Determinants of HbA1c in non-diabetic children and adults

Jansen, Hanneke

Chapter 4

Change in HbA_{1c} levels between the age of 8 years and the age of 12 years in non-diabetic Dutch children The PIAMA birth cohort study

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H. Jansen
A.H. Wijga
S. Scholtens
G.H. Koppelman
D.S. Postma
B. Brunekreef
J.C. de Jongste
H.A. Smit
R.P. Stolk

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ABSTRACT

Aims: HbA_{1c} is associated with cardiovascular risk in persons without diabetes and cardiovascular risk accumulates over the life course. Therefore, insight in factors determining HbA_{1c} from childhood onwards is important. We investigated (lifestyle) determinants of HbA_{1c} at age 12 years and the effects of growth on change in HbA_{1c} between the age of 8 and 12 years.

Methods: HbA_{1c} was measured in 955 non-diabetic children aged around 12 years participating in the PIAMA birth cohort study. In 363 of these children HbA_{1c} was also measured at the age of 8 years. Weight, height, waist and hip circumference were measured when blood samples were taken. Data on parents and children were collected prospectively by questionnaires.

Results: We found no significant association between known risk factors for insulin resistance and HbA_{1c} at age 12 years. The mean(SD) change in HbA_{1c} was 0.6(0.7) mmol/mol per year. HbA_{1c} was fairly stable over time: 68.9% of the children remained in the same quintile or had an HbA_{1c} one quintile higher or lower at age 8 years compared to age 12 years. Anthropometric measures at age 8 and their change between age 8 and 12 years were not associated with the change in HbA_{1c}.

Conclusions: HbA_{1c} in non-diabetic children is fairly stable over time. The lack of association between known risk factors for insulin resistance and HbA_{1c} suggest that HbA_{1c} in non-diabetic children is relatively unaffected by factors associated with glycaemia and is mainly determined by factors which are fairly constant over time.

INTRODUCTION

Several studies have shown an association between HbA_{1c} and cardiovascular risk in people without diabetes[1,2] and it is known that cardiovascular risk accumulates over the life course. Since HbA_{1c} is used as measure of the risk for cardiovascular complications, and probably will be used as the diagnostic test for diabetes[3], it is important to get better insight in all the factors determining HbA_{1c} from childhood onwards.

In a previous study we investigated determinants of HbA_{1c} in 788 non-diabetic Dutch children aged 8-9 years from the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohort[4]. In the current study, we explore anthropometric measures and life-style factors as determinants of HbA_{1c} around the age of 12 years in the same children. In a subgroup of the study population, we investigate the change in HbA_{1c} levels between the age of 8 and 12 years. Between the age of 8 and 12 years children go through a phase of important growth. The aim of the current study is to investigate the effects of this growth on the change in HbA_{1c} levels. And additionally, we study the stability of HbA_{1c} over time in this population of non-diabetic children.

METHODS

The study population consisted of 955 Dutch children born and recruited in 1996-1997 into the PIAMA birth cohort study. Details of the study design have been published previously[5]. A screening questionnaire on maternal allergy[6] was distributed to 10,232 pregnant women visiting one of 52 prenatal clinics in three different regions in the Netherlands (North, Central and West). Based on this screening 7,862 woman were invited to participate in the study; 4,146 agreed and gave written informed consent. 183 participants were lost to follow-up before any data on the child had been obtained, so that the study started with 3,963 newborn children. Questionnaires were sent to the participating parents during pregnancy, at three months and yearly from 1 to 8 years of age and at 11 years of age. Details of the data collected during a hospital-based medical examination at 8 years of age have been described previously[4]. Around 12 years of age, all children who were still participating in the study were invited for a physical examination during a home-visit. In 964 children anthropometric measures were performed and an EDTA blood sample was taken. An HbA_{1c} value could be assessed in 963 samples. Children with diabetes mellitus (n=4) and children treated with growth hormone therapy (n=2) were excluded from the analysis. Two children were excluded from the current analyses since they had unexplained high HbA_{1c} levels of 6.4% and 6.8%, possibly

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due to (still) unknown existence of diabetes mellitus or an analytic error. Finally, we included 955 children (Study population I). In 363 of these children HbA_{1c} levels were also measured at the age of 8 years, in this subgroup we investigated the (determinants of) change in HbA_{1c} (Study population II). Change in HbA_{1c} was defined as the change in HbA_{1c} in mmol/mol per year, to take into account the differences in the interval between both measurements between the children.

