematics and relative risk
Apart from biological differences between women and men, there may also be a mathematical explanation as to why women are observed to have a higher relative risk of diabetes-associated CVD than men. Women generally have a lower absolute risk of CVD than men. Consequently, a similar increase in cardiovascular events in women and men, as a consequence of diabetes, should result in a higher relative effect in women(2, 36). This mathematical explanation is supported by the observation that sex differences in diabetes-associated relative risk for CVD decrease with increasing age, that is, as the overall absolute risk of CVD increases in the general population(2, 37). However, meta-analyses showed no sex differences in the associations of BMI and systolic blood pressure with cardiovascular outcomes, resulting in the same deleterious effects of these risk factors and a comparable relative risk in both sexes(38, 39). Therefore, it seems unlikely that women’s observed higher relative risk of diabetes-associated CVD, is only a consequence of their lower absolute rate, compared with men(2).

Methodological considerations
The findings of the present thesis need to be interpreted in light of its methodological strengths and limitations. The studies in this thesis were based on data from The Maastricht Study, a population-based cohort study that focuses on the etiology, pathophysiology, complications and comorbidities of type 2 diabetes. Strengths and limitations with regard to confounding bias, overadjustment bias,
selection bias and information bias will be discussed in the next section. Additionally, causality and external validity will be discussed.

Confounding and overadjustment bias
Confounding occurs when a third variable affects the association between the variable of interest and outcome(40). A strength of this thesis is that, due to an extensive phenotyping approach of individuals of The Maastricht Study, a large number of important confounders were included in the analyses and consequently the possibility of confounding is minimized. However, in observational studies, it is impossible to fully exclude the possibility of residual confounding, for instance caused by errors in the measurement of confounders and/or unmeasured confounders(41). For example, some confounders are difficult to measure precisely, such as dietary habits assessed by food frequency questionnaires, which are prone to recall bias(42). Additionally, in chapter 4, we observed that, after adjustment for total fat mass, type 2 diabetes was positively associated with lean mass. It might be possible that some residual effects of adiposity, measured by DEXA, underlie this positive association. However, in most studies, we conducted several sensitivity analyses to test the robustness of our findings. We added and/or substituted confounders in the regression models. All these sensitivity analyses gave similar results and only marginally changed regression coefficients when compared to the main regression model. In these associations, it makes it unlikely that residual confounding has influenced the results.

Adjustment for multiple confounders within our studies could lead to overadjustment bias. Overadjustment has been defined as control for an intermediate variable (or a proximal causal variable) on the causal pathway from exposure to outcome(43). To minimize the effects of overadjustment bias, we constructed different regression models, based on potential confounders grouped together according to their putative role. All models, with different groups of potential confounders were compared with each other. In chapter 6, for example, we adjusted in the second model for lifestyle factors in the association between type 2 diabetes and health-related quality of life. These lifestyle factors might mediate this association, results from these models should therefore be interpreted conservatively.

Selection bias
Associations in a population-based cohort study may be biased due to selection, i.e. the observed associations in the study may differ from those observed in the source population(41). Selection bias can be caused by factors that influence study participation (e.g. self-selection bias) and/or procedures used to selectively include participants (e.g. complete-case analysis)(41).

A strength of this thesis is the use of data from a large number of individuals (up to n=7689), sampled from the general population. This reduces the chance of selection bias. Additionally, the oversampling of individuals with type 2 diabetes reduces the chance of selection bias in this clinically relevant subgroup of individuals.

Limitations of population-based studies in general are that individuals with a higher social status, a healthier lifestyle and less disease are more likely to participate(44). The observation that participants of The Maastricht Study have a relatively high educational level and are relatively healthy may indicate a selective non-response bias. However, such a bias would have led to underestimations, rather than overestimations, of the observed associations and the sex differences in chapters 3 to 6. Additionally, we observed that the percentage of women in the type 2 diabetes study population was about 14%-point lower than that in the source population(45). If the apparent underrepresentation
of women with type 2 diabetes was due to health selection, this could have influenced the sex differences. However, the recruitment strategy was the same for women as for men.

