Obesity and insulin resistance

Intermediate hyperglycaemia in populations and ageing tissuers

van Olden, C.C.

Publication date
2023

Chapter 1

Introduction

1.1. History of the Diagnosis of Diabetes Mellitus Type 2

1.1.1. The Name

The first description of two types of diabetes mellitus is attributed to Sushruta and Charaka in the fifth century AD[1].They observed that thin individuals with diabetes developed diabetes at a younger age in contrast to heavier individuals with diabetes, who had a later onset and lived for a longer period of time after diagnosis. Diabetes was diagnosed based on clinical symptoms of hyperglycaemia, and it appears that these different presentations were seen as variations of a single disease and not as entities with a separate pathophysiology[2]. The recognition of these separate phenotypes to be different diseases was first described in 1875 by Bouchardet[3]. He proposed a classification of *diabète maigre* and *diabète gras* to distinguish between two syndromes he deemed to have different prognosis and clinical management. This distinction did not seem to find enough traction and seems to have been lost. In the beginning of the twentieth century the tendency was to consider "mild" and "severe" forms of the disease along with other terms delineating age of onset. During the 1930s the concept of *secondary diabetes* was described to diabetes associated with other diseases like haemochromatosis, pancreatitis etc. This term was used in distinction with *primary* or *idiopathic* diabetes. There were many attempts to stage primary diabetes, however these terms were not used uniformly[2].

The British physician Harold Percival Himsworth was the first to describe a distinction of the various presentations of diabetes based on their physiology. He described in 1936 "insulin sensitive" and "insulin resistant" individuals with diabetes, incidentally also laying the groundwork for the clamp-technique[4]. He went on stating that diabetes mellitus is not one disease but a syndrome encompassing a group of disorders differing in their clinical presentation and pathophysiology[5]. Following this, R.D. Lawrence summarised in his presidential address in 1950 that human diabetics are divided clinically into two main types: those who are probably not insulin deficient and those that are[6].

It was the British respiratory Physician Philip Hugh-Jones who appears two have introduced the terminology "Type 1 and 2 diabetes" in 1955 as a clinical classification[7] (A previous mention of type I and II diabetes was

Table 1.1.: *Diabetes classification based on NDDG, 1979*

Insulin dependent diabetes mellitus (IDDM): Type I	
Non insulin dependent diabetes mellitus (NIDDM): Type II	Nonobese Obese
Other types, including diabetes mellitus associated with certain conditions and syndromes	Pancreatic disease Hormonal Drug or chemical induced Other types
Impaired glucose tolerance (IGT)	Nonobese Obese Associated with certain conditions and syndromes
Gestational Diabetes	

made in 1951[8], these types relate to constitutional make-up or physique of the patient and not to pathophysiology and should therefore not be counted as the first mention). These terms did not gain widespread use until they were revived by Andrew Cudworth in 1976[9], who subsequently popularised its use along with other authors[10]. Up until this period, there was no consensus as to the classification or the appropriate diagnostic criteria. This was a growing concern in the diabetes medical community.

> *Similarly the types of diabetes were loosely divided into juvenile onset and maturity onset, with secondary diabetes, chemical diabetes, borderline diabetes and prediabetes all used in ill-defined ways.* (Alberti and Zimmet 1998[11])

In an effort to harmonise the use for both clinical and research purposes the National Diabetes Data Group (NDDG) in conjunction with the World Health Organization (WHO) revised and published new and unified criteria for the classification and diagnosis of diabetes mellitus using an equivalence of these terms in 1979, see Table 1.1[12,13]. This classification system consisted of mutually exclusive categories based on simple clinical observation.

For the semantics part of the classifications, there remained a feeling that further revision was deemed necessary. The NDDG classified on the basis of clinical descriptors, whereas the authors who popularised the

Table 1.2.: *Aetiologic classification of diabetes mellitus, based on Export Committee report 2003*

I. Type 1 diabetes* (β-cell destruction, usually leading to absolute insulin deficiency)
A. Immune mediated
B. Idiopathic
II. Type 2 diabetes* (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with insulin resistance)
III. Other specific types**
A. Genetic defects of β-cell function
B. Genetic defects in insulin action
C. Diseases of exocrine pancreas
D. Endocrinopathies
E. Drug- or chemical-induced
F. Infections
G. Uncommon forms of immune-mediated diabetes
H. Other genetic syndromes sometimes associated with diabetes
IV. Gestational diabetes mellitus (GDM)

* Patients with any form of diabetes may require insulin treatment at some stage of their disease. Such use of insulin does not, of itself, classify the patient.
** In the article, all subcategories contained specifics

term made the distinction on aetiological grounds. In the subsequent years, with burgeoning of pathophysiological knowledge about diabetes, it was felt that an aetiology-based system could be used. To this end, the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus published a revised classification first in 1997[14], after which some modifications were made regarding the diagnosis of impaired fasting glucose to result in the classifications published in 2003, see Table 1.2[15]. Most notable amongst these changes are the abandonment of the terms insulin-dependend diabetes mellitus and non-insulin-dependent diabetes mellitus, including their acronym.This eliminates classification based on treatment. Also, the Roman numerals were replaced by Arabic numerals.

