Determinants of HbA1c in non-diabetic children and adults

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Chapter 1

General Introduction

Chapter 1
General Introduction

BACKGROUND

Glycated haemoglobin – HbA_{1c}

About forty per cent of the human blood consists of erythrocytes. The major function of erythrocytes is to transport haemoglobin, which in turn carries oxygen from the lungs to the tissues. Human adult haemoglobin (Hb) usually consists of HbA (adult haemoglobin, 97% of the total), HbA_2 (normal variant of haemoglobin A, 2.5%), and HbF (fetal haemoglobin, 0.5%). HbA is made up of four polypeptide chains, two α- and two β-chains.

In 1958, Allen et al. reported that with cation-exchange chromatography human haemoglobin could be separated into at least three minor components that had more negative charges than HbA_1. These minor haemoglobins, or also called "fast haemoglobins" (because they migrate more rapidly than HbA in an electrical field), were all named HbA_1, and further defined as HbA_{1a}, HbA_{1b}, and HbA_{1c}, in order of their elution from the column. All these types of HbA_1 appeared to have a carbohydrate moiety (glucose or a derivative) attached to one of the globin chains. Carbohydrate may be attached to the N-terminal amino acid residue (valine) of the α- or β-chains, or to lysine residues within each chain[2]. The process of non-enzymatic addition of a sugar residue to amino groups of proteins is called glycation. And therefore, HbA_{1a}, HbA_{1b}, and HbA_{1c} are collectively referred to as glycated haemoglobins. HbA_{1c} is the major fraction, constituting approximately 80% of HbA_1.

In 1968, Rahbar et al reported an elevation of the minor haemoglobin fractions in patients with diabetes mellitus[3]. About eight years later, Koenig et al. demonstrated that the concentration of HbA_{1c} was proportional to fasting blood glucose and glucose tolerance[4]. This crucial observation led to the use of HbA_{1c} as a method of assessing diabetic control.

HbA_{1c} is formed in two stages (Figure 1)[5]. First, glucose combines with the α amino group of the valine residue at the N-terminus of β-chains to form an aldimine compound, also called Schiff base. This first reaction is reversible, and dissociation to native haemoglobin and glucose occurs readily. The second stage is the internal rearrangement of the aldimine intermediate by the Amadori reaction, which yields a stable ketoamine derivative, called $HbA_{1c.}$ This reaction is essentially irreversible.

Glycation of HbA begins during erythropoiesis and continues slowly throughout the lifespan of haemoglobin in the circulation. Because, erythrocytes are freely permeable to glucose, the level of HbA_{1c} in a blood sample provides a glycaemic history of the previous 120 days, the average erythrocyte lifespan.

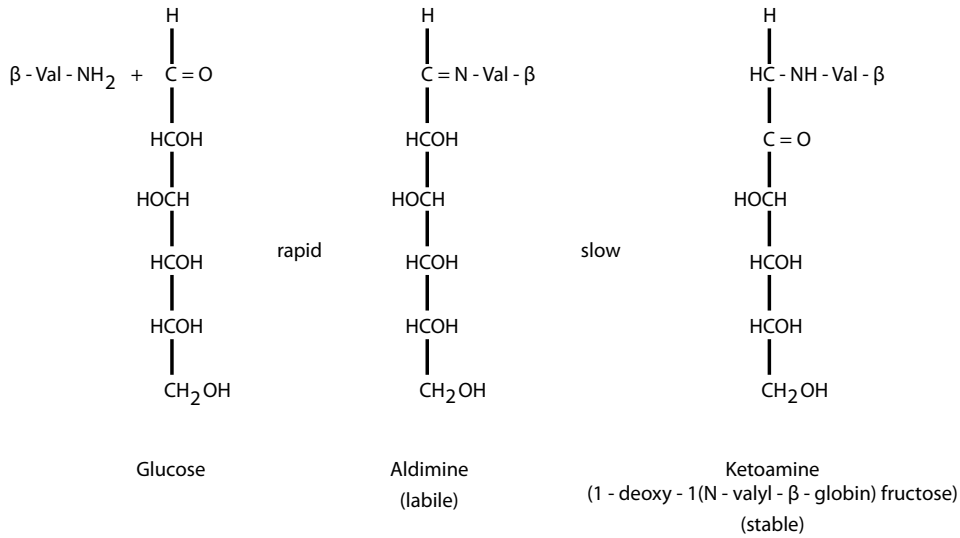

Figure 1: Reactions leading to the formation of HbA_{1c}[2]

