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Impaired insulin secretion in non-diabetic offspring of probands with latent autoimmune diabetes mellitus in adults

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Abstract

Aims/hypothesis. This study was undertaken to investigate metabolic and genetic characteristics of latent autoimmune diabetes in adults (LADA).
Methods. We evaluated insulin secretory capacity with oral and intravenous glucose tolerance tests (early insulin response) and hyperglycaemic clamp (insulin secretory capacity) and the rates of whole body glucose uptake with the euglycaemic clamp, HLA-*DQB1* genotypes (time-resolved fluorescence) and islet cell antibodies (ICA) (immunofluoresence) and antibodies to glutamic acid decarboxylase (GAD) (radio-immunoprecipitation) in 36 non-diabetic offspring (LADA-offspring) of patients with Type II (non-insulin-dependent) diabetes mellitus who tested positive for ICA and/or GAD antibodies during the 10-year follow-up from the diagnosis and in 19 healthy control subjects without a family history of diabetes.
Results. The early insulin response during the first 10 min of an intravenous glucose tolerance test was about 40 % lower in the LADA-offspring than in the control group ($p = 0.008$). Insulin secretory capacity in the hyperglycaemic clamp was also about 30 % lower in the LADA-offspring ($p = 0.048$). The rates of whole body glucose uptake were similar in both groups. The frequency of low risk HLA-*DQB1* genotypes was higher in the LADA-offspring than among Finnish healthy blood donors ($p = 0.033$). The risk conferring genotypes were associated with the lowest tertile of insulin secretory capacity in the LADA-offspring ($p = 0.032$). There were no associations between the autoantibodies and early insulin response or insulin secretory capacity within the study groups.
Conclusion/interpretation. We conclude that LADA is a familial disease involving most likely gene defects leading to a slow progressive beta-cell destruction and insulin deficiency. [Diabetologia (2000) 43: 69–78]

Keywords Islet cell antibodies, glutamic acid decarboxylase antibodies, HLA-*DQB1*, relatives of Type II diabetic patients, insulin sensitivity.

Type II (non-insulin-dependent) diabetes mellitus is a genetically determined disease with clinical onset usually after the age of 40 years [1–2]. It is a heterogeneous disorder representing at least two major phenotypes: insulin resistant and insulin deficient phenotypes [1, 3–4]. Moreover, about 10 % of patients initially diagnosed as having Type II diabetes have detectable serological autoimmune markers of beta-cell destruction [5–20]. These subjects have a latent autoimmune diabetes in adults (LADA).

There is no precise definition of LADA. Recently LADA has been suggested to be defined as patients with diabetes diagnosis after the age of 35 years, who do not need insulin treatment during the first 6 months after the diagnosis and who have antibodies to glutamic acid decarboxylase (GAD)

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Abbreviations: LADA, Latent autoimmune diabetes in adults; ICA, islet cell antibodies; GAD, glutamic acid decarboxylase; LADA-offspring, offspring of the patients with latent autoimmune diabetes in adults; TFM, total fat mass; LBM, lean body mass; VO_{2max}, maximum attainable oxygen uptake.

and/or to islet cell antigens (ICA) [18]. These autoimmune markers predict impaired insulin secretion and a requirement of exogenous insulin [6–20]. On the other hand, not all patients with LADA develop insulin deficiency [7, 9, 11–12, 14, 16–17, 19–20] and furthermore, autoantibodies are not always persistent, but can be of a fluctuating or evanescent nature [16, 19, 20]. Patients with LADA also have an increased frequency of HLA-*DR3* and *DR4* alleles [10, 14] as well as HLA-*DQB1* genotypes [13, 18] predisposing to Type I (insulin-dependent) diabetes mellitus.

This study was undertaken to investigate metabolic and genetic characteristics of LADA. With this aim, we did detailed metabolic studies and determined HLA-*DQB1* genotypes in non-diabetic offspring of LADA probands and control subjects.

Methods

Subjects. The subjects for the study were offspring of the patients with newly diagnosed Type II diabetes who were originally studied in 1979–1981. The formation and representativeness of the study population have been described earlier in detail [21–22]. We have followed these patients for more than 10 years and done repeated oral glucose tolerance tests (baseline, 5 years, 10 years). Originally, the study population consisted of 133 patients with newly diagnosed Type II diabetes, aged 45–64 years and 144 non-diabetic control subjects of the same age group. The probands for the present study were Type II diabetic patients who tested positive for ICA [11] or GAD antibodies [16] or both during the follow-up. Exclusion criteria for the selection of the offspring were: (1) diabetes mellitus in both parents or in the offspring, (2) drug treatment or any disease that could potentially modify carbohydrate metabolism, (3) pregnancy, (4) overt psychiatric disease and (5) age under 30 or over 55 years.