In all our analyses we used complete-case analyses. This may have led to underestimations in the associations and the observed sex differences in chapters 3 to 6, as individuals who were excluded had a somewhat more adverse cardiovascular risk profile.

To conclude, in our opinion, findings from this population-based study provide a representative cross-section of the general populations, and therefore findings can be considered valid in the general population.

Measurement error and information bias

Errors in the measurement of determinants and outcomes (measurement bias) may result in biased associations between determinants and outcomes (information bias)(41). A strength of this thesis is that we used state-of-the-art measurement techniques, which in general reduces the chance of information bias. In this section we will further discuss the validity of the main measures used in this thesis.

In chapters 3 to 6, glucose metabolism status (prediabetes and type 2 diabetes, with NGM as reference category) was the determinant. Glucose metabolism status was assessed with use of the gold-standard method (i.e., a standardized OGTT)(46). Nevertheless, a single OGTT may misclassify GMS, and has been identified in individuals with NGM, prediabetes and type 2 diabetes(47). Misclassification will occur in all directions, of prediabetes as type 2 diabetes and vice versa and of prediabetes as NGM and vice versa. Direct misclassification of NGM as type 2 diabetes or vice versa could be neglected(47). Misclassification of type 2 diabetes as prediabetes may have caused overestimation of the observed associations and sex differences in the prediabetes group. Misclassification of prediabetes as type 2 diabetes may have caused an underestimation in the type 2 diabetes group. Misclassification of prediabetes as NGM or NGM as prediabetes may have caused an underestimation in the prediabetes group. So, the net result of the possible misclassification is likely to be an underestimation. Additionally, in our study, the size of the NGM group is much larger than the prediabetes and type 2 diabetes group, so the quantitatively largest misclassification will therefore be of NGM as prediabetes, which might have resulted in a underestimation of the associations and sex differences.

In chapter 3 microvascular complications and function were assessed. State-of-the-art techniques were used to assess nephropathy, neuropathy and retinal measures. For example, urinary albumin excretion was measured in two 24-hour urine samples, which is the gold standard(48). Additionally, the neurothesiometer, used to assess neuropathy, has observed to be a reliable technique(49). Fundus photography of both eyes is performed to determine retinal arteriolar and venular diameters(50). Retinal microvascular dilation in response to flicker light was measured with the Dynamic Vessel Analyzer. These methods are reproducible and valid measures of the microcirculation(51, 52). However, the dilation response was only measured once (randomly left or right eye) due to time constraints, which prevented us from reducing the effect of biological variability by, for example, averaging repeated measurements.

In chapter 4 we used dual-energy X-ray absorptiometry (DEXA) and MRI to assess measures of body composition. Both techniques are observed to have a high accuracy and reproducibility(53, 54). A possible disadvantage, which might have created bias, is that the DEXA analysis assumes a constant hydration of lean soft tissue, which is not always true as hydration varies with age, sex and
disease(54). Additionally, MRI of obese participants are typically challenging because of the effects of a large field of view image quality(55), which might have caused measurement errors.

In chapter 5 we assessed several cardiovascular risk factors. All of these health outcomes were measured according to well-accepted methods and clinical standards(56). In order to investigate the robustness of the associations with blood pressure (and minimize information bias), we substituted office blood pressure (primarily used for statistical efficiency) for 24-hour ambulatory blood pressure, which is the gold standard.

