# Beta-Cell Dysfunction in Nondiabetic HLA Identical Siblings of Insulin-dependent Diabetics

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**SUMMARY**

**B-cell function was tested in siblings of insulin-dependent diabetics (IDD). From previous studies, it is now recognized that the risk of developing IDD is highest in those sharing both haplotypes ($S_{2H}$) and lowest in those sharing neither haplotype ($S_{0H}$) with the diabetic. Insulin secretion in response to intravenous arginine and glucose was evaluated in $S_{2H}$, $S_{0H}$, and matched controls. Intravenous arginine and glucose elicited an exaggerated acute phase of insulin secretion in $S_{2H}$ compared with controls when analyzed as incremental insulin area 0–10′, peak level attained, and mean insulin levels postinjection. Insulin responses to arginine and glucose in $S_{0H}$ and matched controls were identical. We hypothesize that the increased beta-cell activity found in $S_{2H}$ predisposes their beta-cells to damage by environmental factors and may be part of the mechanism conferring the increased risk of IDD in $S_{2H}$. DIABETES 31:149–153, February 1982.**

Study of the human major histocompatability system (HLA) has increased our understanding of the role genetics plays in insulin-dependent diabetes (IDD). Family studies have shown that siblings of insulin-dependent diabetics have a 5–10% chance of developing diabetes compared with the approximate 0.15% prevalence in the general population.[1,2] HLA typing of families with insulin-dependent diabetes can identify those family members who show the greatest risk of developing IDD, namely, siblings HLA identical with the diabetic, and those at least risk, siblings who share neither haplotype with the diabetic.[2]

Previous reports have suggested that there are abnormalities in beta-cell function and/or glucose metabolism in relatives of diabetics, but the different observations are not in agreement.[3–21] In some of the studies, insulin-dependent diabetes was not evaluated separately from non-insulin-dependent diabetes, methods of defining the high risk individuals were variable and/or were performed before recognition of the relationship between HLA and susceptibility to IDD. Several of the studies employed oral glucose tolerance tests. The many factors that influence this test make precise evaluation of insulin secretion during an oral glucose tolerance test very difficult. Stimulation of insulin secretion by the intravenous route avoids many of these problems and allows distinct measurement of first and second phase secretion. Therefore, we have evaluated beta-cell function poststimulation with intravenous arginine and glucose in HLA identical and nonidentical siblings of insulin-dependent diabetics. We postulate that the beta-cell abnormality we observed in the HLA identical group is related to their increased risk.

## MATERIAL AND METHODS

Subjects were recruited from HLA-typed families where one sibling had classic IDD, defined as presenting in ketoacidosis or with the acute onset of polyuria, polydypsia, and/or weight loss and subsequently requiring insulin treatment. Nine Caucasian, HLA identical siblings of patients with insulin-dependent diabetes ($S_{2H}$) and five Caucasian siblings of insulin-dependent diabetics who shared neither HLA haplotype with their diabetic siblings ($S_{0H}$) were studied. Oral glucose tolerance tests were performed in $S_{2H}$ and $S_{0H}$ to insure that they did not have mild diabetes. Descriptive data for each of the siblings is provided in Table 1. Controls for the siblings consisted of unrelated Caucasian, nondiabetic subjects with no family history of diabetes in their first degree relatives. Each control was selected to individually match for age, sex, height, and body weight with a sibling who shared both or neither haplotype with their diabetic sibling.

All studies were performed after the subjects were admitted to the hospital. Subjects were maintained on a normal diet with no food for 12 h before the test. Informed consent

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TABLE 1
Characteristics of siblings who shared both ($S_{2H}$) and neither ($S_{0H}$) haplotype with the diabetic*

