General Discussion
Main findings and methodological considerations
The main aim of the studies described in this thesis was to investigate different causes and consequences of hyperglycemia. We compared the prevalence of glucose metabolism disorders in the Netherlands in 1989 and 2006 and examined adiposity, genetic factors and family history of type 2 diabetes as risk factors for type 2 diabetes. Moreover, we explored the agreement between the three different markers of hyperglycemia; fasting glucose, postload glucose and HbA1c, and investigated their role in the diagnosis of type 2 diabetes and the development of cardiovascular morbidity and mortality.

In this section, we will describe the main findings of the present thesis, and some methodological issues in relation to our results will be discussed.

**MAIN FINDINGS**

**Prevalence and risk factors of glucose metabolism disorders**

Glucose metabolism disorders (intermediate hyperglycemia and type 2 diabetes) are diagnosed based on standard guidelines (1), shown in Table 1. The prevalence of type 2 diabetes and intermediate hyperglycemia in 1989 and 2006 in the Caucasian population between 50 and 65 years of age in the Netherlands was described in Chapter 2. Between 1989 and 2006, the prevalence of intermediate hyperglycemia increased significantly from 14.4% to 17.6%. The prevalence of type 2 diabetes remained stable at 8%. This coincided with a higher prevalence of hypertension and adiposity, while an improvement in physical activity and lipid profile was observed. In Chapter 3, we further investigated the role of adiposity, as defined by body mass index (BMI) and waist circumference, in the development of type 2 diabetes. Results showed that 16.3% of the participants of the Hoorn Study with adiposity at baseline developed type 2 diabetes within 6 years of follow-up, compared to 6.8% of those without adiposity at baseline. Although this imposes a high relative risk of developing type 2 diabetes due to adiposity, still 83.7% of those...
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with adiposity at baseline did not develop type 2 diabetes. We concluded that not solely adiposity should be used to estimate the individual risk of type 2 diabetes, but that other risk factors should also be taken into account. One of these other risk factors is a family history of diabetes. Our analysis in the Nurses’ Health Study, including 73,227 US women, showed that women with a family history of diabetes have a 2.27 higher risk of developing type 2 diabetes as compared to women without a family history of diabetes (Chapter 4). It is often thought that this increased risk in those with a family history of type 2 diabetes is related to genetic susceptibility. The clustering of obesity within families, which might be related to both shared genetic and environmental factors, might play a role. In Chapter 4 we observed that adiposity, as defined by BMI, explained 21.2% of the association between a family history of diabetes and incident type 2 diabetes. This implies that the higher risk of incident type 2 diabetes in those with a family history of diabetes might not be the result of genetic factors solely. The risk of type 2 diabetes is most likely to be the result of both genetic as well as environmental factors. In Chapter 5, we used a case-control study, including 2628 type 2 diabetes patients and 2041 controls with normal glucose metabolism to investigate the association of 4 genetic variants [glucokinase (GCK), glucokinase regulatory protein (GCKR), islet-specific glucose 6 phosphatase catalytic subunit-related protein (G6PC2) and melatonin receptor type 1B (MTNR1B)] with the susceptibility of type 2 diabetes. The contribution of each individual variant to the risk of type 2 diabetes was found to be very low. However, a risk allele score in which the number of risk alleles in the genes for GCK, GCKR, G6PC2 and MTNR1B were combined, showed that carriers of more than five risk alleles have increased susceptibility to type 2 diabetes (odds ratio of 2.05) compared with the most common risk allele group of four risk alleles (Chapter 5). These results show that genetic variants have a combined effect on type 2 diabetes susceptibility, although the contribution of each individual variant to the risk of type 2 diabetes is very low or undetectable.
Table 1. Glucose metabolism disorders according to the World Health Organisation (WHO) 2006 Criteria (1).

<table>
<thead>
<tr>
<th>Fasting glucose level</th>
<th>Normal glucose metabolism (NGM)</th>
<th>Intermediate hyperglycemia (IH)</th>
<th>Newly-diagnosed type 2 diabetes (NDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AND/Fasting glucose level</td>
<td>&lt;6.1 mmol/L (&lt;110 mg/dL)</td>
<td>6.1-6.9 mmol/L (110-125 mg/dL)</td>
<td>≥7.0 mmol/L (≥126 mg/dL)</td>
</tr>
<tr>
<td>AND/Postload glucose level</td>
<td>&lt;7.8 mmol/L (&lt;140 mg/dL)</td>
<td>7.8-11.0 mmol/L (141-199 mg/dL)</td>
<td>≥11.1 mmol/L (≥200 mg/dL)</td>
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**Markers of hyperglycemia**

Hyperglycemia is defined by three markers, fasting glucose, postload glucose and HbA1c, of which fasting and postload glucose are used to diagnose type 2 diabetes (1). HbA1c is thought to reflect the average glucose level over a previous period of approximately 2-3 months (2,3) and is used to monitor glycemic control in type 2 diabetes patient. In Chapter 6,7 and 8, we investigated diagnostic and prognostic properties of these three markers.