Offspring of the probands with GAD antibodies or ICA positivity or both (LADA-offspring). There were 12 probands who tested positive for GAD antibodies at the baseline examination [16] and 15 probands with ICA positivity at the baseline or at the 5-year follow-up examination or both [11]. Three subjects were positive for both GAD antibodies and ICA. Among these 24 subjects 9 had no children. The remaining 15 subjects had altogether 54 offspring who met the inclusion criteria and were invited to take part in the study. Of the offspring 18 did not reply or were unwilling to participate (participation rate: 67 %). Accordingly, the LADA-offspring consisted of 36 subjects (20 men and 16 women) who represented the offspring of 12 probands. Of the subjects 13 had a parent who tested positive for GAD antibodies (5 probands), 14 subjects a parent who tested positive for ICA (4 probands) and 9 subjects had a parent who tested positive for ICA and GAD antibodies (3 probands). From each family 2 to 5 offspring were included. The probands for this study were treated at the baseline examination with diet only and none required insulin during the first 6 months after the diagnosis. The mean age of the probands at the baseline examination was 55.7 years and their mean BMI was 27.2 kg/m^2. At the 5-year follow-up examination 3 (2 probands positive for ICA and GAD antibodies and 1 proband positive for ICA) of the 12 probands were treated with insulin.

Control group. The control group was recruited from the same follow-up study [21–22]. Originally the control group consisted of 144 subjects among whom 53 had repeatedly normal glucose tolerance according to the World Health Organisation criteria [23] determined by glucose tolerance test (OGTT) (baseline, 5 years, 10 years). Among these 53 subjects 2 had low GAD antibody levels, 9 had no offspring and 10 had a diabetic spouse. From the remaining 32 subjects available 16 were randomly selected to serve as probands. These 16 probands had 37 children to whom a questionnaire was sent including questions about drug treatment, chronic diseases and family history of diabetes and hypertension. The control subjects had to fulfill the following inclusion criteria: (1) age from 30 to 55 years, (2) no diabetes, (3) first-degree relatives without a history of diabetes, (4) no drug treatment or any disease that could potentially modify carbohydrate metabolism and (5) no history of hypertension. Eventually, the control group comprised 19 offspring (10 men and 9 women) of 12 probands. From each family 1 to 3 subjects were examined. The mean age of the probands at the baseline examination was 54.9 year and their mean BMI was 26.2 kg/m^2.

Study protocol. The subjects were admitted to the metabolic ward of the Department of Medicine, Kuopio University Hospital for 2 days. On the first day the bioelectric impedance measurement was done after 12-h fasting followed by an OGTT and the hyperglycaemic clamp, respectively. On the second day an intravenous glucose tolerance test (IVGTT) followed by the hyperinsulinaemic euglycaemic glucose clamp was carried out. The validation of measuring insulin sensitivity by the hyperinsulinaemic euglycaemic clamp after an IVGTT has been recently reported elsewhere [24]. Within 1 month after these examinations a computed tomography of the abdominal fat and a cardiopulmonary exercise test were carried out.

The protocol was approved by the ethics committee of the Kuopio University Hospital and University of Kuopio. Informed consent was given by all the subjects studied.

Oral glucose tolerance test. In a 2-hour OGTT (75 g of glucose) samples for blood glucose, plasma insulin and plasma C peptide were drawn at 0, 30, 60, 90 and 120 min to evaluate the degree of glucose tolerance and the beta-cell response to the oral glucose load.

Hyperglycaemic clamp. At 120 min immediately after the 2-h oral glucose tolerance test blood glucose was rapidly increased to 20 mmol/l by a constant glucose infusion and clamped at 20 mmol/l until 180 min by infusing 20 % glucose at varying rates according to blood glucose measurements done at 5 min intervals applying the hyperglycaemic clamp technique. At 150, 165 and 180 min samples were taken for the measurement of plasma insulin and C peptide. In the present study we use the term "insulin secretory capacity" for the plasma insulin response measured during the last 30 min of the hyperglycaemic clamp although it is possible that insulin concentrations would have been even higher if glucose concentrations had been clamped at a concentration exceeding 20 mmol/l.

Intravenous glucose tolerance test (IVGTT). An IVGTT was done to determine the early insulin secretory capacity. At 0800 hours after a 12-h overnight fast, an intravenous catheter was placed in the antecubital vein for the infusion of glucose. Another cannula for blood sampling was inserted into a wrist vein surrounded by a heated box (40 °C). After baseline blood collection and measurement of gas exchange (see "Indirect Calorimetry"), a bolus of glucose (300 mg/kg in a 50 % solution) was given (within 30 s) into the antecubital vein to rapidly

increase the blood glucose concentration. Samples for the measurement of blood glucose and plasma insulin were drawn at –5, 0, 2, 4, 6, 8 and 10 min.