For HbA_{1c} analysis, erythrocytes were stored at -20 °C for a mean period of 149 days (range 46-364) prior to assay. A 5 µl cell mass was lysated and HbA_{1c} was measured by ion-exchange chromatography using the Adams A_{1c}, HA-8160 HPLC (Menarini Diagnostics Benelux, Valkenswaard, The Netherlands). This analyser was standardized on Diabetes Control and Complications Trial (DCCT) standards. Between-batch imprecision (coefficient of variation) was 1.1% for a mean HbA_{1c} of 5.9% and 0.8% for a mean HbA_{1c} of 11.4%. Results were given as DCCT percentages as well as the new values of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in mmol/mol. New IFCC values were calculated with this equation: new IFCC value (mmol/mol) = (10.93 * old DCCT value) – 23.5[7]. All HbA_{1c} values were adjusted for storage time.

During the medical examination, children were weighed and measured in their underwear. Weight was measured to 0.1 kg and height to 0.1 cm by trained research staff using calibrated measuring equipment. Body mass index (BMI) was calculated as weight/height squared (kg/m^2). 'Overweight' and 'obesity' were defined according to age and gender specific international standards[8]. We use the term 'overweight' for the group of children who are overweight but not obese. Waist-circumference, to the nearest 0.1 cm, was measured midway between the lowest rib and the top of the iliac crest at the end of gentle expiration. Hip-circumference, to the nearest 0.1 cm, was measured off the trochanter major. Waist-circumference as well as the hip-circumference was measured twice. The mean of the two measurements was used in the analysis. Standard deviation scores (SDS) of BMI, waist circumference, hip circumference and waist-to-hip circumference were calculated using Growth Analyser 3.5 (Growth Analyser B.V., Rotterdam, the Netherlands), based on Dutch reference values[9,10].

Data on diet were obtained from a food-frequency questionnaire filled out by the parents when the child was aged around 11 years. Consumption frequency was categorized for the intake of products with a high saturated fat content (i.e. butter, candy bars, fried snacks, fries, chips and chocolate) and high vitamin C content (i.e. fresh fruit, uncooked vegetables and cooked vegetables). In addition, the consumption frequency of fish and nuts was categorized in 3 categories. We compared HbA_{1c} levels in these different categories for each food product.

Data on physical activity were obtained from a questionnaire filled out by the children when they were around the age of 11 years. We calculated the time spent on walking or cycling to school

and screen time (i.e. time spent on computing and watching television), in hours per week, and categorized both in 3 categories. In addition, we determined if they fulfilled the criteria of the Dutch physical activity guideline (at least one hour a day of active behaviour on every day of the week).
Data on maternal BMI and parental educational level (defined as the highest educational level of father and mother and categorized in three categories) were obtained by a questionnaire filled out by the parents when the children were 1 year old. Data on gestational diabetes were obtained by a questionnaire filled out by the parents of the children in whom an HbA_{1c} levels was assessed at age 8 years.

Statistical methods

We used multiple linear regression, with only region and age at time of blood sampling as covariates, to investigate the relation of all separate potential determinants with the change in HbA_{1c} and with HbA_{1c} at 12 years. A level of significance of $p < 0.05$ was applied for all analyses, which were performed with SPSS version 16.0 (SPSS Inc., Chicago, IL, USA).

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RESULTS

The characteristics of the study populations are displayed in Table 1.