In Chapter 6, we studied the correlation between fasting glucose, postload glucose and HbA1c in a random sample of 2753 men and women. In the type 2 diabetes patients in this sample, we found that the correlations between fasting glucose and HbA1c and between postload glucose and Hba1c were high, 0.71 and 0.79 respectively. However, in the general population without type 2 diabetes, these correlations were only 0.46 and 0.33 respectively, indicating that other factors next to glucose might substantially influence HbA1c levels in people without type 2 diabetes (Chapter 6). In Chapter 5, some genetic variants on HbA1c and fasting glucose were investigated in 2041 non-diabetic individuals. We observed that some genetic variants (GCK and G6PC2) were cross-sectionally associated with both fasting glucose and HbA1c, whereas others were only associated with fasting glucose (MTNR1B). The sum of individual risk alleles of GCK, GCKR, G6PC2 and MTNR1B was associated with a 0.05 mmol/l higher fasting glucose and 0.03% higher HbA1c per risk allele respectively.
Recently, it was proposed to use an HbA1c level of ≥ 6.5% for the diagnosis of type 2 diabetes (4). The American Diabetes Association decided to adapt this advice in the 2010 guidelines for the diagnosis of type 2 diabetes (5). In Chapter 6, we compared the diagnostic properties of HbA1c for the diagnosis of type 2 diabetes as compared to the 75-gr OGTT, which is considered to be the gold standard for the diagnosis of type 2 diabetes. Results showed that in a random sample of the Dutch Caucasian population between 40 and 65 years of age, 93% of the participants with HbA1c levels above 6.5% had diabetic glucose levels. However, of all patients with type 2 diabetes, only 24% had HbA1c levels above 6.5%. Therefore, in most of the people, the OGTT would still be necessary to verify the diagnosis. In Chapter 7, we studied trajectories of fasting glucose, postload glucose and HbA1c in the Hoorn Study population, using measurements 6.3 years and 10 years after baseline in those who did and did not develop type 2 diabetes, with diagnosis based on high glucose and/or high HbA1c levels during follow-up. In patients who developed type 2 diabetes after 10 years, glucose levels increased slightly between baseline and 6.3 years of follow-up. This increase was not significantly different from the slight increase in those who did not develop type 2 diabetes after 10 years. In the last 3.7 years of follow-up, future type 2 diabetes patients showed a rapid increase in glucose, which was not observed in those who did not develop type 2 diabetes. HbA1c levels of future type 2 diabetes patients already started to increase 10 years before diagnosis, with a significantly higher slope as compared to those who did not develop type 2 diabetes. As with glucose, a rapid increase in HbA1c in the last 3.7 years of follow-up was observed in future type 2 diabetes patients. These results imply that HbA1c and glucose might display different developmental patterns before the diagnosis of type 2 diabetes.

The relationship of fasting glucose, postload glucose and HbA1c with the risk of developing fatal and/or non-fatal cardiovascular disease (CVD) was studied in 1674 non-diabetic individuals from the Hoorn Study who were
followed with respect to morbidity and mortality for 10 years (Chapter 8). Adjusted for traditional CVD risk factors, high levels of HbA1c were found to be significantly related to the risk of developing non-fatal CVD, in women, but not in men, with a 2.3 higher risk of developing non-fatal CVD in women in the upper 10% of the range of HbA1c levels (HbA1c ≥ 6.0%) as compared to women in the lowest quartile of HbA1c levels (HbA1c ≤ 5.1%). The association between glucose, either fasting or postload, and incident CVD events was explained by traditional CVD risk factors in both sexes.

METHODOLOGICAL CONSIDERATIONS

Introduction: observational study designs
In epidemiological research, which formed the basis of this thesis, different observational study designs can be used, three of which have been applied in this thesis. Firstly, in a longitudinal cohort design (Chapters 3, 4, 7 and 8), causes of disease (determinants) are measured before the onset of disease (outcomes) in a cohort of individuals whom are followed over time. Due to the prospective nature, a longitudinal cohort study is considered to be the best observational study design to study a causal relationship. However, causal relationships cannot be proven based on a prospective relationship only, other factors like biological plausibility are also of importance (6). Secondly, a cross-sectional study design (Chapters 2, 5 and 6) consists of the measurement of determinants and outcome in a population at the same time. This limits the possibility to study causal relationships. However, cross-sectional studies are less time- and money-consuming as compared to cohort studies, which makes them efficient to use in generating hypotheses on relationships, which could then be studied more extensively in a prospective design. Finally, a case-control design was used in Chapter 5. A case-control study has a retrospective nature, in which a disease is diagnosed and afterwards possible determinants
in those diagnosed with the disease (cases) and those without the disease (controls) are studied. Controls are in general matched to the cases on some basic characteristics like age and gender. Due to the retrospective nature of the study, conclusions on causality are limited in a case-control design. However, case-control studies can be effective when the outcome studied has a low incidence rate (which would imply a long follow-up in a cohort design) and when large numbers of individuals with the disease are needed to answer the research question (for example in studies investigating genetic associations).

The largest threat of all three study designs used in this thesis are systematic errors, also known as bias, and random errors. Bias, in the form of selection bias, information bias and (unexplained) confounding, can harm the validity of the study. Moreover, random errors, largely the results of small sample sizes and measurement error, are of influence on the precision of the reported data. In the following paragraphs, we will further discuss the different studies used in this thesis and the threats on validity and precision which might have been of influence on the results reported in this thesis.