Euglycaemic clamp. The degree of insulin resistance was evaluated with the euglycaemic hyperinsulinaemic clamp technique [25]. After IVGTT, a priming dose of insulin infusion (Actrapid 100 U/ml, Novo Nordisk, Gentofte, Denmark) was given during the initial 10 min to rapidly raise plasma insulin to the desired concentration, where it was maintained by a continuous insulin infusion at a rate of 80 $mU \cdot m^2$ body surface $area^{-1} \cdot min^{-1}$. Blood glucose was clamped at 5.0 mmol/l for the next 180 min by infusing 20 % glucose at varying rates according to blood glucose measurements done at 5-min intervals (mean coefficient of variation of blood glucose was < 4 % in both groups). The data were calculated for each 20-min interval; the mean value for the period from 120 to 180 min was used to calculate the rates of whole body glucose uptake. Samples for the measurement of plasma lactate, insulin and serum free fatty acids were drawn in the fasting and at 120, 140, 160 and 180 min.

Indirect calorimetry. Indirect calorimetry was carried out with a computerized flow-through canopy gas analyser system (Deltatrac, TM Datex, Helsinki, Finland) as described previously [26] in the fasting state and in connection with the euglycaemic clamp. Consumption of O_2 and CO_2 production were used for the calculation of glucose and lipid oxidation [27]. Protein oxidation was calculated on the basis of the urinary non-protein nitrogen excretion rate [27]. The fraction of carbohydrate non-oxidation during the euglycaemic clamp was estimated by subtracting the carbohydrate oxidation rate (determined by indirect calorimetry) from the glucose infusion rate (determined by the euglycaemic clamp).

Body fat composition and fat distribution. Body composition was determined by bioelectrical impedance (RJL Systems, Detroit, Mich., USA) in the supine position after a 12-h fast [28].

Abdominal fat distribution was evaluated by computed tomography (Somatom Plus S, Siemens, Germany) according to the method described previously [29]. Briefly, the scanning was done with 120 kV and the slide thickness was 10 mm. The fourth lumbal vertebra (L4) was mapped with a radiograph of the vertebral spine and one scan from that level was obtained. Subcutaneous and intra-abdominal fat areas were calculated as described previously [4, 29–30]. The computed tomography examination was done on 32 LADA-offspring and on 17 control subjects.

Cardiopulmonary exercise test. The cardiopulmonary exercise test was done with a bicycle ergometer (Siemens Elema 380, Solna, Sweden) in the upright position until exhaustion [31]. The initial workload was 20 W with subsequent increments of 20 W/min. During the test a 12-lead ECG, blood pressure, heart rate, subjective symptoms and perceived exertion were recorded. Respiratory gas exchange was analysed continuously during the exercise test with a computer based system (Sensor Medics 2900, Metabolic Measurement Cart/System, Yorba Linda, Calif., USA). The average values of oxygen uptake measured during the last 30 s of exercise were used for maximum attainable oxygen uptake (VO_{2max}). The cardiopulmonary exercise test was carried out on 32 LADA-offspring and on 17 control subjects.

Assays and calculations. Blood glucose and plasma lactate in the fasting state, during clamp studies and blood glucose during the oral glucose tolerance test were measured by glucose and lactate oxidase methods, respectively (Glucose & Lactate Analyzer 2300 Stat Plus, Yellow Springs Instrument Co., Ohio, USA). For the determination of plasma insulin and C peptide, blood was collected into EDTA tubes. After centrifugation, the plasma for the determination of insulin and C peptide was stored at –20 °C until the analysis. Plasma insulin and C peptide were determined by radioimmunoassay (Phadeseph Insulin RIA 100, Pharmacia Diagnostics AB, Uppsala, Sweden and C peptide of insulin by 125 JRIA kit, Incstar Co.; Stillwater, Minn., USA). Insulin assay also detects proinsulin and proinsulin conversion products with a cross-reactivity of 47 %. Serum free fatty acids were determined by an enzymatic method from Wako Chemicals (Neuss, Germany). Non-protein urinary nitrogen was measured by an automated Kjeldahl method [32].

The incremental insulin and glucose areas under the curve were calculated by the trapezoidal method. The insulin and C peptide secretions during the hyperglycaemic clamp study were calculated as mean insulin and C-peptide values measured at 150, 165 and 180 min. Plasma C peptide/plasma insulin ratio was calculated as an index of the hepatic insulin extraction in the fasting state and during the hyperglycaemic clamp (mean value of three measurements during the last 30 min of the clamp).