(Lifestyle) determinants of HbA_{1c} at 12 years

We found no relation between anthropometric measures at 12 years and HbA_{1c} levels at 12 years (Table 2). HbA_{1c} levels were significantly higher in children of mothers with gestational diabetes compared to their counterparts and significantly lower in children who did not fulfil the criteria from the Dutch physical activity guideline compared to their counterparts. We included the food products of which the intake frequency was significantly associated with HbA_{1c} in Table 2. We found neither a significant association between the frequency of intake of the other food products high in saturated fat and vitamin C content and HbA_{1c} nor a significant association between the frequency of intake of nuts and fish and HbA_{1c}.

Change in HbA_{1c} between the age of 8 and 12 years

The mean (SD) difference in HbA_{1c} between the age of 8 and 12 years was 2.4 (3.1) mmol/mol ($p<0.001$). The mean (SD) increase was 0.6 (0.7) mmol/mol per year. HbA_{1c} at age 8 years was significantly and positively associated with HbA_{1c} at age 12 years, with an increase of 0.34 mmol/mol (95% CI 0.27 – 0.40) per 1 mmol/mol increase in HbA_{1c} at age 8 years ($p<0.001$). HbA_{1c} at age 8 years contributed 22% to the explained variance of HbA_{1c} at age 12 years. This positive association remained significant in the multiple linear regression model, adjusting for age at blood sampling around age 12 years and region, with an increase of 0.38 mmol/mol (95% CI 0.31 – 0.44) in HbA_{1c} at 12 years per 1 mmol/mol increase in HbA_{1c} at 8 years ($p<0.001$). The explained variance of this model was 28%. As a result, HbA_{1c} was fairly stable over time: 68.9% (n=250) of the children remained in the same quintile (n=114) or had an HbA_{1c} level in one quintile higher (n=70) or lower (n=66) at age 8 years compared to age 12 years (Table 3).

Neither anthropometric measures at age 8 nor the change in anthropometric measure SD-scores per year between 8 and 12 years were associated with the change in HbA_{1c} (Table 4). Also after adjustment for baseline anthropometric measure SDS (i.e. at age 8 years), the change in anthropometric measure SDS per year between 8 and 12 years was not associated with the change in HbA_{1c}.

Table I. Characteristics of the study populations

	Study population I			Study population II		
	Total n	Mean±SD	n (%)	Total n	Mean±SD	n (%)
General						
Gender: Girl	955		470 (49.2)	363		183 (50.4)
Age medical exam. 8yrs. (yrs.)	861	8.2 ± 0.4		363	8.1 ± 0.3	
Age medical exam. 12 yrs (yrs.)	955	12.5 ± 0.2		363	12.5 ± 0.2	
Δ Age (yrs.)	861	4.3 ± 0.5		363	4.4 ± 0.4	
HbA_{1c}						
HbA_{1c} at 12 yr. (%)	955	5.1 ± 0.2		363	5.1 ± 0.2	
HbA_{1c} at 8 yr. (mmol/mol)	363	29.8 ± 3.3		363	29.8 ± 3.3	
HbA_{1c} at 12 yr. (mmol/mol)	955	32.2 ± 2.4		363	32.2 ± 2.4	
Δ HbA_{1c} (mmol/mol)	363	2.4 ± 3.1		363	2.4 ± 3.1	
Change in HbA_{1c} (mmol/mol/yr)	363	0.6 ± 0.7		363	0.6 ± 0.7	
Anthropometry at age 12 years						
BMI (kg/m^2)	955	18.8 ± 2.7		363	18.8 ± 2.6	
BMI SDS	955	0.19 ± 1.0		363	0.19 ± 1.0	
Δ BMI SDS	861	0.10 ± 0.6		363	0.07 ± 0.6	
BMI	955			363		
Normal			829 (86.8)			316 (87.1)
Overweight			115 (12.0)			44 (12.1)
Obese			11 (1.2)			3 (0.8)
Waist circumference (cm)	955	66.2 ± 6.7		363	66.1 ± 6.4	
Waist circumference SDS	955	0.18 ± 1.0		363	0.18 ± 1.0	
Δ Waist circumference SDS	859	-0.19 ± 0.7		363	-0.22 ± 0.7	
Waist/hip ratio	955	0.82 ± 0.0		363	0.82 ± 0.0	
Waist/hip ratio SDS	955	0.07 ± 0.8		363	0.08 ± 0.8	
Δ Waist/hip ratio SDS	858	0.00 ± 0.8		362	0.00 ± 0.8	
Parental factors						
Maternal atopic constitution: Yes	955		303 (31.7)	363		229 (63.1)
Gestational diabetes: Yes	367	10 (2.7)		359	10 (2.8)	
Maternal BMI (kg/m^2)	910	23.0 ± 3.4		345	23.1 ± 3.5	
Parental educational level	954			362		
Low		103 (10.8)			35 (9.7)	
Intermediate		344 (36.1)			130 (35.9)	
High		507 (53.1)			197 (54.4)	