**Study designs**

*The Hoorn Study*

In Chapter 2, baseline data from the Hoorn Study were used. The Hoorn Study is a longitudinal cohort study which started in 1989. Participants were randomly selected from the municipal registry of the town of Hoorn, the Netherlands. Of the 3553 men and women who were invited, 2540 (71.5%) agreed to participate and after exclusion of 56 non-Caucasian participants, the Hoorn Study consisted of 2484 participants. Due to the high attendance rate, the Hoorn Study population is highly representative of the general Dutch Caucasian population of the same age category. In Chapter 3 and 7, we used data from the follow-up measurements of the Hoorn Study population. A mean of 6.4 year after baseline, in 1996-1998, the first re-examination was performed. A total of 398 patients were lost to follow-up. Of the remaining 2086
participants, 1513 persons (72.5%) participated in the follow-up examination. Although the participation rate is relatively high, selective non-response could have occurred. Participants were younger and relatively more healthy at baseline (less hypertensive, more favourable lipid profile) compared to the non-participants. As a result, we might have underestimated the burden of type 2 diabetes in the Hoorn Study population as compared to the general population of that same age. In 2000-2001, the second follow-up examination was conducted. For this examination, a subsample of the Hoorn Study population was invited, including all patients with type 2 diabetes or intermediate hyperglycemia at the previous visit, and a random sample of the participants with normal glucose metabolism at the previous visit. Of the 1074 persons who were invited, 647 (60%) agreed to participate. Since participants of the follow-up in 2000 were selected based on their glucose level at the previous visit, selection bias might have occurred. It could be speculated that the change of glucose and HbA1c over time might be different in those who participated in 2000-2001 compared to those who participated only in 1989 and 1996-1998. This could have affected the results presented in Chapter 7, in which developmental patterns of HbA1c and glucose between 1989, 1996-1998 and 2000-2001 are described. However, the patterns in glucose observed in our study are in line with other studies who used a comparable time frame, which suggests that we were able to detect proper patterns using this population.

In Chapter 8, we used data obtained from a continuous registry of morbidity and mortality of all Hoorn Study participants who gave permission for this registration. The registry of morbidity and causes of death is done by use of data from general practitioners and hospital files. Vital status is obtained from the municipal registry. Because permission to be followed with respect to morbidity and mortality was not obtained at the initial visit but later on, follow-up information of 561 participants was not available. Loss-to-follow up can affect internal validity when it is associated with the outcome of interest.
(in this case cardiovascular disease). However, a comparison of participants and non-participants did not show major differences in cardiovascular risk factors and markers of hyperglycemia at baseline between the populations with and without information on follow-up (10). Therefore, we do not expect that the loss-to-follow up has affected the association between markers of hyperglycemia and CVD risk presented in Chapter 8.

The New Hoorn Study

Data from the New Hoorn Study were used in Chapter 2, 5 and 6. The New Hoorn Study was initiated in 2006. A random sample of 6180 inhabitants of the town of Hoorn, between 40 and 65 years of age was drawn from the population registry. A total of 2807 people (45.5%) agreed to participate. Due to the relatively low participation rate, selection bias might have occurred, resulting in a less representative sample of the general population in the Netherlands in that same age range. This could have had an effect on the reported prevalence of type 2 diabetes and intermediate hyperglycemia in Chapter 2. For example, we suspected that people with a low socio-economic status, who are expected to have a higher prevalence of type 2 diabetes and type 2 diabetes risk factors, participated less. To evaluate to what degree this may have affected our results, we conducted a ‘worst-case scenario’ analysis in which we estimated the prevalence of type 2 diabetes we would have found if all current non-responders were of a low socio-economic status and would have participated (increasing our study sample with 3373 participants, all grouped into the low educational level). This revealed that the prevalence of intermediate hyperglycemia might have been slightly underestimated (17.5% in current analysis, 18.7% in sensitivity analysis), but that no difference would have been found in the prevalence of type 2 diabetes (Chapter 2).

In Chapter 5, the population of the New Hoorn Study formed the basis of a case-control design. All individuals with normal glucose metabolism in the New Hoorn Study were used as controls. Participants with type 2
Main findings and methodological considerations

diabetes formed the case-group, supplemented with type 2 diabetes patients from the Diabetes Care System West-Friesland, the VU University Medical Center and the Leiden University Medical Center. A possible source of bias of a case-control design is the selection of the cases and controls. To assure comparability between cases and controls, the most effective way is to include cases and controls out of the same population. In Chapter 5, people with normal glucose metabolism (controls) and type 2 diabetes (cases) from the same population, the New Hoorn Study, were used. However, to increase power, type 2 diabetes patients from three healthcare facilities were added to the study. This might have influenced the comparability of the cases and controls and, as a result, influenced the validity of the results presented in Chapter 5. In addition, in contrast to the type 2 diabetes patients from the New Hoorn Study, whom represent both patients with diagnosed and undiagnosed type 2 diabetes, the added type 2 diabetes patients from three care facilities were all diagnosed with type 2 diabetes and treated accordingly. Therefore, the population used in Chapter 5 might over-represent type 2 diabetes patients with co-morbidities. In addition, those whom have died (due to for example severe diabetes complications) are lacking in the study, possibly resulting in a healthy survivor effect. If these type 2 diabetes patients lacking in the current analysis would have had a different genetic risk profile, this would have had an effect on our results.

In Chapter 2, the baseline measurements of the Hoorn Study and New Hoorn Study were compared in the overlapping age group of participants between 50 and 65 years of age. A problem when interpreting the results of the comparison of two populations over time is the ‘age-period-cohort-effect’: individual aging, period influences, and cohort influences together are thought to form the basis for a change over time (11). When individual age is kept constant (both populations were between 50 and 65 years of age), the differences found can be the result of a cohort-effect and/or period-effects. The cohort in 1989 might differ from the cohort in 2006 with respect to their
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risk of developing glucose metabolism disorders, due to differences in socio-economic and behavioral factors (cohort-effect). In addition, the upcoming westernized lifestyle (including an unhealthy diet and a lack of physical activity, which are important risk factors for type 2 diabetes) might have affected the society in 2006 more as compared to the society in 1989 (period-effect). These two effects might form the basis of the differences in (risk factors of) glucose metabolism disorders between 1989 and 2006 reported in Chapter 2.