In this definition, diabetes is classified into four general categories, into

Table 1.3.: *Types of diabetes as described by the WHO 2019*

Type 1 diabetes
Type 2 diabetes
Hybrid forms of diabetes
Slowly evolving immune-mediates diabetes of adults (previous LADA)
Ketosis prone type 2 diabetes
Other specific types
Monogenic Diabetes -Monogenic defects of β-cell function -Monogenic defects in insulin action
Diseases of the exocrine pancreas
Endocrine disorders
Drug- or chemical-induced
Infections
Uncommon specific forms of immune-mediated diabetes
Other genetic syndromes sometimes associated with diabetes
Unclassified diabetes
This category should be used temporarily when there is not a clear diagnostic category especially close to the time of diagnosis of diabetes
Hyperglycaemia first detected during pregnancy
Diabetes mellitus in pregnancy
Gestational diabetes mellitus

which the specific aetiological subtypes can be divided. This system is by and large still in place today. In clinical care, it is of course possible for patients to suffer from two or more forms of diabetes simultaneously or consecutively. This has sprouted the term 'atypical diabetes', denominating a wide range of phenotypic forms of diabetes of uncertain pathophysiology which can not easily be classified in the existing categories[16]. These developments led to the addition of two extra categories to the most recent WHO consultation on the diagnosis of diabetes, with some other minor changes see Table 1.3[17]. Adding to this complexity is the worldwide epidemic of obesity, which has superimposed the pathophysiology of type 2 diabetes across all other types.

1.1.2. Glucose Criteria

The 1979 classification was also the first harmonization of glucose criteria. Prior to this classification, at least six different sets of criteria diagnosed diabetes[18]. The NDDG aimed to create a system of mutually exclusive classes that required only simple clinical measurements. Diabetes was classified if the patient exhibits obvious diabetes symptoms as polyuria, polydipsia, ketonuria and rapid weight loss, together with gross and unequivocal elevation of plasma glucose (PG). In the absence of these signs and symptoms, quantitative measurements of PG are prescribed for making a clinical diagnosis of diabetes. This can be done by measuring the fasting plasma glucose (FPG), if this is not elevated an oral glucose tolerance test (OGTT) should be performed. If the FPG meets the diabetes criteria, an OGTT is not necessary. The authors stress that PG should be measured under controlled conditions, and for clinical diagnostic purposes, more than once. Factors other than diabetes that elevate PG should be carefully considered. They also state that there is 'abundant evidence' of a large intra subject variability of the OGTT. Whereby, individuals who classified as diabetic on one test could classify as normal on another[12]. Is does seem that the average PG glucose for the group as a whole stays the same over time, suggesting the OGTT can be used for epidemiological measurements of insulin resistance.

The substantial differences in the diagnostic criteria used in practice results from the fact that there is no clear division between diabetics and non-diabetics in their FPG concentration or their response to a glucose load. The PG in almost all populations follows a continuous unimodal distribution with a rightward skew[19,20]. No features of this curve allows a good distinction between diabetics and non-diabetics. The extensively studied Pima Indian[21] and Nauruan population[22] exhibit a bimodal distribution because of a very high prevalence of diabetes. In this distribution, a distinction can be found between-non diabetics and diabetics, where a FPG of 7.8 mmol/l distinguishes between the two modes. For the OGTT the 2-h PG of non-diabetics are below 11.1 mmol/l and of diabetics above 13.3 mmol/l. In a systematic survey of the Pima Indians, it was found that the presence of diabetic symptoms and complications were largely confined to the second mode. Alongside this information, the NDDG had four long-term prospective studies on which to base their criteria[23–26]. These studies followed participants whose initial glucose intolerance was between what they termed normal and overt diabetes.

