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Glucose-induced insulin response and insulin sensitivity is not related to HLA-type but to age in young siblings of Type 1 (insulin-dependent) diabetic patients

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Summary. Glucose-induced insulin response and insulin sensitivity were studied in 32 HLA-identical, 38 haplo-identical and 24 non-identical, islet-cell-antibody-negative, healthy siblings of young Type 1 (insulin-dependent) diabetic patients (age range 10–28 years). No significant differences were obtained between HLA-identical, HLA-haplo-identical siblings and HLA-non-identical siblings in insulin response using an i.v. glucose infusion test even when the insulin sensitivity as estimated by the somatostatin-insulin-glucose infusion test was taken into account. A significant inverse correlation to age was found for both insulin response ($r = -0.24$, $p = 0.02$) and insulin sensitivity ($r = -0.36$, $p < 0.01$) in the young siblings studied.

Key words: Type 1 (insulin-dependent) diabetes mellitus, etiology, siblings, HLA-types, glucose tolerance test, insulin resistance, age.

Studies of monozygotic twins have shown that if one of the twins has acquired Type 1 (insulin-dependent) diabetes mellitus, then the co-twin runs an approximate 50% risk of developing Type 1 diabetes [1]. Similarly, HLA-identical siblings of Type 1 diabetic patients run an approximately 30% risk of developing Type 1 diabetes, whereas the risk for haplo-identical siblings is only 5%. HLA-non-identical siblings run the same risk as the normal population [2].

Many groups have been trying to shed some light on mechanisms responsible for the increased susceptibility to Type 1 diabetes in HLA-identical siblings. An interesting observation was made by Hollander et al. [3], who in a small group of siblings of Type 1 diabetic patients found an exaggerated early insulin response to intravenous arginine and glucose infusion in HLA-identical siblings of Type 1 diabetic patients. Similarly, an exaggerated insulin response to oral glucose was described by Schober et al. [4] in HLA-identical but not in HLA-haplo-identical and HLA-non-identical siblings of Type 1 diabetic patients. It was suggested in these studies that the possibility of a functional disturbance of the B-cell occurs before the appearance of B-cell autoantibodies and it was speculated that the increased B-cell activity could predispose the cells to damage by environmental agents. On the other hand, the appearance of B-cell antibodies in peripheral blood seems to be associated with a decrease of the early insulin response to glucose [5–7]. Although insulin sensitivity was not measured, the findings in the study by Hollander et al. [3] suggested that a slight peripheral insulin resistance might be present in the HLA-identical siblings, contributing to the increased glucose-induced insulin response.

The aim of this study was to analyse the kinetics of insulin release and insulin sensitivity in three subgroups of siblings of Type 1 diabetic-patients: HLA-identical, HLA-haplo-identical and HLA-non-identical. In order to identify a possible modulation of insulin responses by B-cell antibodies, islet-cell antibodies (ICA) have been analysed.

Subjects and methods

Subjects

The study was approved by the Ethical Committee of the Karolinska Institute in Stockholm. Informed consent was obtained from all the patients and their parents. Altogether, 159 children with Type 1 diabetes mellitus and 197 healthy siblings from 156 families were typed for HLA-ABC-antigens. Siblings who were HLA-ABC-identical with the proband were further analysed using the mixed lymphocyte culture (MLC) test to ascertain identity. Among the 197 siblings, 38 were HLA-identical, 102 were haplo-identical and 57 were HLA-non-identical. All HLA-identical and groups of age-, sex-, and weight-matched HLA-haplo- and HLA-non-identical siblings, respectively, were invited to further studies of insulin response and insulin sensitivity. Details of the siblings who took part in the study are

Table 1. Characteristics of siblings subjected to the glucose infusion test (GIT) and the somatostatin-insulin-glucose infusion test (SIGIT)