The Nurses’ Health Study

The Nurses’ Health Study is a prospective cohort study which started in 1976 when 121,700 married female registered nurses aged 30-55 years received a questionnaire on health status and potential risk factors for major chronic diseases. Ever since, participants were sent questionnaires biennially and the response rates have been approximately 90% (12). The possibility of selection bias was limited due to the high response rates for the follow-up questionnaires.

The nurses who participated in the Nurses’ Health Study, represent a specific population of females with a certain degree of education and socio-economic status in the United States. In Chapter 4, we observed that adiposity explained 21% of the association between family history of diabetes and incident diabetes. In more diverse populations, SES, lifestyle factors, and adiposity may explain a greater proportion of the association between family history of diabetes and type 2 diabetes risk (Chapter 4). The representativity of this specific population of nurses could be questioned in some cases. Firstly, populations of the United States might differ in the prevalence and combination of diabetic risk factors as compared to other populations (for example European or Asian populations). For example, Asian people with a normal BMI (<25 kg/m²) more often develop type 2 diabetes compared to Caucasian people with a normal BMI (13;14), possibly affecting the role of...
BMI in the association between family history of diabetes and incident type 2 diabetes. Therefore, extrapolation of the results presented in Chapter 4 to other populations should be considered with caution. Secondly, exploration of the results to populations with a lower socio-economic status should also be considered with caution, since socio-economic status might be of influence on the prevalence of adiposity and the incidence of type 2 diabetes. Last, since sex-specific differences have been found in risk factors for incident type 2 diabetes (15) and more specifically in the role of adiposity in the occurrence of type 2 diabetes (16), the results presented in Chapter 4 might not be representative for a male population.

Confounding and effect-modification

Systematic errors are the result of bias, for example selection bias as mentioned in the previous section, and confounding and effect-modification. Confounders are factors which are associated with the determinant of interest, and are also markers for the outcome of interest. For example, when adiposity is the determinant of interest and type 2 diabetes the primary outcome, physical activity might be a confounding factor, since it is associated with adiposity and also with the risk of type 2 diabetes. In this thesis, relationships between determinants and outcome were studied in Chapter 4, 5 and 8. In these Chapters, confounders could be of influence on the association studied, leading to an underestimation or overestimation of the reported effect between the determinant and outcome. Therefore, we used analysis techniques in which adjusting for confounders is possible. Some difficulties could arise when adjusting for confounders. Firstly, when potential confounders are not measured at all or without enough accuracy, results must be considered with caution due to residual confounding. Secondly, a confounder can in fact be a mediator involved in the causal pathway between the determinant and outcome. Including a mediator in the analysis would result in an underestimation of the effect between determinant and outcome.
In all multivariate analysis, we have on forehand tried to gain insight into the mechanism behind the relation of the determinant with the outcome of interest and the role of the possible confounders in this relation to avoid including mediating variables in our analysis. However, some over-adjusting might have occurred. For example, in Chapter 8, the association between markers of hyperglycemia and cardiovascular disease was adjusted for known cardiovascular risk factors (i.e. cholesterol, blood pressure, adiposity). If one of these factors is in fact involved in the causal pathway between hyperglycemic measures and cardiovascular disease, this would lead to an underestimation of the true effect.

Next to confounding, effect-modification, which is the phenomenon that the effect of a determinant on the outcome of interest is different for categories of a second factor, is a possible source of systematic error, which should be investigated (by entering an interaction term into the statistical model) and, if present, eliminated (by stratifying the analysis according to the categories of the effect-modifier). For example, the association between glucose levels and the risk of cardiovascular complications might be different for those with type 2 diabetes compared to those without type 2 diabetes, indicating effect-modification by type 2 diabetes status. In Chapter 8, we discovered effect-modification by gender, implying that the association between hyperglycemic markers and cardiovascular disease is different for men and women. Therefore, the results of Chapter 8 were stratified by gender.

**Measurement methods**

When interpreting the results of epidemiological research, the measurement methods used should be taken into account. Below, the different measurements used in the studies of this thesis will be discussed. In addition, (prevention of) random errors (which affects precision) and systematic errors (which affects validity) as a result of these measurement methods will be discussed.
Main findings and methodological considerations

Diagnosing type 2 diabetes

The gold standard to diagnose type 2 diabetes is the use of an 75-gram OGTT (1). With the use of an OGTT, the large proportion of people with type 2 diabetes based on postload glucose solely (17) can be distinguished, and those with undiagnosed type 2 diabetes can be detected. Still, reporting type 2 diabetes prevalences based on known type 2 diabetes or fasting glucose only is a common feature in epidemiological research (18-22), which is justifiable since this is often used in clinical practice also. In the Hoorn Study and New Hoorn Study, an OGTT was used to diagnose type 2 diabetes, increasing the accuracy of the data. In addition, the general practitioners of participants reporting to be already known with type 2 diabetes were contacted to verify the diagnosis. In the Nurses’ Health Study, type 2 diabetes was based on self-report. A sub-study within the Nurses’ Health Study examined the validity of the self-reported diagnosis and revealed that 98% of the self-reported diagnosis were verified by medical records (23). However, the prevalence of undiagnosed type 2 diabetes is known to be high (24-26) and self-report of type 2 diabetes could therefore have resulted in an underestimation of the true incidence of type 2 diabetes. Moreover, since high levels of BMI have been found to be strongly associated with type 2 diabetes based on isolated postload hyperglycaemia (17), not using an OGTT for the diagnosis of type 2 diabetes might have resulted in an underestimation of the role of BMI in the association between family history of diabetes and risk of type 2 diabetes presented in Chapter 4.