Autoantibody tests. The ICA status of the probands was determined based on ICA analyses in the Research Laboratory, Department of Paediatrics, University of Oulu in 1990, and the ICA status of the offspring was assessed based on ICA testing in the same laboratory in 1997. The ICA assay was done as described previously [33]. The results were expressed in Juvenile Diabetes Foundation units (JDF units) based on a standard curve. The cut-off value for ICA was 5 JDF units. The substrate used in 1997 gave a standard curve identical to that obtained with the substrate used in 1990. The laboratory has participated in the international workshops on the standardisation of the ICA assay with a sensitivity of 100 %, a specificity of 98 % and a validity of 98 % in the most recent round.

Glutamic acid decarboxylase antibody tests for evaluating the GAD antibody status of the probands were performed with a radio-immunoprecipitation assay [34] in serum taken at the baseline examination between years 1979–1981. This method has been described previously in more detail [16]. GAD antibody tests for evaluating the GAD antibody status of the offspring in the present study were measured by a radio-immunoprecipitation method using ^{35}S-labelled recombinant human GAD65 produced by in vitro transcription/translation [35–36]. The *E. coli* clone with full-length human GAD_{65} complementary DNA was a kind gift from Dr. A.E. Karlsen and C.E. Grubin, University of Washington (Seattle, Wash., USA). The results are expressed as relative units, RU = (sample cpm – mean cpm of three negative controls) / (cpm of a positive internal reference serum – mean cpm of three negative controls) × 100. Antibody levels exceeding 5 RU which represents mean + 3 SD of 296 Finnish healthy control subjects were considered positive. In the Combined Autoantibody Workshop (Orvieto; Italy, 1995), the specificity of the assay was 99 %, and the sensitivity 75 %.

HLA-DQB1 genotyping. HLA-*DQB1* typing was done by a previously described method based on time-resolved fluoresence [37–38]. We used four sequence-specific oligonucleotide probes to identify the following *DQB1* alleles known to be significantly associated with either susceptibility or protection against Type I diabetes in the Finnish population: DQB1*0302, DQB1*02, DQB1*0602 or 0603 (DQB1*0602–3) and DQB1*0301 [39]. The recently described simplified classification of *DQB1* genotypes into high-risk (DQB1*02/0302), mod-

Table 1. Clinical and Biochemical Characteristics of the Study Groups

	Control (n = 19)	LADA-offspring (n = 36)	p value
Age (years)	41.2 ± 1.4	42.9 ± 0.9	NS
Sex (men/women)	10/9	20/16	NS
Body mass index (kg/m^2)	25.8 ± 0.9	25.4 ± 0.5	NS
Total fat mass (kg)	18.4 ± 2.1	20.2 ± 1.2	NS
Lean body mass (kg)	54.1 ± 2.6	57.1 ± 2.3	NS
Fasting blood glucose (mmol/l)	4.6 ± 0.1	4.8 ± 0.1	NS
Fasting insulin (pmol/l)	55.1 ± 4.4	49.0 ± 3.3	NS
Fasting C peptide (pmol/l)	513 ± 36	568 ± 35	NS
VO_{2max} (ml · LBM^{-1} · min^{-1})	47.6 ± 2.3	46.5 ± 1.3	NS
Subcutaneous fat area (cm^2)	215 ± 29	233 ± 21	NS
Intra-abdominal fat area (cm^2)	96 ± 18	100 ± 11	NS

The results are given as means ± SEM

erate-risk (DQB1*0302/x; x indicates 0302 or a non-defined allele), low-risk (DQB1*0301/0302, DQB1*02/0301, DQB1*02/x, DQB1*0302/0602–3; x indicates 02 or a non-defined allele) and decreased-risk (protection) (DQB1*x/x, DQB1*0301/x, DQB1*02/0602–3, DQB1*0301/0602–3, DQB1*0602–3/x, x indicates a non-defined allele) genotypes was used [39].

Statistical analysis. All calculations were done with the SPSS for Windows program (SPSS, Chicago, Ill., USA). Data are shown as mean ± SEM. The differences between the two groups were analysed by the Student's t test for unpaired samples or by the chi-squared test when appropriate. In the subgroup analyses the differences among the groups were tested by the analysis of one-way variance (ANOVA) for continuous variables and by Mantel-Haenszel's test for dichotomized variables. Only when the p value was significant, less than 0.05 were the two groups compared by Student's t test for unpaired samples or by the chi-squared test. Plasma insulin and C peptide concentrations were analysed after logarithmic transformation.

Results

Clinical and biochemical characteristics of the study groups. Table 1 shows the clinical and biochemical characteristics of the study groups. The groups were similar in age and sex. Furthermore, there were no significant differences in body mass index (BMI), total fat mass (TFM), lean body mass (LBM), fasting blood glucose, fasting plasma insulin, fasting C peptide, VO_{2max}, abdominal subcutaneous fat area or intra-abdominal fat area between the two groups.