Assay methods for the determination of HbA_{1c}

Short overview of most applied methods and their (dis)advantages, derived from a review of Weykamp et al.: A review of the challenge in measuring haemoglobin A_{1c}[6].

A broad range of assay methods has being developed since HbA_{1c} was described in the late 1960s. Two main difficulties regarding the accurate measurement of HbA_{1c} are the large number of variant haemoglobins and glycohaemoglobins, and the fact that HbA_{1c} is not a stand-alone analyte because its quantity is related to the total haemoglobin concentration. As a result of this latter, HbA_{1c} should be expressed as a ratio, i.e. HbA_{1c} / total haemoglobin, and this dual measurement causes dual uncertainty in het outcome of the test.

Roughly, there are two different methods for the measurement of HbA_{1c}: methods based on difference in charge and methods based on structural difference.

Ion-exchange chromatography, capillary electrophoresis, and isoelectric focusing are all based on the difference in electrical charge. At this moment, only the high-performance liquid chromatography (HPLC) is still in use. It is an efficient method; it meets the clinical requirements of reliability; and interpretation and does not suffer from interference by Schiff base or carbamylated haemoglobin.

Chapter 1
General Introduction

Occasional problems with haemoglobin variants still exist, but the detection of these variants is also an advantage compared to methods based on structural difference.
Affinity chromatography and immunochemical assays are the two main used methods based on structural difference. In which the latter is the one that is mostly applied. In immunochemical assays, antibodies targeted against the β N-terminal glycated tetrapeptide or hexapeptide group, are used to determine HbA_{1c} levels. Advantages of this assay are that it is not affected by problems related to electrical charge and can be adapted easily in the routine medical laboratory. The major challenge for immunochemical tests is to achieve acceptable imprecision.

Worldwide standardization

Though the assay methods advanced during the years, it quickly became apparent that results from different laboratories gave widely different results, particularly if different assay methods were used. This led to several national initiatives for harmonisation and certification of the HbA_{1c} assay.
For example the National Glycoheamoglobin Standardisation Program (NGSP) in the USA based on the Diabetes Control and Complications Trial (DCCT) work, and standardization schemes in Japan and Sweden. But, though these efforts decreased the inter-laboratory variation in HbA_{1c} considerably, due to the lack of real international standardization, HbA_{1c} values still varied substantially nationally as well as internationally. In 1995 the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) formed a study group to address this issue, called the IFCC WG HbA_{1c}. This study group developed a true reference method to which all other assays can be anchored and standardized. In addition, regression equations were established between the IFCC method and het NGSP, Swedish and Japanese harmonized assays[7]. Because of the lack of specificity of the "old" three harmonization methods, the results of the IFCC reference method appeared to be lower than those obtained with the "old" assays.
These lower numbers would have been confusing to patients and clinicians. In addition, the previous unit (%) was not aligned with the international system of measurement (SI). To overcome these two difficulties, the introduction of a new unit (mmol/mol) was proposed. In a consensus statement, the International Diabetes Federation, the American Diabetes Association and the European Association for the Study of Diabetes recommended that: HbA_{1c} test results should be standardised worldwide, including the reference systems and results reporting; that the new IFCC reference system for HbA_{1c} represents the only valid anchor to implement standardization of the measurement; and that the same HbA_{1c} values should be reported worldwide, in both IFCC (mmol/mol) and DCCT units (%), using the IFCC-DCCT master equation to relate these two values[8].