This state is now often termed intermediate hyperglycaemia (IH). There were no uniform criteria between the studies, but in general the FPG was below 7.8 mmol/l and the 2-h PG after OGTT (also non-standardised glucose load) was between 7.8 and 11.1 mmol/l. Overt diabetes was considered with very high glucose values (>16.7mmol/l) and/or clinical symptoms of diabetes and/or microvascular complications alongside hyperglycaemia. The general findings in these studies were that the overwhelming majority of individuals with IH should constitute a separate category. The development to overt diabetes occurs at a rate of 1%-5% per year. While a large proportion of individuals spontaneously revert to normal glucose metabolism (NGM) or stays in the IH stage. These four studies totalled a number of 1166 participants with a follow-up between 1-12 years. Based on these results combined with the bimodal distribution cut-offs found in the Pima Indians population, and the information that in a few research populations the 2-h PG values plotted on a log/log scale indicated a second distribution curve starting around 11.1 mmol/l, the NDDG concluded that the term diabetes mellitus should be restricted to individuals who have;

1. Overt diabetic symptoms and unequivocal hyperglycaemia or
2. FPG levels higher than 7.8 mmol/l on more than one occasion or
3. if FPG is not elevated, PG levels during OGTT that exceed 11.1 mmol/l both at 2 h after administration and at some other time point between time 0 and 2 h on more than one occasions

They further concluded with an anticipated change of these criteria with future research advances, specifically stating that FPG levels between 6.9 mmol/l and 7.8 mmol/l indicate a degree of abnormal glucose metabolism that has not been fully assessed yet. The WHO endorsed the current criteria with the adjustment of rounding the glucose values tot the nearest whole mmol/l[13].

The above criteria remained the same, apart from some minor modifications by the WHO in 1985[27], until in 1997, with the adjustment of the nomenclature, the Expert Committee convened by the ADA re-examined the diagnosis of diabetes in light of new information[14]. They revised the glucose criteria for diagnosing diabetes as shown in Table 1.4, in addition to the criterium of symptoms with a casual plasma glucose $\geq$11.1 mmol/l.

Table 1.4.: *Criteria for the diagnosis of diabetes mellitus formulated by the Expert Committee in 1997*

Test	NGM	Impaired- fasting glucose (IFG)/glucose tolerance (IGT)	Diabetes mellitus*
FPG (mmol/l)	<6.1	≥6.1 and <7.0	≥7.0
2-h postload glucose from OGTT (2h OGTT)	< 7.8	≥7.8 < 11.1 mmol/l	≥11.1

* Diagnosis of diabetes needs to be confirmed, on a subsequent day, by any one of the methods given.

One of the members of this Expert Committee stated that a leading motivation in lowering the FPG criterium was to reduce the discrepancy in diagnosis between the OGTT and the FPG[28]. This motivation is described in the report of the Committee. They reported the findings from a study in Pima Indians[29] where the FPG level equivalent to the 2h OGTT criterium of ≥11.1 mmol/l was shown to be 6.8 mmol/l. In a study encompassing 13 Pacific populations the FPG criterium that gave an equal prevalence to the ≥11.1 mmol/l OGTT was found to be 7.0 mmol/l[30]. The same method was applied to a cohort in the Third National Health and Nutrition Examiniation Survey (NHANES III) in which the cutpoint was 6.7 mmol/l.

They further justified this criterium by linking levels of glycaemia with diabetic retinopathy in populations of Pima Indians (n = 960)[29], , Egyptians (n = 1,081)[31] and a cohort in NHANES III (n = 2,821)[14]. For these studies they reported the FPG, 2h OGTT and glycated heamoglobin (HbA1c) levels, which they divided into deciles for each study. For the studies in the Pima Indians and NHANES III they found a dramatic increase in the prevalence of retinopathy in the highest decile (7.6 mmol/l and 6.7 mmol/l respectively) and in the Egyptians in the second highest decile (7.2 mmol/l). These values are somewhat misleading as a justification for the new criterium as they are the lowest glycaemic level in each decile in which the prevalence increased. The Expert Committee recognizes that the estimates of these 'thresholds' for retinopathy are somewhat imprecise. However, more precision could not be easily obtained because of the limited number of cases of retinopathy in each

sample (32 cases in Pima Indians, 146 in the Egyptian study and 111 in NHANES III).

Further evidence for lowering the FPG criterium was obtained form a letter from researchers of the Paris Prospective Study[32]. These researchers reported, in light of the oncoming review of the diabetes criteria, the incidence of fatal coronary heart disease to be related to both FPG and 2h OGTT at a baseline examination. Incidence rates were markedly increased at FPG $\geq$6.9 mmol/l or 2h OGTT $\geq$7.8 mmol/l. The Expert Committee also reported a personal communication with researchers involved with the Baltimore Longitudinal Study of Aging. Here the incidence of coronary disease and the all-cause mortality rates increased markedly and almost linearly above FPG levels in the range of 6.1-6.7 mmol/l[14]. They concluded that both FPG and 2h OGTT provided important information regarding risk of both micro- and macrovascular disease and that the approximate thresholds for increased risk correspond with those for retinopathy.