In Chapter 2, the prevalence of type 2 diabetes in 1989 and 2006 in the Netherlands was compared. In between this time period, the cut-off point for the diagnosis of type 2 diabetes based on fasting glucose was lowered from 7.8 mmol/l to 7.0 mmol/l (27;28). Since general practitioners in the Netherlands mostly use fasting glucose to diagnose type 2 diabetes (29), people with a fasting plasma glucose between 7.0 and 7.8 mmol/l will have been diagnosed with type 2 diabetes in 2006, but not in 1989. In our 1989 population, of the 123
type 2 diabetes patients, 45 (36%) had fasting glucose levels between 7.0 and 7.8 mmol/l, and could therefore not have been diagnosed with type 2 diabetes by their primary care practitioner in 1989. If the threshold had already been lowered to 7.0 mmol/l in 1989, it is to be expected that not all 45 people would have had such a diagnosis. Therefore, we can not estimate the extent to which this influenced the reported prevalence data in Chapter 2.

Measuring anthropometry and blood pressure

In the Hoorn Study and New Hoorn Study, anthropometry (weight, height and circumference measures) and blood pressure were determined. To minimize errors due to differences in operational procedures, the standard operational procedures from the baseline measurement of the Hoorn Study were used in the follow-up measurements of 1996-1998 and 2000-2001, and in the data collection of the New Hoorn Study. However, blood pressure was measured with a random-zero sphygmomanometer (Hawksley-Gelman Ltd, Lancing, UK) in the Hoorn Study, and with a Colin Press BP 8800p Non-Invasive Blood Pressure Monitor (Colin Medical Technology Corporation, USA) in the New Hoorn Study. Since research has shown that monitors tend to overestimate systolic blood pressure and underestimate diastolic blood pressure as compared to sphygmomanometers (30), this differences in devices between 1989 and 2006 might have influenced the results on blood pressure reported in Chapter 2.

Poor precision in measurement of anthropometry or blood pressure may lead to underestimating the effect in relation to an outcome of interest (31). We tried to optimize precision by several procedures. Firstly, all equipment was calibrated on a regular basis. Secondly, well designed operational procedures were developed to optimize the comparability between measurements performed by different research assistants. Finally, to reduce within-person variation, measures of waist circumference and blood pressure were determined twice in every participant of the Hoorn Study and New Hoorn Study. Final data contained the mean of two measurements to use for analyses.
The use of questionnaires

In all studies included in this thesis, questionnaires were used to obtain self-reported data on lifestyle and family history of disease. In the Nurses’ Health Study, questionnaires were used as main source of data collection. The medical knowledge of this population of nurses is high, and their relatively high degree of education and motivation to participate have increased the validity of the questionnaire data. In addition, the team of the Nurses’ Health Study conducted several studies to determine the validity of the data used in Chapter 4. For example, subsamples of the Nurses’ Health Study were used to compare self-reported weight and circumference measures with measured weight and circumference measures (32), and self-reported dietary intake with diet records and biomarkers (33;34). These validation studies reported high correlations, which indicates a reasonable precision of the questionnaire data.

However, under-reporting or over-reporting cannot be fully excluded. This also applies for the questionnaire data of the Hoorn Study and New Hoorn Study, in which information on smoking behavior and physical activity were obtained by questionnaires. The validity and reliability of self-reported physical activity is known to be poor (35) and this might have been on influence on the precision of our estimates on physical activity behavior presented in this thesis.

Biochemical determinants

In the Hoorn Study and the New Hoorn Study, blood samples from all participants were drawn for the determination of cholesterol, triglycerides, glucose and HbA1c. The determination of biochemical indicators in both studies and all follow-up measurements were performed in one certified laboratory using standardized tests. The analytical and biological variation, expressed as the within-person covariant of variation, of fasting glucose, postload glucose and HbA1c are 5-7%, 15-18% and 3-4% respectively (36). A
high within-person variation could influence the precision of measurements and should therefore be taken into account when interpreting the results. For example, it could be speculated that the rather high variability of glucose compared to HbA1c is of influence on the low correlation between glucose and HbA1c observed in Chapter 6. It would have strengthened our results if we would have had the availability of multiple measurements of glucose. However, although the short-term variability of HbA1c is lower compared to the variability of fasting or postload glucose levels, the variability over a longer period of 1 year is higher than for glucose (37). In addition, the correlations between glucose and HbA1c in patients with known type 2 diabetes found in Chapter 6 are comparable to the correlations found in the study by Nathan et al. in which continuous glucose monitoring, which has a lower variability due to multiple within-person measurements, was used (38). Therefore, it is expected that the low correlation between glucose and HbA1c observed in the general population is not entirely the result of the high variability of glucose.