Group *(n)*	Age (years) Mean ± SD (range)	Sex ratio (male/female)	Body mass index (kg/m^2) Mean ± SD	Comments on SIGIT
HLA-identical (33)	16.8 ± 3.9 (11.0 – 24.3)	17/16	19.9 ± 2.4	6 boys, 5 girls did not perform SIGIT 9 of them younger than 15 years
HLA-haplo-identical (39)	16.8 ± 4.2 (10.5 – 27.8)	23/16	19.8 ± 2.8	5 boys, 6 girls did not perform SIGIT 9 of them younger than 15 years
HLA-non-identical (24)	17.1 ± 4.1 (11.7 – 24.6)	11/13	19.6 ± 2.5	4 boys, 3 girls did not perform SIGIT 4 of them younger than 15 years

summarised in Table 1. The mean interval between onset of diabetes of the proband and the tests performed on the siblings was 6 years (range 3-18).

Methods

Glucose infusion test (GIT) and somatostatin-insulin-glucose infusion test (SIGIT) were performed on the morning after an overnight fast. The subjects were instructed to avoid vigorous exercise and alcoholic drinks the day before the test. Indwelling catheters were inserted into both cubital veins. The subjects remained supine during the experiments.

Glucose infusion test (GIT). GIT was performed as described by Cerasi et al. [8]; 500 mg of glucose/kg-body weight was injected rapidly and followed by a glucose infusion at a rate of 20 $mg \cdot kg^{-1} \cdot min^{-1}$ over 60 min. Venous blood samples were drawn at 5-20 min intervals during 120 min. All blood samples were kept on ice until centrifugation. Plasma was frozen at −20 °C until analysed. Insulin response was calculated as insulin (incremental) areas.

Somatostatin-insulin-glucose infusion test (SIGIT). SIGIT was performed according to Harano [9] as modified by Wajngot et al. [10]; glucose (6 $mg \cdot kg^{-1} \cdot min^{-1}$), insulin 0.4 $mU \cdot kg^{-1} \cdot min^{-1}$ - Actrapid, Novo, Copenhagen, Denmark) and somatostatin (4.5 µg/min - Stilamin, Serono, Rome, Italy) were infused simultaneously. Human albumin (20% Albumin, Kabi Vitrum, Stockholm, Sweden) was added to avoid absorption of insulin to the bottle wall. Subjects less than 50 kg of weight received reduced amounts of somatostatin (3.3 µg/min). Venous blood samples were drawn every 30 min for determination of glucose, insulin and C-peptide.

During the test, the C-peptide values were substantially depressed. The mean values ± SD for all individuals before and at 90, 120, 150 min were: 0.37 ± 0.16, 0.08 ± 0.05, 0.08 ± 0.05, 0.09 ± 0.08 pmol/l. During the test, circulating insulin levels were stable. The mean values ± SD for all individuals before and at 90, 120, 150 min were: 16 ± 5, 24 ± 4, 25 ± 4, 25 ± 5 mU/l. Insulin and C-peptide levels were similar in the groups studied, e.g. plasma insulin concentrations at 90 min were 24, 24, 25 mU/l, respectively.

Since endogenous insulin secretion, was continuously depressed during the test, the blood glucose levels at the end of the test could be used as an estimate of insulin sensitivity. The mean of blood glucose levels at 90, 120, 150 min is referred to as SIGIT ($\bar{x}$).

Chemical methods

Plasma glucose was determined using a commercially available glucose oxidase method - GLOX, Kabi Vitrum, Stockholm, Sweden [11]. Insulin and C-peptide were determined using RIA-methods, commercially available by Novo Company, Copenhagen, Denmark [12, 13]. HLA-ABC-typing was performed by means of the standard NIH technique and HLA-identity was confirmed using the MLC test [14]. ICA was analysed using a two-colour immunofluorescence technique [15].

Statistical analysis

As insulin-area and the quotient insulin-area/SIGIT ($\bar{x}$) showed skewed distribution, results are expressed as median, quartiles and ranges. Statistical methods for the evaluation of non-parametric data (Mann-Whitney U-test and Spearman's rank correlation coefficient) were used. For the other variables, Student's t-test was used. A p value less than 0.05 was considered to be statistically significant. A power test was performed for insulin sensitivity showing that with a power of 80% and a p value of 0.05, a 25% difference could be detected using sample sizes of 30 individuals in each group.