This lowering of the FPG values was not without controversy and has been debated in subsequent years[11,33]. Among the criticism is the fact that different individuals may be classed depending on which method is used, and that more people may have diabetes in toto using the FPG rather then the 2h OGTT WHO test[34,35].

The diagnostic cut-off point for the 2h OGTT was retained because the reasons for it's original adoption were deemed to be valid:

1. 11.1 mmol/l has been found to approximate the cutpoint separating the two components of the bimodal distribution of 2h OGTT.
2. In several studies the prevalence of microvascular disease sharply increased above 2h OGTT levels of 11.1mmol/l.
3. An enormous body of clinical and epidemiological data has been collected based on the 11.1 mmol/l limit.

Also, they considered changing the criterium to be "very disruptive, and add little benefit".

Furthermore, they concluded that OGTT, although an invaluable tool in research, is not recommended for routine use. Because of it's inconvenience to patients, it's infrequent use in ordinary practice and it's poor reproducibility[36,37]. Also, the measurement of HbA1c was not recommended for diagnosis. Although it's use is widespread in the monitoring

of treatment of diabetes, at the current time there were many different methods for measurement and standardization had only just begun[38].

The WHO published an update to their Diabetes classification and criteria in 1999[39]. They endorsed the new diagnostic value of a FPG $\geq$7.0 mmol/l, also motivating the correspondence with the 2h OGTT value, the evidence of increased risk of microvascular disease and the fact that it represents an optimal cut-off point to separate the components of bimodal frequency distributions of FPG seen in several populations. Both parties agree that the diagnosis in asymptomatic individuals can only be made on the basis of at least two abnormal results. In the WHO criteria if a casual blood glucose value lies in the uncertain range (i.e. between the levels that establish or exclude diabetes, being $\geq$11.1 mmol/l), an OGTT needs to be performed, measuring *both* FPG 2-h and OGTT. This is in contrast with the ADA criteria where diabetes can be diagnosed on the bases of multiple increased FPG measurements. The WHO does make a distinction between the requirements for the diagnosis in clinical setting and for population studies, in the latter using both FPG and 2h OGTT is still highly recommended. but they can be used on their own when circumstances prevent execution of an OGTT. So where the ADA Expert Committee makes a strong recommendation that the FPG can be used on its own and that in general the OGTT need not be used. The WHO by contrast argues strongly for the retention of the OGTT.

The WHO also recognizes IFG and IGT, whereby they state that these categories are not interchangeable and represent different abnormalities of glucose regulation. The criteria for these categories is the same in the WHO classification as in the ADA.

Only 4 years later the ADA came with a follow-up report on the diagnosis of diabetes[40]. This update was in light of several new data published in these years. Among which a growing body of evidence which shows that the criteria for IFG and IFG not only identify different individuals[34,41], but also that the IGT category has a stronger association with cardiovascular disease (CVD) events and CVD mortality[42,43]. The National Glycosylated Hemoglobin Standardization Program had ensured that most laboratories in the U.S. perform the assays using standardized controls and report results in a manner traceable to the assay used in the Diabetes Control and Complications Trial (DCCT)[44]. Finally data from major clinical trials proved that the progression from IGT to diabetes could be delayed or prevented by several methods with different efficacy: in-

tensive lifestyle modification (nutritional and exercise interventions)[45,46], metformin[46,47] and acarbose[47,48]. The major update of this follow-up report was the lowering of the FPG criterium for diagnosing IFG from 6.1 mmol/l to 5.6 mmol/l. The committee indicated that a selection of a lower limit for the IH categories would be somewhat arbitrary as studies show that no real threshold for FPG exist for cardiovascular risk factors, all-cause mortality or future diabetes[43,49]. Data from the Prima Indians however show that the risk of diabetes does increase markedly at FPG concentrations above 5.6 mmol/l. The rationale for establishing the intermediate categories was based on their ability to predict future diabetes. However, the 6.1-6.9 mmol/l IFG criterium includes a much lower proportion of the population than is included in the IGT criterium[50]. Also, the sensitivity of IFG as originally defined is less than that of IGT in most populations, but the specificity maybe somewhat greater[49,50]. The Expert Committee analysed the receiver operator characteristic curve (ROC) of the ability of various baseline levels of FPG to predict diabetes and there findings suggested that the lower limit of 6.1 mmol/l for IFG was inappropriately high[40]. By lowering the limit there was once again a harmonization of the prevalence between FPG and 2h OGTT. It also leads to a much higher percentage of people classifying in the IH group without at the moment being clear what the benefit or cost to an individual would be. This point has been heavily criticized[51,52].