In the sub-group analyses there were no significant differences in age, BMI, TFM or in the rates of whole body glucose uptake between the offspring of ICA positive and the offspring of GAD antibody positive probands and the offspring of ICA and GAD antibody positive probands and the control group (ANOVA; p = NS for all comparisons).

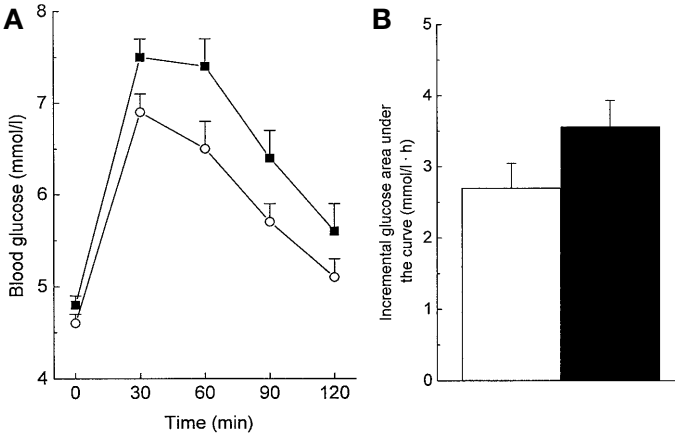


Fig. 1. A. Blood glucose concentrations during the oral glucose tolerance test: the control group (○); the LADA-offspring (■). **B.** The incremental blood glucose area under the curve; the control group (□); the LADA-offspring (■)

Glucose tolerance. Figure 1 depicts the blood glucose response in the OGTT. The blood glucose concentrations tended to be higher in the LADA-offspring compared with those in the control group at all time points measured after the oral glucose load (30 min: 6.9 ± 0.2 vs 7.6 ± 0.2 mmol/l, p = 0.100; 60 min: 6.5 ± 0.3 vs 7.4 ± 0.3 mmol/l, p = 0.118; 90 min: 5.6 ± 0.2 vs 6.4 ± 0.3 mmol/l, p = 0.110; 120 min: 5.1 ± 0.2 vs 5.6 ± 0.2 mmol/l, p = 0.190; the control group and the LADA-offspring, respectively). Oral glucose response, when expressed as an incremental glucose area under the curve tended to be higher in the LADA-offspring (3.56 ± 0.37 mmol/l · h) compared with that in the control group (2.70 ± 0.35 mmol/l · h; p = 0.139).

Figure 2 shows the plasma insulin response in the OGTT. There were no significant differences in plasma insulin concentrations at any time point (0 min: 55.8 ± 4.6 vs 55.0 ± 4.5 pmol/l; 30 min: 283.2 ± 27 vs 256.8 ± 18.3 pmol/l; 60 min: 275.4 ± 31.8 vs 260.4 ± 19.2 pmol/l; 90 min: 211.2 ± 19.8 vs 226.8 ± 15.6 pmol/l; 120 min: 172.8 ± 22.8 vs 181.8 ± 16.2 pmol/l; the control group and the LADA-offspring, respectively). Plasma insulin response expressed as an incremental insulin area under the curve (IIAUC) was also similar in the two groups (the control group: 330.6 ± 35.4 pmol/l · h; the LADA-offspring: 321.6 ± 21.6 pmol/l · h). The plasma C-peptide response in the OGTT was of the same magnitude in both study groups (0 min: 513 ± 36 vs 568 ± 35 pmol/l; 30 min: 1650 ± 101 vs 1694 ± 112 pmol/l; 60 min: 1948 ± 141 vs 2065 ± 111 pmol/l; 90 min: 1823 ± 114 vs 2029 ± 104 pmol/l; 120 min: 1642 ± 109 vs 1817 ± 113 pmol/l; the control group and the LADA-offspring, respectively). Likewise, the incremental C-peptide area under the curve was similar between the study groups (the control group: 2222 ± 150 pmol/l · h; the LADA-offspring: 2353 ± 144 pmol/l) There was no

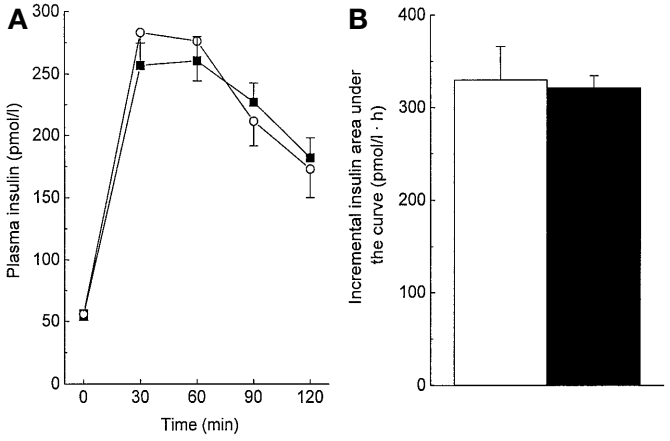


Fig. 2. A. Plasma insulin concentrations during the oral glucose tolerance test: the control group (○); the LADA-offspring (■). **B.** The incremental plasma insulin area under the curve; the control group (□); the LADA-offspring (■)

significant difference in hepatic insulin extraction (pmol C peptide/pmol insulin) in the fasting state (the control group: 9.87 ± 0.62; the LADA-offspring: 10.79 ± 0.35).