Results

Prevalence of islet cell antibodies (ICA)

All siblings subjected to the glucose infusion test were analysed for ICA. All were negative except for two siblings - one HLA-identical boy and one HLA-haplo-identical girl. When comparing insulin response and insulin sensitivity between the different HLA-groups as described below, these two subjects were excluded.

Insulin response and HLA-type

The insulin responses and glucose levels in the three different groups are shown in Figure 1 and the exact values for glucose and insulin are given in Table 2. Insulin responses measured as insulin area during the early 0-10 min, late 10-60 min and total phases did not differ between HLA-identical siblings and HLA-haplo- or HLA-non-identical siblings respectively. The individual values, however, varied markedly. Median, quartiles and ranges are given in Table 3.

Insulin sensitivity and HLA-type

The insulin sensitivity measured as SIGIT ($\bar{x}$) for the three groups are given in Figure 2. No significant differences were found between the groups. Again, a considerable individual variation is notable.

Insulin response during the glucose infusion test was related to the prevailing insulin sensitivity by calculating the quotient insulin area/SIGIT ($\bar{x}$). This quotient was given as early, late and total phase respectively. Again, no significant differences were found be-

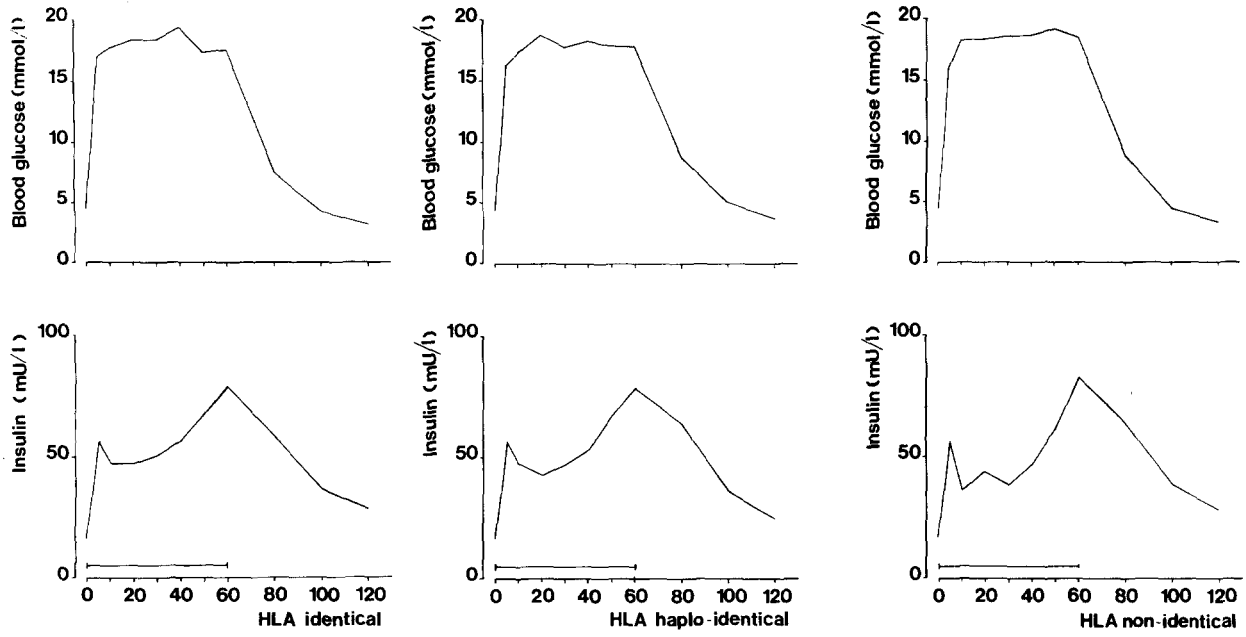

Fig. 1. Glucose and insulin response to the glucose infusion test. Median values in three groups of siblings to Type 1 diabetic patients. Glucose infusion ⟵⟶

tween HLA-identical compared to HLA-non-identical siblings and HLA-haplo-identical compared to HLA-non-identical siblings respectively (Table 4).