Δ = variable at age 12 years minus variable at age 8 years

Abbreviations: BMI, body mass index; SD, standard deviation; SDS, standard deviation score

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Table II. Study population I. Determinants of HbA_{1c} at the age of 12 years

	HbA_{1c} (mmol/mol)						
	n	Mean*	SD	Difference**	CI	p-value	R2
Gender							0.04
Girls	470	32.2	2.5	-	-		
Boys	485	32.2	2.2	0.01	- 0.28 – 0.31	0.93	
BMI							0.04
Normal	829	32.2	2.4	-	-		
Overweight	115	32.1	2.2	- 0.10	- 0.56 – 0.35	0.66	
Obese	11	32.4	3.2	0.24	- 1.15 – 1.63	0.73	
Gestational diabetes							0.04
No	357	32.1	2.3	-	-		
Yes	10	33.8	3.4	1.53	0.04 – 3.01	0.04†	
Parental educational level							0.04
Low	103	32.3	2.6	-	-		
Intermediate	344	32.2	2.4	- 0.10	-0.61 – 0.42	0.72	
High	507	32.2	2.3	- 0.04	-0.53 – 0.46	0.89	
Candy bars							0.05
No	232	32.1	2.4	-	-		
< 1 day per week	522	32.2	2.3	0.10	-0.26 – 0.46	0.60	
≥ 1 day per week	173	32.7	2.4	0.57	0.11 – 1.03	0.02†	
Chocolate							0.04
0-1 day per week	197	32.5	2.5	-	-		
2-5 days per week	467	32.1	2.3	-0.44	-0.83 – -0.05	0.03†	
> 5 days per week	270	32.3	2.4	-0.21	-0.64 – 0.22	0.34	
Cooked vegetables							0.05
< 3 days per week	56	32.9	2.2	-	-		
3-5 days per week	508	32.1	2.3	-0.89	-1.53 – -0.25	0.01†	
> 5 days per week	362	32.4	2.4	-0.56	-1.22 – 0.09	0.09	
Active transport to school							
< 1 hours/week	356	32.3	2.3	-	-		0.04
1 – 1.5 hours/week	308	32.3	2.4	0.09	-0.26 –	0.45	0.61
> 1.5 hours/week	268	32.1	2.5	- 0.17	-0.55 – 0.20	0.36	
Dutch physical activity guideline:							0.04
No	729	32.1	2.3	-	-		
Yes	205	32.6	2.5	0.42	0.05 –	0.78	0.03†
Screen time							0.04
< 10 hours/week	398	32.3	2.3	-	-		
10-20 hours/week	377	32.2	2.4	- 0.18	-0.51 –	0.15	0.28
> 20 hours/week	158	32.1	2.4	- 0.25	-0.68 – 0.18	0.25	
HbA_{1c} at 8 years (mmol/mol)	363			0.38	0.31 – 0.44	<0.001§	0.28
Weight SDS	955			0.05	- 0.10 – 0.20	0.50	0.04
Height SDS	955			0.03	- 0.12 – 0.18	0.73	0.04
BMI SDS	955			0.05	- 0.10 – 0.19	0.52	0.04
Waist SDS	955			0.03	- 0.13 – 0.18	0.73	0.04
Hip SDS	955			0.04	- 0.12 – 0.21	0.60	0.04
Waist-to-Hip SDS	955			0.02	- 0.17 – 0.21	0.85	0.04
Maternal BMI (kg/m^2)	910			0.02	- 0.03 – 0.06	0.47	0.04