| $S_{2H}$ | Age | Sex | Weight (kg) | Height (cm) | IBW (%) | FPC | 2 h PP | Genotype |
|---|---|---|---|---|---|---|---|---|
| BB | 15 | M | 56 | 170 | 87 | 96 | 143 | AX,Bw22/A2,B8 |
| CC | 22 | F | 73 | 170 | 122 | 87 | 117 | A3,Aw31,B7,Bw44† |
| SF | 27 | F | 81 | 168 | 139 | 64 | 94 | A3,Aw31,B7,Bw44† |
| DH | 21 | M | 62 | 170 | 103 | 91 | 63 | A1,B8/A2,B15 |
| PM | 25 | F | 68 | 178 | 104 | 90 | 143 | A2,B18/A1,B8 |
| JS | 23 | M | 87 | 185 | 115 | 97 | 158 | A1,B7/A2,B27‡ |
| RS | 21 | M | 84 | 184 | 112 | 84 | 90 | A1,B7/A2,B27‡ |
| DW | 38 | M | 80 | 185 | 105 | 82 | | A1,A2,B18,Bw21§ |
| ML | 15 | M | 52 | 168 | 76 | 79 | 106 | A1,B8/Aw24,BX |
| $S_{0H}$ | | | | | | | | |
| SB | 26 | F | 60 | 162 | 112 | 62 | 67 | A1,B17/A1,BW51‖ |
| MC | 19 | F | 50 | 155 | 100 | 67 | 59 | A1,B17/A1,Bw51‖ |
| GS | 31 | F | 54 | 160 | 102 | 77 | | A9,B40/A2,B15 |
| DB | 22 | F | 52 | 158 | 101 | 83 | 125 | A1,B8/A2,B7¶ |
| CB | 22 | F | 52 | 158 | 101 | 76 | 81 | A1,B8/A2,B7¶ |

* Oral glucose tolerance tests were not performed in DW and GS.
† Father not available.
‡ Siblings.
§ Identical twin of IDD, parents not typed.
‖ Siblings.
¶ Siblings.

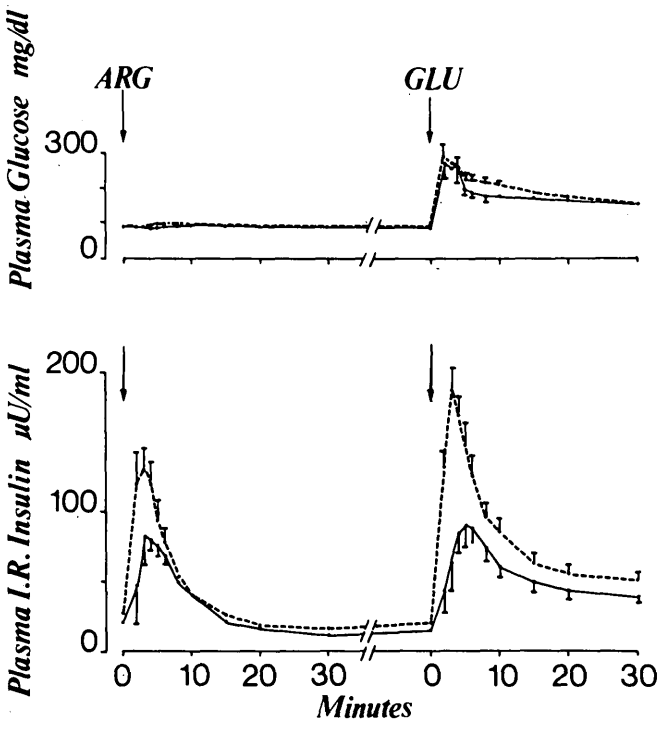
was obtained from all subjects before participation. Studies began at 8:00 a.m., with the patient at rest in bed during the entire procedure. A needle was placed in each antecubital vein and was maintained patent by the slow infusion of normal saline. After the baseline samples were drawn at −45, −30, −15, and 0 min, 5 g of arginine was injected intravenously over a 30-s interval. Blood samples were obtained at 2, 3, 4, 5, 6, 8, 10, 15, 20, 30, 45, 60, 70, 80, and 90 min post-arginine. The patient was then given 25 g of glucose intravenously over a 30-s period. Blood samples were taken at the same time intervals as above for 60 min after the glucose injection. All blood samples were heparinized and kept on ice until separated by centrifugation. The plasma was frozen at −20°C until analyzed. Plasma insulin (IRI) was measured by radioimmunoassay[22] and plasma glucose by the glucose-oxidase technique (Beckman Glucose Analyzer, Beckman Instruments, Fullerton, California). HLA-A and -B locus typing was performed with the standard NIH two-stage microcytotoxicity assay using a well-characterized panel of 140 antisera.[23]

First phase insulin secretion is calculated as the incremental area under the insulin curve for the first 10-min post-intravenous administration of arginine and glucose. Second phase IRI is calculated as the IRI incremental area from 10 to 30 min poststimulation. Glucose disposal rate (kg) is expressed as the slope of the semilogarithmic decline of blood sugar over the 10–30-min period following the intravenous glucose injection. Statistical analyses were performed by standard nonparametric and parametric methods.[24,25] Statistical significance is taken as $P < 0.05$.