Analysing all the siblings, insulin sensitivity was inversely correlated to insulin area, i.e. individuals with a high SIGIT ($\bar{x}$) value (low sensitivity) tended to exhibit high insulin responses measured as insulin areas. The correlation between insulin area, early phase and SIGIT ($\bar{x}$) were $r_s = 0.31$, $p = 0.01$ and between insulin area total phase and SIGIT ($\bar{x}$) were $r_s = 0.35$, $p < 0.01$.

Insulin response, insulin sensitivity, and sex

There were no significant differences between sexes when comparing the variables insulin area or the quotient insulin area/SIGIT ($\bar{x}$) respectively. However, for SIGIT ($\bar{x}$) a significant difference was found ($p = 0.01$) showing a higher SIGIT ($\bar{x}$) for girls compared to boys when analysing all the siblings.

Insulin response, insulin sensitivity and age

Insulin area was significantly inversely correlated to age in the total material of siblings: insulin area early phase $r_s = -0.25$, $p = 0.02$; insulin area total phase $r_s = -0.24$, $p = 0.02$. Insulin sensitivity, SIGIT ($\bar{x}$) was also significantly inversely correlated to age $r_s = -0.36$, $p < 0.01$. A 95% confidence interval for SIGIT by age in low risk individuals (i.e. HLA-non-identical siblings) was constructed (Fig. 3). The values for the HLA-identical siblings are plotted in the same figure and all values fell within the confidence limits of the low risk individuals.

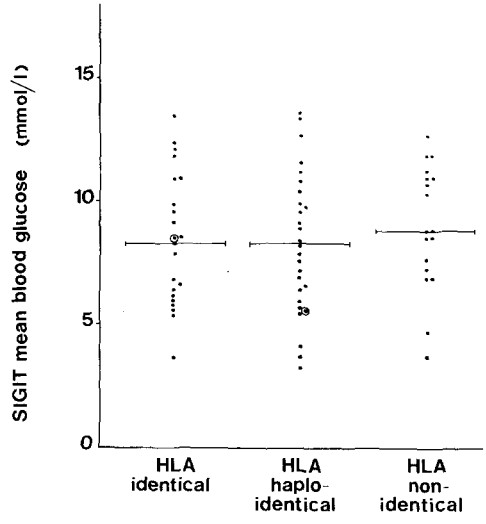

Fig. 2. Insulin sensitivity measured as SIGIT ($\bar{x}$) in the three groups of siblings of Type 1 diabetic patients. ICA-positive siblings are indicated by 0

Diabetes emerged in one sibling

The HLA-identical sibling who was ICA-positive was a twin brother of a boy who developed Type 1 diabetes at 11 years of age. The healthy co-twin was examined at the age of 16 years when he had a normal fasting blood glucose as well as normal responses to GIT and SIGIT. The early insulin area was 193 and the total insulin area was 1603 mU·min/l, i.e. close to the lower quartiles of the total material (Table 3). His SIGIT ($\bar{x}$) value was normal as indicated in Figures 2 and 3. At the age of 18 years, the boy had postprandial glucosuria but no clinical symptoms of diabetes. A new GIT was performed which now showed definite pathologi-

Table 2. Glucose and insulin values during the glucose infusion test in 3 groups of healthy siblings of Type 1 diabetic patients. Glucose values are given as means ± SD. Because of skewed distribution insulin values are given as medians and quartiles (within brackets)