Between 1989 and 2006, the assay used in our laboratory for determination of HbA1c changed twice. Between 1989 and 1998, HbA1c levels were determined by ion-exchange high performance liquid chromatography (HPLC) on a modular diabetes monitoring system (Bio-Rad, Veenendaal, the Netherlands). In 1998, a slightly different ion-exchange HPLC procedure was introduced, based on separation on a Mono-S column (Pharmacia, Uppsala, Sweden) using previously described methodology (39). Last, in 2003, reversed-phase cation exchange chromatography (HA 8160 analyzer, Menarini, Florence, Italy) was introduced, accompanied by the use of a method to standardize HbA1c across laboratories (DCCT-standardization) (40;41). Though after both changes validation reports concluded that this did not affect the clinical values used in the care for type 2 diabetes patients, the changes could have affected the results presented in Chapter 2 (comparison of HbA1c in 1989 and 2006) and Chapter 6 (change in HbA1c between 1989, 1996 and 2000). Therefore, the results of the validation reports, in which linear regression models were
provided which described the associations between the different methods, were used to recalculate the HbA1c values into comparable estimates and, as a result, increase the validity of the data presented in Chapter 2 and 6.

Registration and classification of morbidity and mortality
Since the start of the Hoorn Study, a continuous registration of morbidity and mortality of the participants who gave permission to be followed over time has been conducted. Events are abstracted from the medical records at the general practitioner, local hospital and/or local nursing home. Events are then coded using the International Classification of Diseases, Injuries and Causes of Death, ninth version (ICD-9) (42) using a ICD-coding program designed for the Hoorn Study. For example, to code a myocardial infarction, the coding program uses the available information on specific blood enzymes, ECG’s and pain. The coding was validated against the coding of a certified nosologist in a subsample of 52 deceased Hoorn Study participants. Good validity was observed, with a kappa-value of 0.85 for cardiovascular disease (43). Since it has been found that death certificates can be incorrect when determining causes of death (44;45), the use of a coding program might have increased the validity of our morbidity and mortality data.
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Mechanisms, clinical implications and future research

In this section, the results presented in this thesis will be evaluated in relation to the mechanisms behind the causes and consequences of hyperglycemia. Moreover, some clinical implications will be evaluated and ideas for future research will be postulated.

MECHANISMS BEHIND THE RESULTS PRESENTED IN THIS THESIS

Type 2 diabetes is a complex trait
Type 2 diabetes is among the large number of diseases which are caused by multiple risk factors, both genetic and environmental. For type 2 diabetes, a large number of genetic and environmental risk factors have been identified so far, some of which are described in this thesis. In Chapter 4, we described family history of diabetes as a risk factor for the development of type 2 diabetes. Moreover, we observed that adiposity, also a risk factor for type 2 diabetes, explains a part of the association between family history of diabetes and incident type 2 diabetes, which suggests the combination of shared genetic and shared environmental factors in the development of type 2 diabetes. In Chapter 5, we observed associations of 4 genetic variants with the susceptibility of type 2 diabetes, and found that the analysed loci have a combined effect on type 2 diabetes susceptibility, although the contribution of each individual variant to the risk of type 2 diabetes was very low or undetectable. This is a common feature of complex traits, in which multiple risk factors act together in the occurrence of a disease and individually have a relatively weak effect on the disease (46). Due to the multifactorial nature of type 2 diabetes, the prediction of type 2 diabetes is complex and it is to be expected that accurately predicting the individual risk of type 2 diabetes remains a challenge (46). As a result, the paper risk score presented in Chapter 3 is able to estimate a maximum likelihood of only 20%, and not 100%, of
developing type 2 diabetes within 5 years. Even risk predictions that combine genetic and environmental data (47), or risk predictions which combine a paper risk score with information on blood levels of for example glucose (48), only marginally improve prediction. There will always be unidentified risk factors, complex interactions between risk factors and multiple pathways of disease evolution which need further research and which presently hamper the prediction of future type 2 diabetes.

Differences between markers of hyperglycemia
It has been recognized since long that high fasting glucose and high postload glucose are the result of different processes. Both are related to insulin resistance and impaired beta-cell function. However, high fasting glucose is thought to be mainly the result of hepatic insulin resistance, while high postload glucose is associated with peripheral (muscle) insulin resistance (49;50). Indeed, the low correlation between fasting glucose and postload glucose which was found in Chapter 6 of this thesis, strengthens the concept of a partially different pathophysiological background between elevated fasting and postload glucose. Earlier studies also showed that elevated levels of fasting glucose and postload glucose display different risks of future cardiovascular disease and mortality (7;51;52). We did not observe major differences in the developmental patterns of fasting glucose and postload glucose before the onset of diabetes (Chapter 7) or major differences between fasting and postload glucose in the risk of future CVD (Chapter 8) in the general population of the Hoorn Study. Studies in which specific groups with isolated impaired fasting glucose, isolated impaired glucose tolerance or a combination of both are prospectively investigated, could be helpful in further investigating the risks of future diabetes and cardiovascular disease associated with high levels of fasting or postload glucose.
HbA1c is thought to reflect average glucose levels over the past 2-3 months. Indeed, earlier research observed high correlations between glucose and HbA1c in patients with type 2 diabetes (38;53;54). This corresponds with our results in Chapter 6, in which we observed high correlations between glucose and HbA1c in known type 2 diabetes patients. This justifies the role of HbA1c in monitoring glycemia in type 2 diabetes patients. However, we observed a low correlation between glucose and HbA1c in a random sample of the general Dutch population, mainly including people without type 2 diabetes (Chapter 6). This is in accordance with the accumulating evidence that HbA1c might also be determined by other factors not related to glycemia. Several of these factors have been described in previous research. Firstly, HbA1c is known to differ between ethnic groups. Mean levels of HbA1c have been found to be higher among Hispanics, Asian Americans, American Indians and African Americans as compared to Caucasian Americans (55). Secondly, several studies in both healthy and diabetic twins have shown that HbA1c levels are largely genetically determined, independently of the genes influencing fasting glucose (56;57). In Chapter 5, we identified loci’s associated with fasting glucose and HbA1c. Earlier studies also reported on shared loci’s for glucose and HbA1c (58;59), however, loci’s only related to HbA1c have also been identified (60). More research into the heritability of different glycemic measures in patients with type 2 diabetes as well as healthy individuals is needed to further unravel the role of genetics in the formation of HbA1c. Thirdly, aging has been found to be of influence on HbA1c. In the non-diabetic populations of the Framingham Offspring Study and NHANES, a 0.014 and 0.010 unit increase in HbA1c per year was observed respectively (61). Last, levels of HbA1c are determined by factors related to erythrocyte environment, leading to the so called ‘glycation gap’, which represents the difference between protein glycation occurring in the intracellular space (HbA1c) versus in the extracellular space (measured as fructosamine) (62,63). Factors of influence on the ‘glycation gap’ are interindividual heterogeneity.
in the erythrocyte life-span and in the erythrocyte transmembrane glucose gradient (3;64).