## RESULTS

The IRI response to arginine (Figure 1) was larger in the HLA identical siblings with significantly elevated mean IRI levels at 3 and 4 min and larger first phase release compared with controls, 557 ± 74 versus 359 ± 42 μU/ml/10 min ($P < 0.02$). During the intravenous glucose tolerance test (Figure 1), significantly higher mean insulin concentrations were seen in $S_{2H}$ compared with the controls at 2, 3, 4, 5, 6, 8, and 10 min postinjection ($P < 0.01$ for all time points) and the insulinogenic index (IRI/glucose) was significantly elevated in $S_{2H}$ at all time points between 2 and 15 min. The acute or first phase insulin response to glucose was significantly higher in $S_{2H}$ when compared with their controls, 980 ± 83 versus 393 ± 36 μU/ml/10 min ($P < 0.01$), whereas second

**FIGURE 1. Plasma glucose (top) and plasma insulin (bottom), post-arginine (arg) and glucose (glu) injection in HLA identical siblings of diabetics (----) and matched controls (——).**



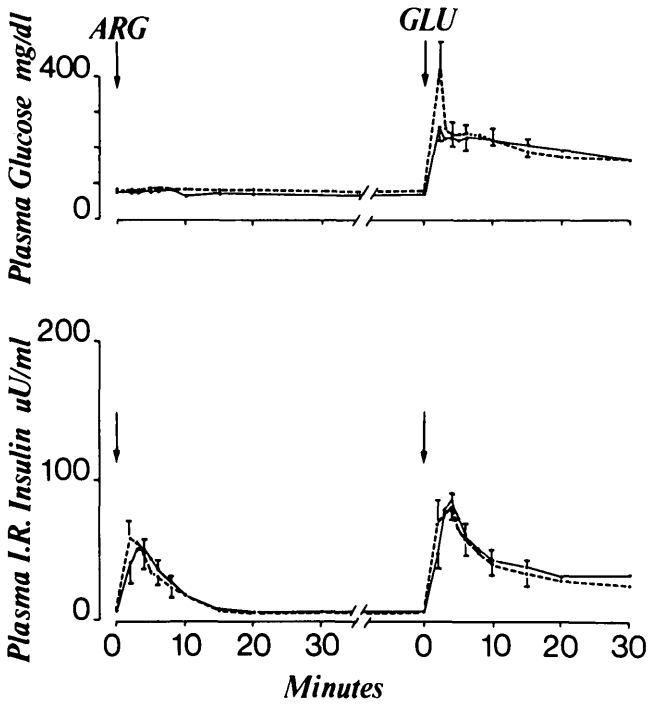



**FIGURE 2. Plasma glucose (top) and plasma insulin (bottom) post-arginine (arg) and glucose (glu) injection in individuals who share neither haplotype with their diabetic siblings (----) and matched controls (——).**

phase IRI secretion was similar (812 ± 112 versus 724 ± 85 μU/ml/30 min, respectively). The HLA identical siblings had significantly higher glucose levels than the controls at 5, 6, 8, 10, and 15 min post-glucose injection but glucose disposal rate (kg) was the same in both $S_{2H}$ and their controls (1.55 ± 0.22 and 1.72 ± 0.18, respectively). The IRI responses to arginine and glucose stimulation were virtually identical in the siblings who shared no haplotype with the diabetic and their controls (Figure 2).

Figure 3 shows the individual acute phase insulin responses for each control and HLA typed sibling ($S_{2H}$ and $S_{0H}$) during the arginine stimulation test and the intravenous glucose tolerance test. There was considerable overlap between the insulin responses in the groups during the arginine stimulation tests, but virtually complete separation of the HLA identical siblings from controls during the intravenous glucose tolerance tests.