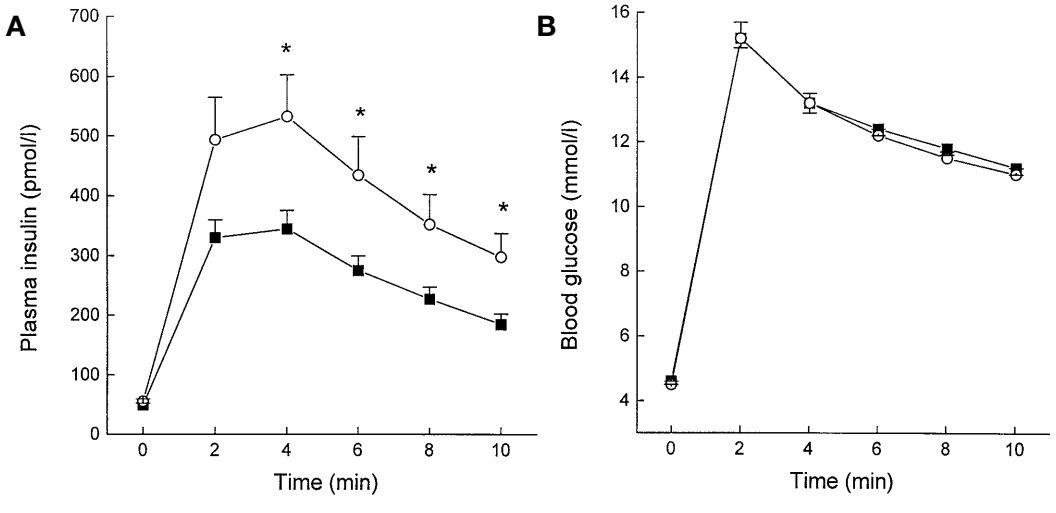
Insulin secretion

Early insulin response. Figure 3 shows the early plasma insulin response in the IVGTT. Fasting plasma insulin concentrations were similar in the study groups (the control group: 55.1 ± 4.4 pmol/l; the LADA-offspring: 49.0 ± 3.3 pmol/l). Plasma insulin concentrations were lower in the LADA-offspring compared with those in the control group at all time points measured after the intravenous glucose load (2 min: 493.8 ± 71.4 vs 330 ± 30.4 pmol/l, $p = 0.028$; 4 min: 532.8 ± 69.6 vs 345.0 ± 30.6 pmol/l, $p = 0.016$; 6 min: 433.8 ± 65.4 vs 274.8 ± 24.6 pmol/l, $p = 0.024$; 8 min: 352.2 ± 49.8 vs 226.8 ± 20.4 pmol/l, $p = 0.023$; 10 min: 297.6 ± 40.2 vs 184, 8 ± 17.4 pmol/l, $p = 0.016$; the control group and the LADA-offspring, respectively). The early insulin response during the first 10 min of an intravenous glucose tolerance test was about 38 % lower in the LADA-offspring than in the control group (incremental plasma insulin area under the curve: 3428 ± 502 vs 2097 ± 206 pmol/l · min for the control group and for the LADA-offspring, respectively; $p = 0.008$). The glucose responses in the IVGTT were similar between the study groups (Fig. 3). The incremental glucose area under the curve was similar in both study groups (74.8 ± 2.4 mmol/l · min for the control group, and 75.2 ± 1.8 mmol/l · min for the LADA-offspring). The early insulin response divided by the early glucose response was lower in the LADA-offspring (28.1 ± 2.7 pmol insulin/mmol glucose) than in the control group (44.4 ± 6.2 pmol insulin/mmol glucose, $p = 0.005$).

In the sub-group analyses the offspring of ICA positive probands had lower incremental plasma insulin area under the curve (1482 ± 318 pmol/l · min) than the control group ($p < 0.001$) and the offspring of GAD antibody positive probands (2616 ± 318 pmol/l · min, $p = 0.016$) (Fig. 4). Furthermore, the offspring of ICA and GAD antibody positive probands tended to have lower incremental plasma insulin area under the curve (2208 ± 378 pmol/l · min) than the control group ($p = 0.122$) (Fig. 4).