The Committee believed it still premature to add HbA1c to the group of tests used to diagnose diabetes and continues the recommendation that it be used to monitor the effectiveness of glycaemic therapy. The new criteria are shown in table 1.5

In view of these developments the WHO and the International Diabetes Federation (IDF) released new report on the diagnosis of diabetes and IH in 2006[53]. For the first time they deviate from the glycaemic criteria that are established by the ADA. The WHO agrees with the finding that existing evidence does not provide a clear threshold for normal or non-normal plasma glucose or even diabetes per se. However, they did not endorse the lowering of the limit of IFG to 5.6 mmol/l. They argued that te various approaches for deriving the most appropriate cut-off point do not provide a consistent and unequivocal answer and felt that consideration should be given to a risk score combining known risk factors which include a measure of FPG as a continuous variable. They were of the opinion that a cut-off point should include clinical and public health considerations and not be based on statistical ones (i.e. maximization of

Table 1.5.: *Criteria for the diagnosis of T2DM and IH formulated by the ADA in 2003 and the WHO in 1999/2006*

Category	ADA 2003 FPG	2h OGTT	WHO 2006* FPG	2h OGTT
Normal	<5.6 mmol/l	<7.8 mmol/l	<6.1 mmol/l	<7.8 mmol/l
IFG	5.6-6.9 mmol/l	–	6.1-6.9 mmol/l	<7.8 mmol/l
IGT	–	7.8-11.1 mmol/l	<7.0 mmol/l	7.8 - 11.1 mmol/l
Diabetes**	≥7.0 mmol/l	≥11.1 mmol/l	≥7.0 mmol/l	≥11.1 mmol/l

* The WHO recommends performing an OGTT for all categories. For the IGT category if a 2h OGTT is not measured, status is uncertain as diabetes or IGT cannot be excluded. Diabetes can be diagnosed by either a FPG or OGTT

** A diagnosis of diabetes needs to be confirmed on a separate day.

the sum of sensitivity and specificity). There were concerns about the significant increase in IFG prevalence that would result form lowering the criteria. Whilst there was no clear evidence of any benefit in terms of reducing adverse outcomes or progression to diabetes. Also, several studies had reported that people defined by the new ADA criteria have a more favourable cardiovascular risk profile and are less likely in developing diabetes compared with the WHO cut-off point[54,55]. Thus the WHO recommended the cut-off point for IFG should remain at 6.1 mmol/l which is in line to the position statement of the European Diabetes Epidemiology Group that was published around the same time[56].

This deviation in criteria marks a difference in the target group for both organizations. Where the ADA is targeted to health care providers in one country (or suitable for the health care organization in high-income countries), the WHO makes global recommendations and needs to take health care organization in middle- and low-income countries into account. This difference became visible once more in the adaptation of HbA1c as a diagnostic criterium.

In 2009 the ADA published a report on the role of the HbA1c assay in the diagnosis of diabetes by an international expert committee with members appointed by the ADA[57]. As mentioned above, for a long time already HbA1c was considered for the diagnosis of diabetes. It was often measured in population studies and used in monitoring treatment effect in T2DM as it reflects average glycaemia over the preceding 8-12 weeks[58]. Based on the common sense that a measurement that captures the long-

term glycaemic exposure should provide a better marker than single measures of glucose concentration,besides data from multiple studies demonstrating a strong correlation between retinopathy and HbA1c[59,60] and the widely accepted HbA1c treatment goals for diabetes[61]. The reluctance for adaptation as a diagnostic criterium was for a large part standardisation and probably more a hurdle for the WHO than the ADA, world wide access to the assay. Standardisation was more or less completed in the U.S. by 2003 but world wide standardisation was realised in the subsequent years[62,63].This, combined the superiority of the HbA1c assay in terms of pre-analytic stability[64,65], biological variability[66,67] and patient convenience, let to the adoption of HbA1c as a diagnostic criterium for diabetes in 2009.

The optimal cut point for diagnosis was determined on the basis of the same three studies that were previously used for determining the cut point for diabetes together with an analysis from the DETECT-2 studie[57,68]. This analysis included ∼28000 subjects from nine countries and determined the optimal cut point to be 6.5%, based on the fact that below this value diabetes-specific "moderate" retinopathy was virtually non-existent and the indication based on the receiver operating characteristic curve. Diagnosis of T2DM should be confirmed with a repeat test unless clinical symptoms and plasma glucose levels >11.1 mmol/l are present.