## DISCUSSION

Within families where one or more member had insulin-dependent diabetes, HLA typing can identify those members at greatest risk of developing IDD, namely, individuals HLA identical with the diabetic.[2] Exactly how great a risk these individuals have of developing IDD has not been established, although the identical twin studies would suggest that it cannot be greater than 50%.[3] Several factors probably contribute to making this question difficult to answer. Insu-

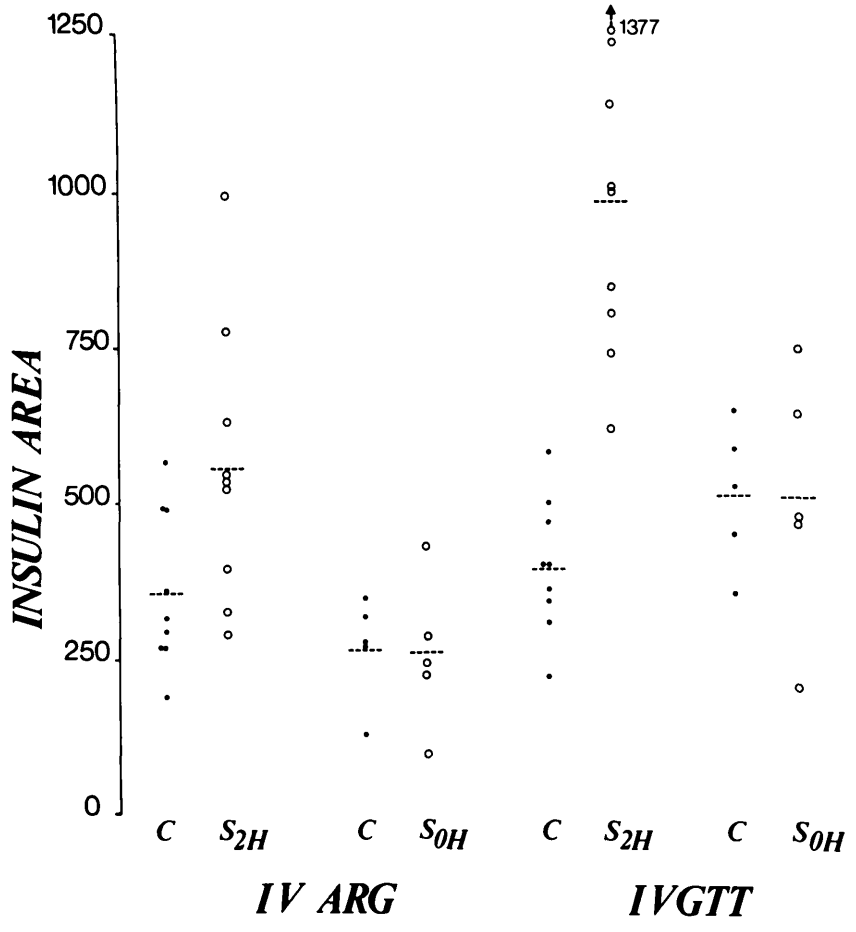



**FIGURE 3. Acute insulin response (μU/ml/10') to intravenous arginine (IV arg) and glucose (IVGTT) in siblings of IDD who share both ($S_{2H}$) and neither ($S_{0H}$) haplotypes with the diabetic and in their respective controls (C). Mean value depicted as ----.**

lin-dependent diabetes is very likely a heterogeneous disorder and different subsets of IDD may have different inheritance patterns and, therefore, different risks.[2] In susceptible individuals, environmental factors including viral infections may trigger the onset of some cases of insulin-dependent diabetes and in these individuals the risk of IDD is in part dependent on the prevalence of the environmental factors.[26] And finally, some of the previously reported inconsistencies in risk may in part be due to nonrandom recruitment techniques and ascertainment problems.[27–29] Because of these considerations, we cannot accurately predict what percentage of our HLA siblings will become diabetic, but would estimate it to be substantially less than 50%. Nonetheless, in families without identical twins, HLA typing provides the opportunity of evaluating islet-cell function in a group of individuals with the greatest relative risk of developing insulin-dependent diabetes as can currently be defined.