In the Netherlands, from April 2010 until January 2011, laboratories reported HbA_{1c} values in DCCT percentages as well as the new IFCC values in mmol/mol. Since January 2011, laboratories should only report the "new" HbA_{1c} values in mmol/mol. One can convert DCCT values to IFCC values with the equation: IFCC-HbA_{1c} (mmol/mol) = (10.93 * DCCT-HbA_{1c} (%)) – 23.5. To convert IFCC values to DCCT values the equation one should use is: DCCT-HbA_{1c} (%) = (0.0915 * IFCC-HbA_{1c} (mmol/mol)) + 2.15.

Chapter 1
General Introduction

Normal distribution of HbA_{1c} in non-diabetic adults

In 1989, Simon et al. reported the distribution of HbA_{1c} in a large, healthy adult population. They found an approximately normal distribution of HbA_{1c} with mean (SD) HbA_{1c} of 5.03% (0.53) in men and a mean (SD) HbA_{1c} of 5.07% (0.55) in women[9]. Gulliford et al. found a mean (SD) HbA_{1c} of 6.34% (0.85) in a general population of 9,772 non-diabetic, white European subjects aged 16 years and older[10]. Figure 2 displays the distribution of HbA_{1c} in 2,921 non-diabetic Dutch adults from the LifeLines cohort study. In general, an HbA_{1c} level below 6% is considered normal.

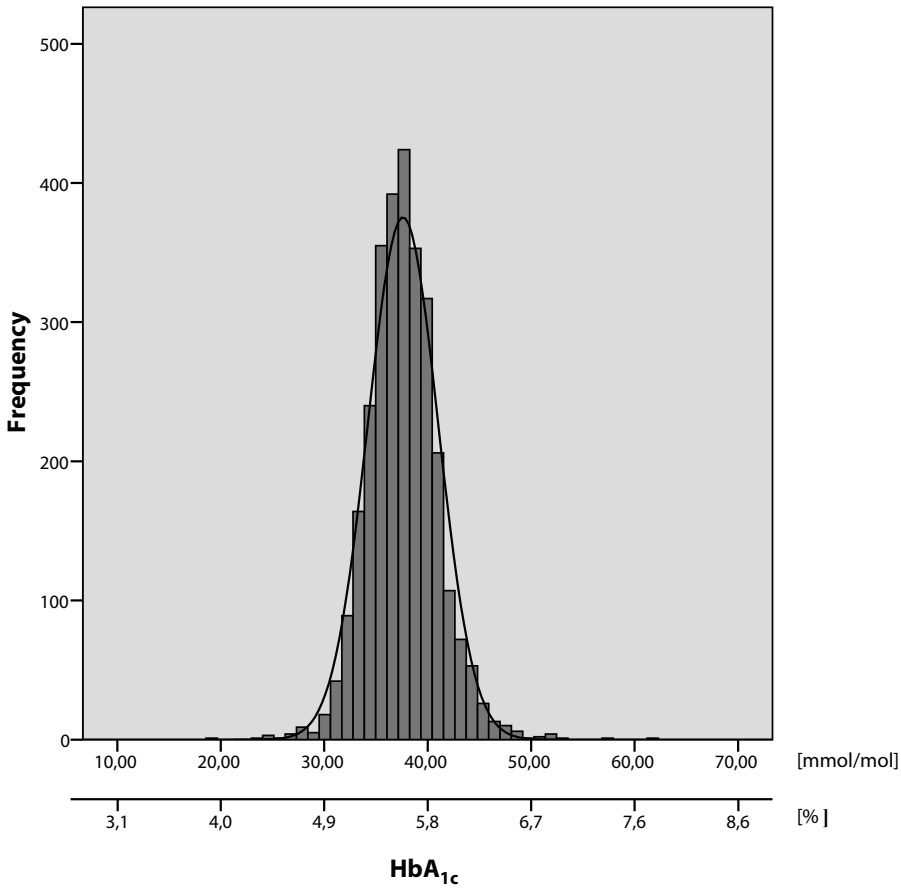

Figure 2: Distribution of HbA_{1c} in 2,921 non-diabetic adults from the LifeLines cohort study. Mean ± SD: 38 ± 3