* Crude mean

** Multiple linear regression model, only adjusted for age at medical examination 12 years and region

† P < 0.05; § P < 0.001

Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation; SDS, standard deviation score

Table III. Number of children shifted between quintiles of HbA_{1c}

Δ Quintile	Frequency	%	Cumulative %
0	114	31.4	
1 or -1	136	37.5	68.9
2 or -2	66	18.2	87.1
3 or -3	40	11.0	98.1
4 or -4	7	1.9	100.0
Total	363	100.0	

Table IV. Study population II. Determinants of the change in HbA_{1c}

	Change in HbA_{1c} (mmol/mol/yr)					
	n	Mean*	SD	Difference**	CI	p-value
Gender						
Girls	183	0.58	0.7	-		
Boys	180	0.53	0.7	- 0.07	- 0.21 – 0.06	0.30
BMI 8 years						
Normal	316	0.57	0.7	-		
Overweight	39	0.40	0.6	- 0.16	- 0.38 – 0.06	0.15
Obese	8	0.74	0.8	0.18	- 0.29 – 0.65	0.45
BMI SDS 8 years	363			- 0.05	- 0.13 – 0.02	0.17
Δ BMI SDS/yr	363			- 0.13	- 0.66 – 0.39	0.61
Waist circumference SDS 8 years	363			- 0.04	- 0.12 – 0.04	0.32
Δ Waist circumference SDS/yr	363			- 0.28	- 0.73 – 0.17	0.21
Hip circumference SDS 8 years	362			- 0.09	- 0.18 – 0.01	0.07
Δ Hip circumference SDS/yr	362			- 0.05	- 0.59 – 0.49	0.86
Waist/hip ratio SDS 8 years	362			0.04	- 0.06 – 0.14	0.40
Δ Waist/hip ratio SDS/yr	362			- 0.27	- 0.64 – 0.10	0.15
Maternal BMI (kg/m^2)	345			0.00	- 0.02 – 0.02	0.85

* Crude mean

** Multiple linear regression, only adjusted for age at medical examination 12 years and region

Abbreviations: BMI, body mass index; CI, confidence interval; SD, standard deviation; SDS, standard deviation score

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DISCUSSION

We found no consistent significant association between life-style factors and HbA_{1c} at the age of 12 years. HbA_{1c} at age 8 years is by far the most important predictor of HbA_{1c} at age 12. Therefore, HbA_{1c} levels appear to be fairly stable over time. We found a mean increase in HbA_{1c} between age 8 and age 12 of 0.6 mmol/mol/yr. Anthropometric variables at age 8 years as well as change in anthropometry between age 8 and 12 were not associated with the change in HbA_{1c}.