A large number of previous studies have investigated beta-cell function in relatives of diabetics. Insulin levels have been reported to be decreased, delayed, normal, or elevated.[3–21] Part of this discrepancy may be related to the failure in some of these studies to evaluate insulin-dependent diabetic families separately from non-insulin-dependent diabetic families. The individuals we evaluated were all siblings of diabetics with classic insulin-dependent diabetes in childhood. Several studies evaluating relatives of insulin-dependent diabetics have found results similar to ours. Johansen, Soeldner, and colleagues studied nondiabetic monozygotic twin siblings of patients with juvenile-onset-type diabetes and found that compared with controls the twins had higher mean serum IRI levels during all tests, but the differences reached statistical significance only during the cortisone-primed oral glucose tolerance test.[15] Other investigators evaluating first degree relatives of insulin-dependent diabetics[30] or HLA identical siblings of insulin-dependent diabetics[5,13,14] have found elevated IRI levels compared with controls at certain time points during an oral glucose tolerance test, but lower IRI values post-oral glucose have also been reported.[19] The many factors that influence this test make evaluation of insulin secretion during an oral glucose tolerance test very difficult. These include variability of gastric emptying, variable absorption and hepatic extraction of glucose, the influence of gastrointestinal hormones, neural influences during the cephalic phase, and the difficulty in separately assessing first versus second phase release. Stimulation of insulin secretion by the intravenous bolus administration of glucose and arginine is not hampered by these factors and in this test we observed separation of controls and haplo-identical siblings.

The increased glucose levels, 5–15 min postinjection in the HLA identical siblings, compared with controls, would suggest that the HLA identical siblings are slightly insulin-resistant. The subsequent normal glucose disposal (kg) with higher insulin levels than in controls would also be compatible with the concept of insulin resistance, but fasting insulin levels as another measure of insulin resistance were not different in $S_{2H}$ and controls. Difference in volume of distribution of the injected glucose is not a likely explanation for the higher glucose levels in $S_{2H}$ since the early glucose levels (2′, 3′, 4′) are not different in the two groups. Also, the controls and siblings were carefully matched and if anything, the $S_{2H}$ were slightly larger than the controls (71.0 versus 70.6 kg, respectively) and therefore a smaller glucose space in $S_{2H}$ would not be likely on this basis.

It is unlikely that the exaggerated acute insulin response to glucose was merely due to the higher glucose levels in the HLA identical siblings. $S_{2H}$ were hyperinsulinemic at 2, 3, and 4 min, times when the glucose levels were equivalent in the two groups and calculating the insulinogenic index as a correction for the increased glucose levels (IRI/glucose) did not diminish the difference between $S_{2H}$ and controls. Also, 10–20 g of glucose intravenously is a maximal stimulating dose for first phase insulin release. Larger doses of glucose, although resulting in higher glucose levels, do not normally release more first phase insulin.[31] The glucose levels achieved in our controls were equivalent to those reported to be a maximal stimulus.[31] We feel that slight insulin resistance and resultant increased sensitivity of the beta-cells in $S_{2H}$ is the most likely explanation for our findings.

Since substantially less than 50% of the HLA identical siblings will develop overt diabetes, it is not appropriate to consider the hyperinsulinemia that we observed in all these individuals to be a manifestation of mild, subclinical diabetes or "prediabetes." Rather, it is more likely to be a manifestation of a genetically determined metabolic state that is associated with an increased risk of IDD. We would like to hypothesize that increased beta-cell activity predisposes these cells to damage by environmental agents or other factors. Several laboratory observations are compatible with this hypothesis. In BL/K mice with the db mutant gene, hyperinsulinemia occurs before the onset of beta-cell atrophy, insulinopenia, and insulin-dependent diabetes.[32] Ventromedial hypothalamic destruction results in increased beta-cell secretion and is associated with increased beta-cell sensitivity to destruction by streptozotocin.[33] Similarly, mice treated with gold thioglucose or glucocorticoids have increased susceptibility to EMC virus-induced beta-cell damage and diabetes.[34,35] Both these treatments are known to increase insulin secretion. Very recently, Craighead and colleagues reported that susceptibility to EMC virus-induced beta-cell injury is increased in mice carrying the ob gene and have suggested that this increased susceptibility was due to increased beta-cell metabolic activity.[36] This hypothesis may also explain certain epidemiologic findings in humans. Viral infections may in some cases directly cause beta-cell destruction and IDD, but the increased insulin demands that commonly occur during infection may predispose the beta-cells to injury from many causes. This could explain the fall and winter seasonal incidence pattern of insulin-dependent diabetes,[37,38] which does not coincide with the incidence pattern of any one virus likely to be directly diabetogenic. Finally, there may be markedly increased insulin demands at puberty which could account for the peak incidence of insulin-dependent diabetes at this time.[39]

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## REFERENCES


[1] Gorwitz, K., Howan, G. G., and Thompson, T.: Prevalence of diabetes in Michigan school-age children. Diabetes *25*:122–27, 1976.