Determinants of HbA_{1c} in non-diabetic humans

Blood glucose levels

Since erythrocytes are freely permeable to glucose, the rate of formation of HbA_{1c} is directly proportional to the ambient glucose concentration. But because the traditional idea that HbA_{1c} reflect the simple mean plasma glucose level during the previous 120 days raised questions, Tahara et al. analyzed the relationship between HbA_{1c} and the preceding plasma glucose levels[11]. They showed that the rate of contribution of the preceding plasma glucose level to HbA_{1c} depends on their time interval. In other words, the HbA_{1c} level should be considered to reflect the weighted mean plasma glucose level in the preceding period. Their results showed that 50% of the HbA_{1c} was determined by the plasma glucose level during the preceding 1-month period, while 25% of its level was determined by the plasma glucose level during the 1 month period before this month, and the remaining 25% was determined by the plasma glucose level during the 2-month period before these 2 months. Thus, HbA_{1c} levels reflect the weighted mean plasma glucose level over the preceding 4 months, with more recent values providing a larger contribution than earlier values.

Since HbA_{1c} provides a retrospective index of the integrated plasma glucose values over an extended period of time, it has been firmly established as an index of long term glucose concentrations in patients with diabetes mellitus. But, despite its standing as the most validated and widely used measure for average glycaemic control over time, it is common to find discordance between HbA_{1c} and other measures of glycaemic control in diabetes patients.

Among 223 adults without diabetes, differences in glucose intolerance explained only one third of the variance found in glycated haemoglobin levels[12]. In addition, Van 't Riet et al. found only moderate correlations of glucose with HbA_{1c} (correlation of 0.26 between fasting plasma glucose and HbA_{1c}, and a correlation of 0.14 between 2-hour postload plasma glucose and HbA_{1c}) in a population of 2,122 randomly selected non-diabetic adults aged 40-65 years[13]. These mismatches, between blood glucose monitoring data and HbA_{1c} levels, also often seen in clinical practice, may imply that HbA_{1c} and glucose partly reflect different processes, especially in the non-diabetic range of glucose tolerance.

One hypothesis to explain the discordance of HbA_{1c} from mean plasma glucose is the notion of a "haemoglobin glycation index". This concept denotes that individuals glycate haemoglobin proteins at different rates. Gould et al. described persistent differences between HbA_{1c} and blood glucose in non-diabetic subjects and categorized these differences as "high glycator" and "low glycator" subsets[14]. Khera et al. tested the hypothesis that interindividual heterogeneity of the intracellular-to-extracellular glucose ratio contributes to this "haemoglobin glycation index". They confirmed the existence of a glucose gradient across the human erythrocyte membrane and demonstrated

Chapter 1
General Introduction

inter-individual heterogeneity in glucose gradients across the human erythrocyte membrane that may affect haemoglobin glycation[15].

Erythrocyte lifespan

Glycation of HbA begins during erythropoiesis and continues slowly throughout the lifespan of haemoglobin in the circulation. Consequently, erythrocyte lifespan determines the duration of exposure of haemoglobin to glucose, and thereby also determines HbA_{1c} levels. Increased erythrocyte turnover, as observed in for example haemolytic anaemia, results in lower HbA_{1c} levels[16]. On the contrary, several studies showed higher HbA_{1c} levels in (diabetic as well as non-diabetic) patients with iron deficiency anaemia[17,18]. Cohen et al. concluded from their study that erythrocyte survival varies sufficiently among haematologically normal persons to cause clinically important differences in HbA_{1c}[19]. Koga et al. examined the relationship between erythrocyte indices and HbA_{1c} in pre- and post-menopausal women[20]. In pre-menopausal women, stepwise multivariate regression analysis demonstrated that of the erythrocyte indices MCH was negatively associated with HbA_{1c}. No significant association was found between any of the erythrocyte indices and HbA_{1c} in the post-menopausal women. Certainly, HbA_{1c} is influenced by factors associated with the lifespan of erythrocytes but more studies are needed to investigate the mechanisms through which erythrocyte indices influence HbA_{1c} levels.