In chapter 6 we assessed cognitive performance, depressive symptoms, depressive disorder and health-related quality of life. Based on previous literature(57), the results of a concise neuropsychological test battery were combined into several cognitive domains, i.e. memory function, information processing speed and executive function. However, it must be noted that the results of a neuropsychological test cannot always be exclusively related to a single domain(58), and some misclassification may have occurred. Nevertheless, this approach is well-established and reduces biological variability. The Mini-International Neuropsychiatric Interview (MINI) was used to assess depressive disorder(59, 60). Although a structured interview is observed as the gold standard(61), older people are known to underreport depressive symptomatology(61), which may have caused bias. Additionally, the Patient Health Questionnaire (PHQ-9)(62) was used to assess clinically relevant depressive symptoms, which has a high sensitivity and specificity (88% and 85%, respectively) compared to a structured interview for the diagnosis of depression(63). For the assessment of health-related quality of life, the 36-Item Short Form Health Survey (SF-36) was used(64). Although SF-36 has been widely used and generally performed well, older individuals with comorbidities may be more susceptible to bias(65). SF-36 is a self-reported measure of health-related quality of life; therefore, items may be interpreted using different frames of reference in a younger versus an older population. In addition, older adults with comorbidities may have undergone a response shift where they may have reconceptualized the importance of certain domains of the SF-36(65).

In chapters 3 to 6, questionnaires were used to assess, for example, smoking status, alcohol consumption, adherence to the Dutch dietary guidelines and postmenopausal status. Assessing variables with a questionnaire is less objective than a biometric measure (e.g., a blood test for alcohol consumption), which may have resulted in some measurement error(41).

To conclude, overall, the impact of measurement errors and information bias is likely small. Therefore, in our opinion, findings of the thesis can be considered as numerically accurate estimates of the investigated associations.

Causality
A cause can be defined as an antecedent event, condition or characteristic that was required for the disease to occur at the moment it did, given that all other conditions are fixed(66). The temporality criterion of this definition can aid in establishing whether the determinant can indeed be the cause of the outcome or whether the opposite (or both) is true. In all chapters, we used cross-sectional data of The Maastricht Study, which did not allow us to disentangle cause and effect, or a potential bidirectional association, e.g., in chapter 6 we cannot determine that type 2 diabetes preceded worse cognitive performance, depression and poorer health-related quality of life. Therefore, any causal inference made from these studies (chapters 3 to 6) should be made with caution. However, our main goal was to investigate differences between women and men and we do not expect this to affect the investigated sex differences.
**External validity**

External validity refers to the extent in which results of a study with a certain study population can be generalized to other populations (41). All studies in this thesis were performed using data from The Maastricht Study. At least, our results are generalizable to individuals with similar characteristics as The Maastricht Study, i.e. middle-aged Caucasian individuals with access to high-quality healthcare. However, it should be kept in mind that the associations and sex difference that we found may differ in populations with a different distribution of determinants, such as age, or in other ethnic or racial groups.

**Conclusions and future directions**

The present thesis contributes to identifying sex differences in causes and consequences of type 2 diabetes, and more specifically, to understanding the sex differential in diabetes-associated CVD. Summarized, we did not find sex differences in the association of (pre)diabetes with early microvascular complications. Previously, a higher relative risk of diabetes-associated CVD has been observed in women compared to men. Supported by our results, this may be caused by a greater deterioration in cardiovascular risk factors, even before the clinical onset of diabetes. Moreover, our data is consistent with the concept that there is a sex-specific effect of body composition on the development of type 2 diabetes. Whether these sex differences explain the observed differences in diabetes-associated CVD requires further study. Additionally, the sex differences observed, however, were generally not reflected in excess effects on health-related quality of life in men or women. Likewise, no sex differences were observed in the association of diabetes with mental health aspects, such as cognitive performance and depression. Nevertheless, signs for a greater relative worsening of the physical aspects of quality of life in women with diabetes should not be ignored.

While progress has been made towards identifying sex differences in causes and consequences of type 2 diabetes, and more specifically, in understanding the underlying mechanisms of the observed sex differential in diabetes-associated CVD, many uncertainties remain. Future longitudinal studies are necessary to understand the interplay between sex, body composition, type 2 diabetes and CVD (risk). Additionally, more research is required to investigate potential sex differences in the effects of type 2 diabetes on more advanced-stage microvascular complications, mental health aspects, and other related consequences. Eventually, this could result in more personalized care and reduce the burden of diabetes in both sexes.

**References**


34. de Jong, M. Thesis Cardiovascular risk and disease management in diabetes: differences between women and men. 2021