[2] Rotter, J. I., and Rimoin, D. L.: The genetics of the glucose intolerance disorders. Am. J. Med. *70*:116–26, 1981.

[3] Tattersall, R. B., and Pyke, D. A.: Diabetes in identical twins. Lancet *2*:1120–25, 1972.

[4] Cudworth, A. G., Gorsuch, A. N., Wolf, E., and Festenstein, H.: A new look at HLA genetics with particular reference to type-1 diabetes. Lancet *2*:389–90, 1979.

[5] Barbosa, J., Chavers, B., Steffes, M., Szalapski, E., Cohen, R. A., et al.: Muscle extracellular membrane immunofluorescence and HLA as possible markers of prediabetes. Lancet *2*:330–33, 1980.

[6] Serrano-Rios, M., Ramos, F., Rodriguez-minon, J. L., and Vivanco, F.: Studies in prediabetes: insulin response to oral glucose, intravenous tolbutamide and rapid intravenous glucose infusion in genetic prediabetes. Diabetologia *6*:392–98, 1970.

[7] Colwell, J. A., and Lein, A.: Diminished insulin response to hyperglycemia in prediabetes and diabetes. Diabetes *16*:560–65, 1967.

[8] Soeldner, J. S., Gleason, R. E., Williams, R. F., Garcia, M. J., Beardwood, D. M., and Marble, A.: Diminished serum insulin response to glucose in genetic prediabetic males with normal glucose tolerance. Diabetes *17*:17–26, 1968.

[9] Pyke, D. A., Cassar, J., Todd, J., and Taylor, K. W.: Glucose tolerance and serum insulin in identical twins in diabetics. Br. Med. J. *4*:649–51, 1970.

[10] Soeldner, J. S., Snoksen, P. H., and Gleason, R. E.: Early Diabetes. New York, *Academic Press*, 1970, p. 297.

[11] Cerasi, E., and Luft, R.: Insulin response to glucose infusion in diabetic and nondiabetic monozygotic twin pairs. Genetic control of insulin responses? Acta Endocrinol. *55*:330–45, 1967.

[12] Gottlieb, M. S., Soeldner, J. S., Kyner, J. L., and Gleason, R. E.: Oral glucose-stimulated release in non-diabetic twin siblings of diabetic twins. Diabetes *23*:684–92, 1974.

[13] Orchard, T. J., Rabin, B. S., Wagener, D. K., Salas, M., Banks, M., and Drash, A.: Metabolic features associated with specific HLA types (B8, B15, B18) and sharing of haplotypes with a diabetic sibling. Diabetes *29*:83A, 1980.

[14] Akerblom, H. K., Ilonen, J., Mustonen, T., Kiovukangas, T., Herva, E., Tiilikainen, A., Kouvalainen, K., and Lautala, P.: The use of HLA identity in the search for predisposition to juvenile-onset, insulin-dependent diabetes mellitus. Excerpta Medica *481*:5–6, 1979.

[15] Johansen, K., Soeldner, J. S., Gleason, R. E., Gottlieb, M. S., Park, B. N., Kaufmann, R. L., and Tan, M. H.: Serum insulin and growth hormone response patterns in monozygotic twin siblings of patients with juvenile-onset diabetes. N. Engl. J. Med. *293*:57–61, 1975.

[16] Tan, M. H., Williams, R. F., Soeldner, J. S., and Gleason, R. E.: Serum insulin response to slow-rise glucose infusion in "genetic prediabetics" (offspring of two diabetic parents). Diabetes *26*:490–99, 1977.

[17] Jackson, W. P. U., van Mieghem, W., and Keller, P.: Insulin excess as the initial lesion in diabetes. Lancet *1*:1040–43, 1972.

[18] Rojas, L., Soeldner, J. S., Gleason, R. E., Kahn, C. B., and Marble, A.: Offspring of two diabetic parents: differential serum insulin responses to intravenous glucose and tolbutamide. J. Clin. Endocrinol. Metab. *29*:1569–79, 1969.