Besides erythrocyte lifespan, a clear clinical variable that determines HbA_{1c}, there are also erythrocyte related analytical variables that may influence the measurement of HbA_{1c} levels. Most important are the haemoglobin variants that can cause spuriously HbA_{1c} levels with some assay methods. The effect of these haemoglobin variants, such as HbF, HbS and HbC, depends on the specific method of analysis; results may be falsely increased or decreased[21].

Other determinants of HbA_{1c}

Besides blood glucose levels and erythrocyte lifespan, there are several other variables that have shown to influence HbA_{1c} levels, in non-diabetic adults and children, but some studies showed contradictory results.

Age and gender

Pani et al examined whether HbA_{1c} was associated with age in non-diabetic persons. They stated that their results establish clearly that HbA_{1c} increases with age, even after multivariate adjustments for sex, fasting, and 2-hour postload glucose and suggested that non-glycaemic factors may contribute to the relationship of HbA_{1c} with age[22]. Other studies confirm the positive association between age and HbA_{1c} in adults[23,24] and children[25,26], though the difference found in HbA_{1c} levels between children of different age are very small.

Faerch et al. and Gulliford et al. both found somewhat higher levels of HbA_{1c} in men compared to women[10,27], but other studies found no sex-related differences in HbA_{1c} [9,28]. In children, most studies found higher HbA_{1c} levels in boys compared to girls, though again the differences are (very) small[25,26,29].

Ethnicity

Race- and ethnicity related differences in HbA_{1c} have been described[30]. Ziemer et al. found higher HbA_{1c} levels in black persons than in white persons across the full spectrum of glycaemia after adjustments for plasma glucose and other characteristics known to correlate with HbA_{1c} levels[31]. And also subjects of South Asian origin showed to have higher HbA_{1c} levels than white subjects independent of fasting and postprandial glycaemia on OGTT[32]. These ethnic differences in HbA_{1c} are confirmed in studies to determinants of HbA_{1c} in non-diabetic children[25,29].

Overweight/obesity

Body mass index (BMI) is acknowledged as an important risk factor for diabetes, with higher BMI causing insulin resistance and thereby higher levels of glycaemia. Consequently, a positive association between BMI and HbA_{1c} can be expected. Simon et al. a found higher level of HbA_{1c} in obese persons (defined as BMI > 28 kmg/m^2), but after adjustment for age, the relation between (deciles of) BMI and HbA_{1c} was no longer significant[9]. Modan et al. found no significant correlation between BMI and HbA_{1c} [28], but in contrast, in the study of Gulliford et al. on determinants of HbA_{1c} in the general population, HbA_{1c} increased with increasing BMI and with increasing waist-hip circumference ratio[10]. And also Boeing et al. found greater obesity to be related with higher HbA_{1c} levels[33]. In children, several studies found a positive relation between measures of obesity and HbA_{1c} [25,29,34], surprisingly Shultis et al. found suggestive evidence of inverse associations between body size and body composition and HbA_{1c} [26].

Chapter 1
General Introduction

Dietary intake and physical activity

High energy and energy-adjusted saturated fat intakes were associated with increased risk of being in the highest tertile of HbA_{1c}, and alcohol, vitamin C, and vitamin E intakes were inversely related to this risk, in a study of Boeing et al. in which the association of diet and other lifestyle factors with HbA_{1c} was examined in a non-diabetic adult population[33]. Also Harding et al. found independent associations between HbA_{1c} concentration across the normal range of HbA_{1c} and both total fat intake and the pattern of dietary fat intake[35]. In contrast, Modan et al. found no correlation between the intake of any specific food component and HbA_{1c}[28]. Metabolic studies in humans suggest that increased saturated fat consumption may increase insulin secretion and possibly lead to insulin insensitivity[36]. The resulting higher levels of glycaemia could explain the relation with higher HbA_{1c} values. There are two proposed mechanisms for the observed inverse association between vitamin C and HbA_{1c}: competition of the ascorbic acid and dehydroascorbic acid with glucose for the reaction with the protein amino group, thereby inhibiting glycation[37] or the anti-oxidant properties of vitamin C[38]. To our knowledge, the association of dietary factors with HbA_{1c} has not been investigated in children yet.