	Time (min)										
	0	5	10	20	30	40	50	60	80	100	120
HLA-identical ($n=33$)											
Glucose (mmol/l)	4.6±0.6	16.8±3.5	17.8±2.6	18.5±3.0	18.6±3.4	18.4±4.4	17.9±4.5	17.4±5.1	9.2±4.3	5.6±3.1	3.9±1.9
Insulin (mU/l)	16 (14, 19)	56 (42, 72)	47 (36, 61)	47 (39, 58)	50 (42, 64)	56 (44, 78)	67 (50, 89)	78 (61, 94)	58 (47, 75)	36 (28, 50)	28 (22, 32)
HLA-haplo-identical ($n=39$)											
Glucose (mmol/l)	4.7±0.4	15.9±3.4	17.7±2.5	18.6±2.4	18.2±2.9	18.2±3.3	18.1±4.0	17.4±4.4	9.4±4.0	5.3±2.4	3.8±1.4
Insulin (mU/l)	17 (15, 19)	56 (42, 86)	47 (36, 69)	43 (36, 58)	47 (33, 64)	53 (44, 89)	67 (50, 94)	78 (61, 111)	64 (44, 100)	36 (28, 50)	25 (21, 33)
HLA-non identical ($n=24$)											
Glucose (mmol/l)	4.5±0.3	16.5±3.2	17.0±3.3	17.8±3.1	18.5±2.9	18.2±3.4	18.6±3.6	18.0±4.1	10.9±4.9	6.1±3.7	4.3±2.9
Insulin (mU/l)	17 (15, 18)	56 (39, 83)	36 (31, 64)	44 (32, 67)	39 (33, 67)	47 (42, 64)	61 (42, 78)	83 (57, 92)	64 (50, 83)	39 (28, 53)	28 (22, 36)

Table 3. Insulin response to the glucose infusion test (GIT) in ICA-negative siblings to Type 1 diabetic patients. Insulin response is calculated as the area under the insulin curve with the base at the 0 min value

Group *(n)*	Insulin area early phase 0–10 min (mU min/l)	Insulin area late phase 10–60 min (mU min/l)	Insulin area total phase 0–60 min (mU min/l)
HLA-identical ($n=32$)			
Median	259	1 945	2 289
Lower q	191	1 400	1 591
Upper q	368	2 900	3 221
Range	71-1 629	600-10 695	671-12 324
HLA-haplo-identical ($n=38$)			
Median	270	1 875	2 352
Lower q	176	1 390	1 566
Upper q	430	3 135	3 504
Range	38-1 626	645-6 335	683-7 961
HLA-non-identical ($n=24$)			
Median	266	1 755	1 966
Lower q	150	1 290	1 502
Upper q	431	2 810	3 211
Range	65-1 018	645-4 325	750-5 179

Table 4. The quotient insulin area/SIGIT ($\bar{x}$) in ICA-negative siblings to Type 1 diabetic patients

Group *(n)*	Insulin area /SIGIT ($\bar{x}$) early phase 0–10 min (mU min/mmol)	Insulin area /SIGIT ($\bar{x}$) late phase 10–60 min (mU min/mmol)	Insulin area /SIGIT ($\bar{x}$) total phase 0–60 min (mU min/mmol)
HLA-identical (n=21)			
median	31	221	252
lower q	20	181	200
upper q	45	324	379
range	12–135	93–883	105–1019
HLA-haplo-identical (n=27)			
median	34	229	260
lower q	18	167	193
upper q	55	366	436
range	3–133	56–1336	59–1469
HLA-non-identical (n=17)			
median	32	199	221
lower q	22	164	208
upper q	45	332	360
range	13–76	90–440	104–483

cal glucose and insulin responses and two months later he developed insulin-dependent diabetes.

The other ICA-positive sibling was a haplo-identical girl examined at the age of 12 years. She then had normal GIT and SIGIT tests.

Discussion

The present study clearly demonstrates that the insulin response to a standardised glucose infusion test in ICA-negative HLA-identical siblings of young Type 1

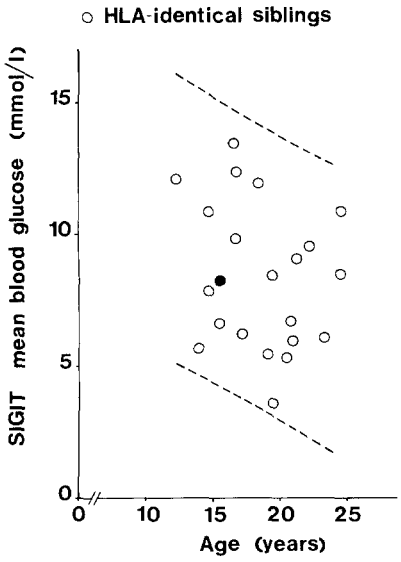

Fig. 3. Insulin sensitivity measured as SIGIT ($\bar{x}$) by age. 95% confidence limits for HLA-non-identical healthy siblings of Type 1 diabetic patients. Regression line: $y = 13.9 - 0.28\ x$, $r = -0.4$. The individual values for HLA-identical siblings are indicated by ○, all falling within the confidence limits of the low-risk groups. ● ICA-positive, HLA-identical sibling, who later developed Type 1 diabetes mellitus