In Chapter 7, we observed that HbA1c levels start to increase earlier in time compared to glucose levels in future type 2 diabetes patients, which also underlines the possibility of non-glycemic factors related to HbA1c levels. However, rapid increases in both glucose and HbA1c shortly before the diagnosis of type 2 diabetes were observed (Chapter 7). It is thought that the rapid increase in glucose a few years before diagnosis, as shown in our study in Chapter 7 and previous studies (8;9), is the result of a long period in which beta-cell function can compensate for insulin resistance, followed by an unstable period in which insulin secretion is no longer sufficient, leading to type 2 diabetes (65;66). Whether the rapid increase in both HbA1c and glucose the last years before the diagnosis of type 2 diabetes is the result of the relation of HbA1c to glycemia or whether other biological processes may also play a role, needs to be further investigated.

Next to their relation to each other, we also observed differences between glucose and HbA1c in their association with future cardiovascular disease (Chapter 8). A stronger association of HbA1c as compared to glucose with fatal CVD was observed in previous studies and replicated by our results in Chapter 8. Moreover, we found a strong association between HbA1c, but not glucose, and non-fatal CVD, indicating a possible role of HbA1c in atherosclerotic processes (Chapter 8). Other studies reported strong relationships between HbA1c and retinopathy risk (67;68), stronger as compared to glucose (67;68). Since type 2 diabetes-related complications like CVD and retinopathy are expected to be the result of chronic glycemia, it is thought that HbA1c is more strongly related to type 2 diabetes-related complications than fasting and postload glucose because it more accurately represents chronic glycemia (4). However, it could also be speculated that the non-glycemic factors of influence on HbA1c mentioned earlier (i.e. age, genetic factors, erythrocyte environment), are also among the explanations
for the differences between glucose and HbA1c in their association with type 2 diabetes-related complications.

**CLINICAL IMPLICATIONS**

In this section, some clinical implications of the results presented in this thesis will be discussed.

**The importance of early detection**

Type 2 diabetes is one of the world’s most common chronic disorders. It is characterized by high disease-related costs and diminished quality of life due to diabetes-related complications. Type 2 diabetes is associated with multiple risk factors, of which slightly elevated glucose levels, age, family history of disease and adiposity are the most common features. Due to the increasing prevalence of intermediate hyperglycemia and adiposity (Chapter 2), which are both threats implying a high risk of future type 2 diabetes (7) as well as diabetes-related complications (Chapter 8), prevention in those at high risk of developing type 2 diabetes in the future is of utmost importance. A challenge is to effectively identify people at high risk. A paper risk score, as mentioned in Chapter 3, might be effective in primary care to identify those at high risk of developing type 2 diabetes as well as cardiovascular disease. Not having to perform laboratory tests and physical examinations in everyone could reduce health care costs and workload for general practitioners. The (cost-)effectiveness of the use of a risk score in the Dutch health care system to jointly identify those at high risk of several diseases needs to be further evaluated. Recently, the Dutch College of General Practitioners in cooperation with the Dutch Type 2 diabetes Research Foundation, the Netherlands Heart Foundation and the Dutch Kidney Foundation, initiated an investigation into the use of a paper risk score to identify individuals at high risk of cardiovascular disease, type 2 diabetes and kidney failure.

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1. http://nhg.artsennet.nl/kenniscentrum/k_implementatie/k_preventie/Preventieconsult-1.htm
Diagnosing type 2 diabetes: the use of HbA1c