Insulin secretory capacity. Fig. 5 illustrates the plasma insulin and C-peptide secretory response in the hyperglycaemic clamp. The mean blood glucose concentration during the hyperglycaemic clamp from 160 to 180 min was similar in both groups: 21.2 ± 0.1 mmol/l in the control group and 21.4 ±

Fig. 3A, B. Plasma insulin concentrations **A** and the blood glucose concentrations **B** during the intravenous glucose tolerance test: the control group (○); the LADA-offspring (■). * $p < 0.05$



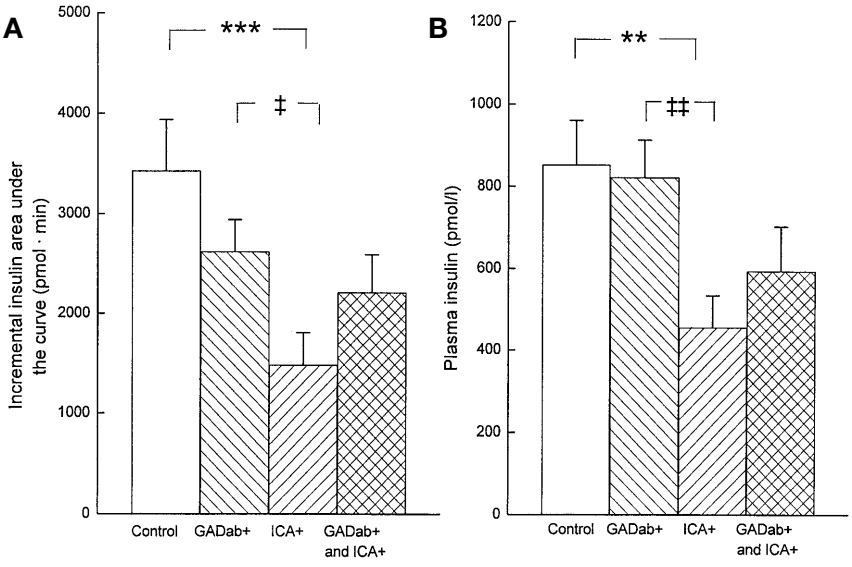


Fig. 4 A, B. The incremental plasma insulin area under the curve during the intravenous glucose tolerance test **A** and the mean plasma insulin concentrations during the hyperglycaemic clamp **B** in offspring with GAD antibody positive probands (GADab +), in offspring with ICA positive probands (ICA +) and in offspring with GAD antibody and ICA positive probands (GADab + and ICA +). *** $p < 0.001$ and ** $p < 0.01$ (ICA + vs control group); ‡ $p < 0.05$ and ‡‡ $p < 0.01$ (ICA + vs GADab +)

0.2 mmol/l in the LADA-offspring. The LADA-offspring had about 30 % lower plasma insulin secretory capacity (602.4 ± 58.2 pmol/l) than the control group (856.8 ± 109.2 pmol/l; $p = 0.048$). The plasma C-peptide secretory capacity, however, did not differ between the two groups (control group: 3858 ± 218 pmol/l, and LADA-offspring: 3587 ± 191 pmol/l). The hepatic insulin extraction was somewhat higher in the LADA-offspring (6.61 ± 0.34) than in the control group (5.57 ± 0.62) but the difference was not statistically significant ($p = 0.112$). The offspring of ICA positive probands had lower plasma insulin secretory capacity (459.0 ± 79.2 pmol/l) than the control group ($p = 0.005$) and the offspring of GAD antibody positive probands (826.8 ± 89.9 pmol/l, $p = 0.004$) (Fig. 4). Furthermore, the offspring of ICA and GAD antibody positive probands tended to have lower plasma insulin secretory capacity (594.6 ± 111.6 pmol/l) than the control group ($p = 0.121$) (Fig. 4). The offspring of ICA positive probands also had lower plasma C-peptide concentrations during the hyperglycaemic clamp (3004 ± 312 pmol/l) than the control group ($p = 0.027$) and the offspring of GAD antibody positive probands (4109 ± 258 pmol/l; $p = 0.012$). There were no significant differences in plasma C-peptide concentrations between the offspring of ICA and GAD antibody positive probands (3665 ± 374 pmol/l) and the control group ($p = 0.635$).