In our studies on determinants of HbA_{1c} in non-diabetic children around 8 years as well as 12 years of age, several (life-style) factors known to be associated with insulin resistance and risk for Type 2 diabetes, appear not to be associated with HbA_{1c}. Other studies on determinants of HbA_{1c} in childhood found varying results. Pettitt et al. found a higher HbA_{1c} in minority boys compared to non-Hispanic white boys in a group of 400 children aged 11 to 13 years, suggested as an early sign of predisposition to Type 2 diabetes among groups known to be at higher risk for Type 2 diabetes[11]. Saaddine et al investigated determinants of HbA_{1c} in 7,968 children and adolescents aged 5 to 24 years[12] and found a higher HbA_{1c} in the 10- to 14-year-old age group, in participants with overweight, in those with lower levels of education, in those with a positive parental history of diabetes and in those with serum glucose ≥ 7.0 mmol/l. They also found consistent differences in HbA_{1c} levels among different racial/ethnic groups studied. Finally, Eldeiwari et al. investigated predictors of HbA_{1c} among 4,928 non-diabetic young people aged 4-17.0 years and concluded that HbA_{1c} was generally associated with other known risk factors for Type 2 diabetes[13]. In contrast, Shultis et al. concluded from their study to determinants of HbA_{1c} in 1,645 children aged 9-11 years that HbA_{1c} is not a good marker of fasting or post-load glucose and insulin measures in healthy children, and that it is not a viable alternative to these measures for investigating the early life and childhood determinants of insulin resistance and Type 2 diabetes in children[14].

In contrast to the studies described above, in the current study we were also able to investigate life-style factors, i.e. dietary intake and physical activity, as determinants of HbA_{1c}. A higher physical activity level is known to decrease insulin resistance[15]. However, we found higher HbA_{1c} levels in physically active children, both at age 8 years[4] and at age 12 years in the current study. Also, at both ages we found no consistent association between dietary factors and HbA_{1c}, in contrast to studies in adults[16]. These results suggest that HbA_{1c} in non-diabetic children is relatively unaffected by level of glycaemia and factors associated with glycaemia. And that HbA_{1c} is determined by life-style factors to a greater extent in adults compared to children or, alternatively, that the differences in life-style factors may be less among children than among adults. Further studies to investigate these differences are warranted.

Puberty is associated with modest insulin resistance[17]. Given the mean (SD) age of our study

population of 12.5 (0.2) small differences in pubertal development could be expected. Unfortunately, we had no good measure to determine the early pubertal development at the moment the blood sample was taken. Therefore, we were not able to investigate the influence of puberty on HbA_{1c} levels.

Three studies previously reported that HbA_{1c} is relatively stable over time in adults[18-20]. Meigs et al. concluded, in their study on tracking of HbA_{1c} over a period of 4-6 years, that HbA_{1c} reliably categorizes the glucose control of non-diabetic subjects over a period of 4-6 years, thereby confirming its value as an epidemiological measure. Our results are in line with these findings. But to our knowledge, we are the first who can confirm these findings in children. The stability of HbA_{1c} over time suggests that the factors causing between-individual variability of HbA_{1c} are fairly constant in an individual.

We investigated determinants of change in HbA_{1c} in a subgroup of only 363 children. We found, though not statistically significant, unexpected inverse associations between the change in anthropometric measures and the change in HbA_{1c}. The found effect of especially the change in waist circumference SDS and waist-to-hip ratio SDS on the change in HbA_{1c} seems to be quite large compared to the mean change in HbA_{1c}, but is, probably due to a lack of power, not significant.

In the study population invited for a hospital-based medical examination at the age of 8 years, offspring of allergic mothers were overrepresented[4]. Consequently, in our study population II also offspring of allergic mothers are overrepresented compared to study population I. We repeated all analyses in the offspring of allergic and non-allergic mothers separately and the results were largely the same in both groups.

In conclusion, we found no significant association between known risk factors for insulin resistance and HbA_{1c} at the age of 12 years. HbA_{1c} at age 8 years is by far the most important predictor of HbA_{1c} at age 12 years. Consequently, HbA_{1c} in non-diabetic children seems to be fairly stable over time. Neither anthropometry at age 8 years nor change in anthropometry between age 8 and 12 years was associated with the change in HbA_{1c}. These results suggest that HbA_{1c} in non-diabetic children is relatively unaffected by level of glycaemia and factors associated with glycemia. It seems to be mainly determined by factors which are fairly constant over time in childhood.

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