The Expert Committee sort of defined the IH range for HbA1c values to be ≥6.0% and <6.5%, advising individuals with levels in that range should receive preventive interventions. They emphasized that the risk for development of T2DM is a continuum and there appears to be no threshold above which risk clearly begins, so there should not be a lower glycaemic threshold. Studies during that time demonstrated a risk of diabetes development in levels well within what was considered "normal"[69]. It does appear that risk increases the closer the values get to the diagnostic level. This no-lower-boundary IH category would be quickly changed in the next update of the criteria.

The report makes a, not explicitly stated, recommendation that HbA1c should be used as the standard for diagnosing diabetes saying the "assay may be a better means of diagnosing diabetes than measures of glucose levels". Hereby arguing that there is no single assay related to hyperglycaemia that can be considered the gold standard as it relates to the risk for micro- or macrovascular complications and that different test

not necessarily identify the same populations. They further conclude care should be taken interpreting HbA1c levels, because values can be effected by clinical conditions like increased erythrocyte turnover and haemoglobin variations. Also, studies have suggested racial disparities in HbA1c[70]. The Committee noted however, that the aetiology and significance of this finding is unclear. These differences in HbA1c values between people from a different ethnic background have been described frequently in literature since the adoption of the HbA1c test. But even until this day the relevance of this finding is still being debated[71,72](also see Chapter 4 of this manuscript).

One year after the publication of the report advising the use of HbA1c as diagnostic criterium the ADA published an update to it's position statement on the diagnosis and classification of diabetes[73]. In this update they properly defined the IH category for HbA1c values between 5.7 and 6.4%. This lowering of the previous, not officially recommended value of 6.0%, was partly because the 6.0-6.5% range fails to identify a substantial number of patients who have IFG and/or IGT. Data from the NHANES shows that de values which most accurately identifies people with IFG or IGT falls between 5.5 and 6.0%[73]. Also, prospective studies indicated that individuals with HbA1c in the range of 5.5-6.0% have a 5-year cumulative incidence of diabetes that ranges from 12 to 25%[74–77]. This, together with data from the Diabetes Prevention program (DPP), wherein the mean HbA1c of 5.9% indicates that preventive interventions are effective in groups of people with HbA1c levels both below and above 5.9%, let the ADA to recommend HbA1c values between 5.7% and 6.5% as the IH category. Whilst once again stating that defining the lower limit of an intermediate category is somewhat arbitrary as risk of T2DM is a continuum.

Another specification was that if the results of multiple glycaemic test in one person are discordant on the diagnostic criteria, the test whose result is above the diagnostic cut point should be repeated and the diagnosis is made on the basis of the confirmed test.

In 2011 the WHO published a report endorsing the value of $\geq$6.5% as a diagnostic criterium for the diagnosis of T2DM[78]. As in the previous reports and the ADA report they advised that the diagnosis should not be made in asymptomatic individuals on the basis of one abnormal test result. They also state that an HbA1c less than 6.5% does not exclude diabetes diagnosed using glucose tests. The WHO consultation

Table 1.6.: *Current criteria for the diagnosis of NGM, IH* and T2DM formulated by the ADA in 2010 and the WHO in 2011*

Test	Heatlhy		IH		T2DM	
	ADA	WHO	ADA	WHO	ADA	WHO
FPG (mmol/l)	<5.6	<6.1	5.6-6.9	6.1-6.9	≥7.0	≥7.0
2h OGTT (mmol/l)	<7.8	<7.8	7.8-11.0	7.8-11.0	≥11.1	≥11.1
HbA1c (%)	<5.7	<6.5	5.7-6.4	–	≥6.5	≥6.5

* The ADA uses the term *prediabetes* for all IH categories, the WHO makes a distinction for IFG and IGT for FPG and 2h OGTT respectively

concluded further that there is insufficient evidence to make any formal recommendation on the interpretation of HbA1c levels below 6.5%. Thus not endorsing the IH category defined by the ADA.

The next, and to this date most recent position statement from the ADA appeared in 2013, and a published correction in 2014[79] In this latest position statement the criteria for the diagnosis remained unchanged. They did further justify the IH criteria based on HbA1c values with studies that were published in the subsequent years since the last position statement[80–82].

In next and latest WHO consultation no changes to the diagnostic criteria have been made. This results to the current criteria for normal glucose metabolism, intermediate hyperglycaemia and type 2 diabetes mellitus being shown in table 1.6, the recommendations for when performing which test have remained unchanged with the previous diagnostic criteria.