The diagnosis of type 2 diabetes has been under debate several times in the past, changing the diagnostic cut-off point for fasting glucose and evaluating the need of the OGTT. Recently, a new debate was started in which the use of HbA1c for the diagnosis of type 2 diabetes was questioned, resulting in an official advice from the American Type 2 diabetes Association to use an HbA1c level of ≥ 6.5% for the diagnosis of type 2 diabetes (5). This advice was mainly based on the strong association between high HbA1c levels and the occurrence of retinopathy. Advantages of using HbA1c for the diagnosis of type 2 diabetes include the rather low within-person variability as compared to glucose (see section ‘biochemical determinants’ in Chapter 9.1), the recent standardization of the laboratory assay (69), the low day-to-day variability, the fact that there is no need for fasting sampling and the strong association between HbA1c and diabetes-related complications like cardiovascular disease and retinopathy (4;10;67;70;71). However, disadvantages include the lack of standardization of HbA1c determination across laboratories in many (developing) countries (72), the effect of iron deficiency or hemoglobin variants on the HbA1c level (72;73) the high costs of the determination of HbA1c as compared to glucose (73) and the variability of HbA1c due to non-glycemic factors like age, ethnicity and erythrocyte environment (see section ‘difference between hyperglycemic markers’ of this Chapter). In this thesis, we observed low correlations between HbA1c and glucose levels in the general population and showed that the sensitivity of the proposed diagnostic cut-off point of 6.5% is low, advocating against using HbA1c for the diagnosis of type 2 diabetes. Furthermore, the effect of treatment based on an HbA1c-diagnosis is unknown and it could be speculated that this might result in complications like hypoglycemia (74). The American Diabetes Association proposed the use of both glucose and HbA1c for the diagnosis of type 2 diabetes in their 2010 guidelines (75). However, determining both glucose and HbA1c might not be as cost-effective as the use of solely glucose and HbA1c. Moreover, it is unclear
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which diagnosis should be made and which treatment should be started in those with high glucose levels but low HbA1c as well as in those with low glucose but high HbA1c. Based on our results and the disadvantages of HbA1c for the diagnosis of type 2 diabetes mentioned above, it is advisable not to use HbA1c for the diagnosis of type 2 diabetes. More research, mentioned in the next paragraph, on the consequences of using HbA1c for the diagnosis of type 2 diabetes is needed first.
FUTURE RESEARCH

Based on the results presented in this thesis, we would suggest the following topics for future research:

1) With the introduction of genome wide association (GWA) studies, evidence on the role of different loci’s in the development of type 2 diabetes is accumulating. However, due to the enormous number of tests needed in GWA studies, the chance of false positive results are high. Therefore, future research should focus on replication of associations found in GWA studies in multiple populations to verify whether there is a true genetic association (76). Moreover, much additional work is needed to further unravel the emergence of type 2 diabetes. It remains a challenge to understand the pathophysiological processes behind such a complex disease as type 2 diabetes and therefore, further studies on gene-environment interaction and gene-gene interaction might be helpful in mapping type 2 diabetes. For example, gene-environmental interaction studies can help to provide insight into the role of adiposity and genes in the association between a family history of disease and the risk of future type 2 diabetes, as well as how an implicated locus functions in the mechanisms of evolving type 2 diabetes (76).

2) Before the recent recommendation to use HbA1c for the diagnosis of type 2 diabetes (4) is implemented, several questions should be answered by additional epidemiological research. Firstly, longitudinal studies are needed to evaluate the effects of treatment in patients who are diagnosed based on an HbA1c level of ≥ 6.5%. As a result of the lack of concordance between glucose and HbA1c, one could expect that a substantial proportion of these patients will have low glucose levels. It is unknown whether treatment in these individuals is effective or will cause side-effects like hypoglycemia. Databases including extensive clinical data of a large number of type 2 diabetes
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patients, for example the General Practice Research Database in the United Kingdom\(^2\) or the recent developed String of Pearls Initiative in the Netherlands\(^3\), can be helpful to answer this question. Secondly, more research is needed into the risk of future complications in people with low glucose/high HbA1c and people with high glucose/low HbA1c, also known as ‘high and low glycators’. An ongoing analysis in the Hoorn Study data revealed that people with high HbA1c, but normal glucose levels, have an increased risk of mild retinopathy, but not microalbuminuria (77). Thirdly, the cost-effectiveness of a combination of HbA1c and glucose for the diagnosis of type 2 diabetes should be assessed, as well as the feasibility of HbA1c for the diagnosis of type 2 diabetes in developing countries in which money and resources are limited. In addition, the cut-off point for the diagnosis in different age- and ethnic groups and groups with conditions that shorten or prolong erythrocyte survival should be evaluated. Last, the official recommendation to use HbA1c for the diagnosis of type 2 diabetes states to be cautious about providing a cut-off point for a group at high risk of developing type 2 diabetes. Using glucose as a diagnostic criterion, intermediate hyperglycemia was defined as the high risk group and indeed, research showed a high risk of type 2 diabetes and complications in this group (7;52), as well as a potential for prevention of type 2 diabetes (78;79). We observed a high risk of cardiovascular complications in people with HbA1c ≥ 6.0% (Chapter 8), indicating that HbA1c levels between 6.0% and 6.5% might be effective to identify those at risk. Further research on the risk of future type 2 diabetes and complications and on the possibility of prevention in people with slightly elevated HbA1c levels could be helpful in determining a cut-off point for a high risk group based on HbA1c levels.

\(^2\)http://www.gprd.com
\(^3\)http://www.parelsnoer.org


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In conclusion, this thesis showed the complex background of hyperglycemia, in which both genetic and environmental factors, with interrelationships, are included. It remains a challenge to effectively map the occurrence of type 2 diabetes and to, as a result, identify those at risk of developing the disease. In addition, this thesis contributed to the ongoing discussion about the agreement between glucose and HbA1c, both markers for hyperglycemia, and the consequences of using HbA1c instead of glucose for the diagnosis of type 2 diabetes. Before HbA1c is implemented as diagnostic tool for type 2 diabetes, several urgent questions need to be answered.
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