diabetic patients was not different from that seen in HLA-haplo-identical and HLA-non-identical siblings. Insulin release, however, showed great individual variation. This variation could not be attributed to the presence of B-cell autoantibodies. Thus, we were unable to confirm the observation of a deranged B-cell responsiveness, i.e. an exaggerated acute phase insulin response to intravenous arginine and glucose as reported by Hollander et al. [3]. The reason for the contradictory results is unclear, though methodological differences may play a role. In the present study insulin secretion was stimulated using a glucose bolus followed by glucose infusion during 60 min, while Hollander et al. gave arginine followed by glucose i.v. as bolus doses. Our method may allow a better separation of the maximum, first and second phase insulin responses. It is, however, noteworthy that the same group of investigators [16] using an intravenous glucose tolerance test (IVGTT) recently reported virtually identical insulin responses in HLA-identical siblings of Type 1 diabetic patients compared to that in unrelated control subjects. In our study, subjects belonging to three HLA-subgroups of siblings of Type 1 diabetic patients exhibited a wide range of insulin responses ranging from low to very high. Thus, one reason for the conflicting results between our and earlier studies may be that HLA-identical siblings of Type 1 diabetic patients are a heterogenous group of individuals, where some subjects could exhibit an early derangement in B-cell responsiveness. Only follow-up studies of large groups of siblings may give the answer.

In order to better evaluate the response of insulin, insulin sensitivity was also tested. This is of particular interest since, as discussed by Wajngot et al. [10], low insulin response in relation to prevailing insulin sensitivity may be an early event in the development of diabetes. It is also of importance since insulin resistance has been shown to vary during the early natural course of Type 1 diabetes [17]. In our study, it was shown that there is a significant correlation between insulin response and insulin sensitivity, but there was no difference between the three groups of HLA-typed siblings. Thus the insulin responses in the three groups of siblings were not different although insulin sensitivity was taken into account.

In contrast to our findings, Raghu et al. [16] have recently reported decreased insulin sensitivity in HLA-identical siblings of Type 1 diabetic patients compared to matched unrelated control subjects. These authors determined the insulin sensitivity index on the basis of an IVGTT using the minimal model of insulin kinetics. There is a good correlation between the sensitivity index as derived from an IVGTT and that derived from an euglycaemic clamp [18], suggesting that the opposite results reached in their and our studies are probably not of a methodological character. The relatively large number of siblings in the HLA subgroups in the present study would be enough to detect a difference of a least 25% of the SIGIT ($\bar{x}$) values with a power of 80% and a probability of 5%. Furthermore, in our study HLA-identity was based not only on HLA-ABC-typing, but also confirmed by the MLC-test. This could be important, as recent studies have shown that the strongest association between HLA antigens and susceptibility of Type 1 diabetes may be present in the DQ region [19]. Again, a possible explanation for the diverging results may be that HLA-identical siblings represent a very heterogenous group, insulin resistance being a characteristic feature of some of these individuals.

An important finding of the present study was the demonstration of an age dependency of both insulin response and insulin sensitivity in young, healthy individuals. However, the correlations are low, indicating low fractions of explained variances. Our observation is in agreement with that of Amiel et al. [20], who studied both non-diabetic and diabetic individuals. Thus, these authors found a lower insulin sensitivity in adolescents compared to both prepubertal and adult subjects, irrespective of the fact if they had diabetes or not. Our findings of an age dependency of both insulin response and insulin sensitivity underlines the importance of taking age into account when interpreting such data.

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