In this Thesis the current criteria recommended by the ADA have been used. This decision is for the largest part based on three arguments. First, we want to be able to compare our results to other large cohort studies, which usually also adhere to the ADA criteria. Second, the studies described in this Thesis focus on the development of hyperglycaemia. The ADA's cut-off points for IH are chosen for the reason that below these values development of insulin resistance in the short term is very low. These values seemed the most logic choice to distinguish the start of the development of hyperglycaemia Third, in our studies we measured FPG and HbA1c but we did not perform an OGTT. The OGTT was not performed because of cost and participant burden in the large studies,

the large intraindividual variability of the test as described above and because it is no longer performed in routine clinical care in the Netherlands.

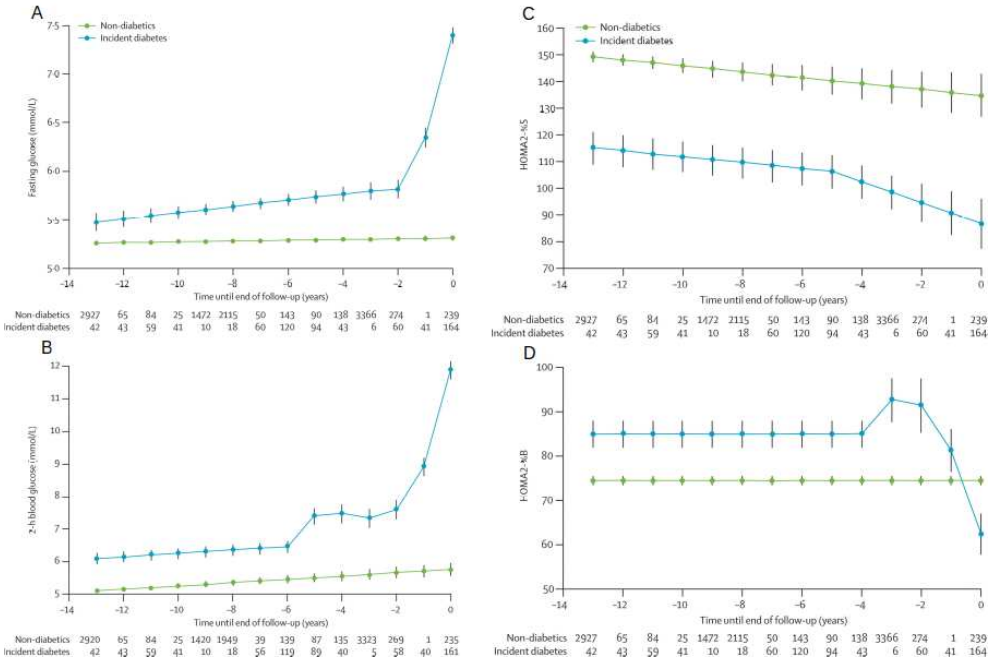
1.2. The Development of hyperglycaemia

One of the most accurate descriptions of the development of hyperglycaemia is reported in the Whitehall II study[83]. The researchers analysed data from a prospective cohort study of 6538 Britisch civil servants without T2DM at baseline. There was an incidence of 505 diabetes during a median follow-up of 9.7 years (49.1% on the basis of an OGTT). The measurements included, among others, FPG, insulin and OGTT, see figure 1.1. They report changes in glycaemic control as much as 3-6 years before diagnosis. These changes occur both for glycaemic control in the fasting state as in the postprandial state.

Multiple environmental factors and genetic factors have been identified that contribute to pathophysiological disturbances that are responsible for impaired glucose homeostasis[84,85]. The pathophysiological changes are characterised by insulin resistance, β-cell dysfunction. As shown in the trajectories of the Whitehall II study the development of insulin resistance in the fasting and postprandial state do not necessarily occur at the same time. Also, these do not necessarily have to occur at the same severity, more on this later. The development of hyperglycaemia closely follows β-cell dysfunction. It has long been debated whether insulin resistance was a result of overproduction by the β-cell, or that it precedes β-cell dysfunction and hyperglycaemia occurs as insulin secretion decreases as a result when the compensatory overproduction starts to fail. The weight of evidence at this time is in favour of the latter hypothesis[86–88].