[19] Ginsburg-Fellner, F., Dobersen, M., Witt, M., Notkins, A., Rubinstein, P., and Rayfield, E. J.: HLA antigens, islet cell antibodies and carbohydrate metabolism in siblings of children with insulin-dependent diabetes mellitus. Diabetes *28* (Suppl.):396, 1979. Abstract.

[20] Ganda, O. P., Soeldner, J. S., Gleason, R. E., Smith, T. M., Kilo, C., and Williamson, J. R.: Monozygotic triplets with discordance for diabetes mellitus and diabetic microangiopathy. Diabetes *26*:469–79, 1977.

[21] Rotter, J. I., and Rimion, D. L.: Heterogeneity in Diabetes Mellitus—Update, 1978. Evidence for further genetic heterogeneity within juvenile-onset insulin-dependent diabetes mellitus. Diabetes *27*:599–605, 1978.

[22] Morgan, C. R., and Lazarow, A.: Immunoassay of insulin: two antibody systems. Diabetes *12*:115–26, 1963.

[23] NIH lymphocyte microcytotoxicity technique. *In* NIAID Manual of Tissue Typing Techniques 1979-1980. U.S. DHEW, NIH Publication no. 80-545:39-41. November 1979.

[24] Snedecor, G. W., and Cochran, W. G.: Statistical Methods. Ames, Iowa State University Press, 1967.

[25] Hollander, M., and Wolfe, D. A.: Nonparametric Statistical Methods. New York, John Wiley & Sons, 1973.

[26] Maugh, T. H., II: Virus isolated from juvenile diabetic. Science *204*:1187, 1979.

[27] Barbosa, J., Chern, M., Anderson, V. E., Noreen, H., Johnson, S., et al.: Linkage analysis between the major histocompatibility system and insulin-dependent diabetes in families with patients in two consecutive generations. J. Clin. Invest. *65*:592–01, 1980.

[28] Barbosa, J., Chern, M., Noreen, H., Anderson, V. E., and Yunis, E. J.: Analysis of linkage between the major histocompatibility system and juvenile, insulin-dependent diabetes in multiplex families. J. Clin. Invest. *62*:492–95, 1978.

[29] Barbosa, J., King, R., Noreen, H., and Yunis, E. J.: The histocompatibility system in juvenile, insulin-dependent diabetic multiplex kindreds. J. Clin. Invest. *60*:989–98, 1977.

[30] Landgraf, R., Landgraf-Leurs, M. M. C., Lander, T., Scholz, S., Kuntz, B., and Albert, E. D.: HLA haplotypes and glucose tolerance in families of patients with juvenile-onset diabetes mellitus. Lancet *2*:1084–85, 1976.

[31] Lerner, R. L., and Porte, D., Jr.: Relationships between intravenous glucose loads, insulin responses and glucose disappearance rate. J. Clin. Endocrinol. Metab. *33*:409–17, 1971.

[32] Coleman, D. L.: Obesity and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. Diabetologia *14*:141–48, 1978.

[33] West, D. B., Seino, Y., Woods, S. C., and Porte, D., Jr.: Ventromedial hypothalamic lesions increase pancreatic sensitivity to streptozotocin in rats. Diabetes *29*:948–51, 1980.

[34] Craighead, J. E.: The role of viruses in the pathogenesis of pancreatic disease and diabetes mellitus. Prog. Med. Virol. *19*:161–214, 1975.

[35] Craighead, J. E.: Viral diabetes mellitus in man and experimental animals. Am. J. Med. *70*:127–34, 1981.

[36] D'Andrea, B. J., Wilson, G. L., and Craighead, J. E.: Effect of genetic obesity in mice on the induction of diabetes by encephalomyocarditis virus. Diabetes *30*:451–54, 1981.

[37] MacMillan, D. R., Kotoyan, M., Zeidner, D., and Hafezi, B.: Seasonal variation in the onset of diabetes in children. Pediatrics *59*:113–15, 1977.

[38] Christau, B., Kromann, H., Andersen, O. O., Christy, M., Buschard, K., et al.: Incidence, seasonal and geographical patterns of juvenile-onset insulin-dependent diabetes mellitus in Denmark. Diabetologia *13*:281–84, 1977.

[39] Christau, B., Kromann, H., Christy, M., Andersen, O. O., and Nerup, J.: Incidence of insulin-dependent diabetes mellitus (0–29 years at onset) in Denmark. Acta Med. Scand. *624*:54–60, 1979.