Also the level of physical activity is known to influence insulin resistance[39], and thereby possibly may influence HbA_{1c}. Gulliford et al. found a 0.180% lower HbA_{1c} in participants who were vigorously active compared with the inactive participants, and HbA_{1c} gradually decreases with increasing level of physically activity (analyses adjusted for possible confounders, e.g. BMI)[10]. But other studies found no association between level of physical activity and HbA_{1c}[28,33]. Owen et al. examined the association between objectively measured level of physical activity and cardiometabolic risk factors (including HbA_{1c}) in 2,049 primary school children from the United Kingdom[40]. They found only a weak inverse association between levels of physical activity and HbA_{1c}, but this relation was no longer significant after adjustment for sum of skinfold thicknesses.

Smoking and alcohol consumption

A negative association between alcohol consumption and HbA_{1c} has been found in at least three studies regarding the association between alcohol consumption and HbA_{1c}[10,33,41]. In contrast, Meyer et al. could not confirm these findings in their study to the relations of alcohol patterns with HbA_{1c} in non-diabetic men[42]. Several studies have documented that smoking is associated with higher HbA_{1c} levels[10,28,43,44], but Koga et al. found no association between smoking and HbA_{1c} levels[45]. Glycotoxins found in cigarette smoke may induce the higher rate of glycation of HbA[46] or the relative higher tissue hypoxia[47] can explain increased HbA_{1c} levels in smokers[48].

Genetic factors

The heritability of HbA_{1c} nears 40% in the general population[49] and 60% in twin studies[50]. Two genome-wide association studies identified fifteen different single nucleotide polymorphisms (SNPs) to associate with HbA_{1c} in non-diabetic adults. Soranzo et al. identified ten genetic loci reproducibly associated with HbA_{1c} in up to 46,368 non-diabetic adults of European descent, from 23 genome-wide association studies (GWAS) and 8 cohorts with de novo genotyped SNPs[51]. Paré et al. performed a GWAS that evaluated 337,343 SNPs in 14,618 non-diabetic female individuals and identified four loci being significantly and independently associated with HbA_{1c}[52].

The physiological mechanisms through which these genetic loci regulate HbA_{1c} levels remain unclear. Some SNPs are considered to modulate glycaemic physiology[53], while others are supposed to regulate non-glycaemic factors like red blood cell function[54]. Thus, despite the lack of knowledge about the exact way the identified SNPs act on HbA_{1c}, it is clear that also genetic factors determine HbA_{1c}.

Distribution and determinants of HbA_{1c} in non-diabetic children

Not until 2002 normal ranges for non-diabetic children were established. Saaddine et al. investigated the distribution of HbA_{1c} in a nationally representative sample of U.S. children, adolescents and young adults aged 5-24 years and found a mean (SD) HbA_{1c} level of 4.99% (0.50)[29]. Additionally, Pettitt et al. established the normal distribution for HbA_{1c} in 400 non-diabetic children aged 11-14 years. In this population, HbA_{1c} was fairly normally distributed with a mean (SD) HbA_{1c} of 4.77% (0.39)[34]. And Shultis et al. found a mean (SD) HbA_{1c} of 4.91% (029) in a population of 1,645 non-diabetic children aged 9-11 years[26]. Data on the distribution of HbA_{1c} in infants are missing. This is of special interest because in the first year of life important changes in haemoglobin synthesis take place. Studies to determinants of HbA_{1c} in children found largely the same variables to be associated with HbA_{1c} as in adults. However, several potential determinants, like growth and early-life, parental and lifestyle factors, have not been investigated thoroughly in children yet.