Chief among the environmental factors in the current world population are ageing, overweight and obesity, sedentary lifestyle and dietary constituents[89]. For all of these factors, except the first, there exist effective preventive strategies that are able to decrease the incidence of T2DM in high risk individuals and improve insulin sensitivity[90]. The first factor, ageing, can be considered to have a special place. It is the single most important predictor of all non-communicable diseases[91]. And, as a species throughout time, we seem obsessed with is modifiability[92]. Even

Figure 1.1.: *Results from the Whitehall II study. Time 0 is diagnosis of incident diabetes or end of follow-up for non diabetics. Panel A shows trajectories of FPG before diagnosis and panel B shows trajectories of 2h OGTT. Panel C shows homeostasis model assessment of insulin sensitivity (HOMA2-%S) and β-cell function (HOMA2-%B). Data are estimates calculated using multilevel longitudinal modelling adjusted for age, sex, ethnic origin and study phase.*



in the presence of other environmental- and to some extend also genetic factors, T2DM at a young age is relatively rare[93]. Ageing can be a described as a progressive loss of physiological integrity, leading to impaired function and increased vulnerability to death as a result of, to a large extent, the passing of time. One of the hallmarks of this process is cellular senescence[94].

Cellular senescence, or senescence, can be defined as a stable arrest of the cell cycle coupled to phenotypic changes[95]. One of these phenotypical changes, is the secretion of a collection of proteins collectively called the senescence-associated secretory phenotype (SASP)[96]. This pro-inflammatory secretome has been suggested to drive age-related tissue dysfunction. It is widely accepted that senescent cells play a role in the ageing of tissues and organisms[97,98]. But to say that senescence and ageing are synonymous is wrong: cells can enter a senescent state regardless of organismal age. Senescence can occur through a number of different processes, like potentially oncogenic mutations activated oncogenes, metabolic insults and damage/danger signals. It plays a major role in tissue remodelling and seems designed to eliminate unwanted cells[99]. However when senescent cell accumulate they can cause pathological manifestations. This thesis reports the accumulation of senescent cells in liver and adipose tissue see, part II, chapters 3 and 4. Cellular senescence has been shown to play an important part in obesity related diseases like NAFLD and T2DM[100,101]. The accumulation of senescent hepatocytes correlates closely with markers of hepatocyte senescence, and the elimination of these cells reduces overall hepatic steatosis[100]. The accumulation of senescent cells in adipose tissue during obesity appears to be a driver of peripheral insulin resistance and hyperinsulinemia[102,103]. In chapter 3 a correlation between hepatocyte senescence, steatosis and high insulin is described in humans. Chapter 4 describes a correlation between high insulin after a meal ingestion and adipocyte senescence in the mesenteric adipose tissue of obese individuals. These data are suggestive of a causal role for insulin in the induction of senescence in tissues of obese individuals.

1.3. Insulin Resistance in the population

As mentioned in the previous section, there are numerous pathophysiological processes that can lead to hyperglycaemia and T2DM. These

different pathways lead to different phenotypes of hyperglycaemia. Because of this heterogeneity, the view of T2DM as a single disorder with a uniform progression has been long since abandoned. T2DM can be seen as the result of processes which drive hyperglycaemia to a point where a high risk of vascular complications occur. There are different ways to measure and describe these phenotypes. The most important measurements are glucose, insulin and HbA1c. Because the first are metabolically active molecules, their measurement in one state is not very informative about individuals metabolic regulation. Various approaches have been developed in order to assess an individuals response to glucose and insulin[104]. These range from intensive direct measures like glucose clamps techniques, to indirect measurements like the OGTT and the mixed meal test, to surrogate indexes like the homeostasis model assessment (HOMA). Each method has it's merits and limitations. Chapter 2 describes the use and reproducibility of a two-hour-seven-sample oral glucose tolerance test to assess glycaemic responses to a meal in obese individuals[105]. Differences in insulin resistance and hyperglycaemia can be seen for the categories of IH based on FPG and HbA1c. It is likely that different pathophysiological processes are dominant in these different categories.

In clinical care in Western medicine measurements of FPG and HbA1c are, combined with anthropometric and clinical observation data, the only parameters on which diabetes diagnosis, and to large extend care, is based. Part III describes investigations in the different categories of IH that can be assessed by these measurements. The chapters are based on data from the HELIUS study which includes individuals living in the greater Amsterdam area and encompasses multiple ethnic groups[106]. There is a great disparity in the prevalence of T2DM in different ethnic groups across the world[107–110]. These disparities are driven by a variety of influences including lifestyle, socioeconomic and possibly genetic factors. There is a great challenge in understanding the inequalities in T2DM between ethnic groups. The chapters in part III of this thesis try to contribute to this further understanding.Chapter 5 describes the difference in prevalence of IH between ethnic groups and investigates the different risks for each category and ethnic group for developing T2DM. Chapter 6 details an attempt to see if, based on clinical, non-glycaemic variables individuals fulfilling a specific IH classification can be identified using a machine learning algorithm called XGBoost[111]. Differences in these identified variables between ethnic groups may lead to a better

understanding of the differences in development of IH.

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