The use of HbA_{1c} for diagnosing diabetes

HbA_{1c} has been firmly established as an index of long term glucose concentrations and as a measure of the risk for the development of complications in patients with diabetes mellitus. Recently, an international expert committee recommended using HbA_{1c} also as indicator for the diagnosis of diabetes[55].

This committee, with members appointed by the American Diabetes Association, the European Association for the Study of Diabetes, and the International Diabetes Federation, pointed out that HbA_{1c}: is better standardized compared to glucose measurements; is a better index of overall

Chapter 1
General Introduction

glycaemic exposure and risk for long-term complications; has substantially less biologic variability and preanalytic instability; needs no fasting or timed samples; is relatively unaffected by acute (e.g. stress or illness related) perturbations in glucose levels; and is already used to guide management and adjust therapy. But, they also recognize the limitations of HbA_{1c} as the recommended means of diagnosing diabetes, e.g. higher costs of the assay compared to glucose measurements, the inference of some haemoglobin traits (such as HbS, HbC and HbF) with some HbA_{1c} assay methods, the influence of any condition that changes red cell turnover (such a haemolytic anaemia and chronic malaria) on HbA_{1c} levels and the effect of age and ethnicity on HbA_{1c} levels. Despite these limitations, they stated that the ultimate goal of identifying individuals at risk for diabetes complications will be accomplished with an HbA_{1c} diagnostic level of 6.5%.

AIMS AND OUTLINE

In the near future, HbA_{1c} might increasingly be used as a diagnostic test for diabetes[55] and it is also proposed to translate HbA_{1c} to an estimated average glucose value[56]. Consequently, an increased use and also a different way of using HbA_{1c} can be expected. In addition, several studies have shown an association between HbA_{1c} and cardiovascular risk in people without diabetes[57],[58] and it is known that cardiovascular risk accumulates over the life course.
Therefore, it is important to:
1. get better insight in the normal distribution of HbA_{1c} in non-diabetic children of all ages;
2. increase the knowledge about all the factors, environmental as well as genetic, determining HbA_{1c} in non-diabetic persons from childhood onwards;
3. learn more about the way these factors act on HbA_{1c} levels. Identifying environmental factors and genetic loci associated with HbA_{1c}, will give further insight in the relative contribution of the different factors, glycaemic vs. non-glycaemic and environmental vs. genetic, to HbA_{1c} levels in non-diabetic persons.

The main aim of this thesis is to investigate determinants of HbA_{1c} in non-diabetic children and adults.
Chapter 2 presents the distribution of HbA_{1c} in 8-12 month-old non-diabetic infants from the GECKO-Drenthe birth cohort study and potential predictors of HbA_{1c} in this age group.
In chapter 3 the distribution of HbA_{1c} in non-diabetic Dutch children aged 8-9 years is provided as well as early-life, parental and lifestyle determinants of HbA_{1c} at this age.

Chapter 4 presents the associations of (lifestyle) determinants with HbA_{1c} at age 12 years and the effects of growth on change in HbA_{1c} between the age of 8 and 12 years. For chapter 3 and chapter 4 we used data from the PIAMA birth cohort study.
Chapter 5 provides the associations of "environmental" factors, genetic loci, and gene-environment interactions with HbA_{1c} in non-diabetic Dutch adults from the LifeLines cohort study.
Chapter 6 comprises the general discussion in which I show that HbA_{1c} is not suitable for diagnosing diabetes.

Obesity is acknowledged as an important determinant of insulin resistance and thereby higher levels of glycaemia and HbA_{1c}, also in children. There is only limited evidence on risk factors and treatment of obesity in children. Chapter 7 and chapter 8 are two supplemental chapters concerning childhood obesity.
Chapter 7 covers a summary of the results of a Cochrane review we conducted to assess the efficacy of a range of interventions designed to treat obesity in children and adolescents.
Chapter 8 provides the results of our study to energy intake and physical activity during treatment for acute lymphoblastic leukaemia (ALL) with intermittent dexamethasone (DEXA), to explain the weight gain seen in children treated for ALL.

Chapter 1
General Introduction

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Chapter 1
General Introduction

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