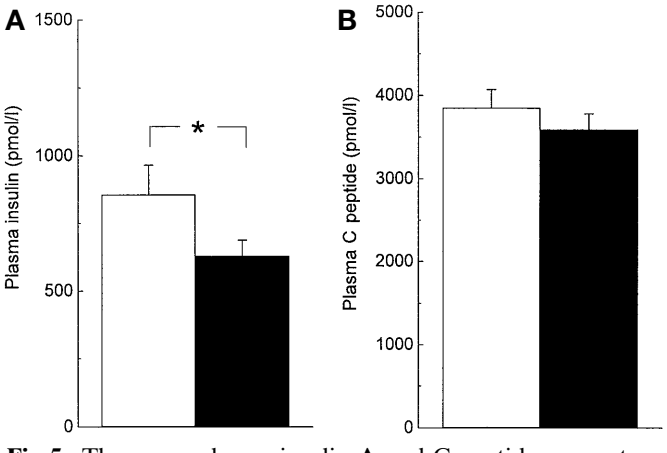


Fig. 5. The mean plasma insulin **A** and C-peptide concentrations **B** during the hyperglycaemic clamp: the control group (□); the LADA-offspring (■). * $p < 0.05$

Insulin sensitivity. The steady-state insulin concentrations during the euglycaemic clamp were similar in the study groups (the control group: 924.6 ± 43.8 pmol/l and the LADA-offspring: 921.6 ± 36.6 pmol/l). The rates of whole body glucose uptake did not differ between the groups (the control group: 83.7 ± 4.3 μmol · LBM^{-1} · min^{-1} and the LADA-offspring: 86.2 ± 6.2 μmol · LBM^{-1} · min^{-1}). Moreover, the rates of glucose non-oxidation (55.7 ± 3.3 μmol · LBM^{-1} · min^{-1} for the control group; 56.7 ± 4.7 μmol · LBM^{-1} · min^{-1} for the LADA-offspring) and glucose oxidation (28.0 ± 1.4 μmol · LBM^{-1} · min^{-1} for the control group; 29.5 ± 1.7 μmol · LBM^{-1} · min^{-1} for the LADA-offspring) were similar in the two groups. In the fasting state the rates of glucose oxidation did not differ between the groups (Table 2).

Energy expenditure, lipid oxidation, serum free fatty acid and plasma lactate concentrations. Energy ex-

Table 2. Glucose oxidation, lipid oxidation and energy expenditure and serum free fatty acid and plasma lactate concentrations in the fasting state and during the euglycaemic clamp

	Control	LADA-offspring	*p* value
Fasting:			
Glucose oxidation ($\mu mol \cdot LBM^{-1} \cdot min^{-1}$)	10.7 ± 1.1	12.1 ± 0.9	NS
Lipid oxidation ($mg \cdot LBM^{-1} \cdot min^{-1}$)	0.99 ± 0.10	0.97 ± 0.09	NS
Energy expenditure (kcal/LBM)	28.7 ± 0.8	29.6 ± 1.6	NS
Serum free fatty acids (mmol/l)	0.47 ± 0.04	0.45 ± 0.03	NS
Plasma lactate (mmol/l)	0.91 ± 0.07	0.97 ± 0.07	NS
Euglycaemic clamp:			
Glucose oxidation ($\mu mol \cdot LBM^{-1} \cdot min^{-1}$)	28.0 ± 1.4	29.5 ± 1.7	NS
Lipid oxidation ($mg \cdot LBM^{-1} \cdot min^{-1}$)	0.17 ± 0.05	0.15 ± 0.05	NS
Energy expenditure (kcal/LBM)	32.9 ± 1.0	34.2 ± 1.9	NS
Serum free fatty acids (mmol/l)	0.06 ± 0.01	0.05 ± 0.01	NS
Plasma lactate (mmol/l)	1.28 ± 0.06	1.32 ± 0.04	NS

The results are given as means ± SEM

Table 3. The frequencies of HLA-*DQB1* genotypes in relation to risk for Type I diabetes among the study groups

DQB1 -genotype	Control (*n* = 18)	LADA-offspring (*n* = 35)	LADA-offspring[a] (*n* = 12)
High risk, *n* (%):	0 (0)	1 (2.9)	0 (0)
02/0302	0 (0)	1 (2.9)	0 (0)
Moderate risk, *n* (%):	1 (5.6)	6 (17.1)	3 (25)
0302/x	1 (5.6)	6 (17.1)	3 (25)
Low risk, *n* (%):	2 (11.1)	16 (45.7)	6 (50)
0301/0302	1 (5.6)	0 (0)	0 (0)
02/0301	1 (5.6)	1 (2.9)	1 (8.3)
02/x	0 (0)	15 (42.9)	5 (41.7)
0302/0602-3	0 (0)	0 (0)	0 (0)
Decreased risk, *n* (%):	15 (83.3)	12 (34.3)	3 (25)
x/x	2 (11.1)	3 (8.6)	1 (8.3)
0301/x	0 (0)	2 (5.7)	0 (0)
02/0602-3	0 (0)	1 (2.9)	0 (0)
0301/0602-3	1 (5.6)	2 (5.7)	1 (8.3)
0602-3/x	12 (66.7)	4 (11.4)	1 (8.3)

x means either a homozygous allele or a non-defined allele. [a] Only the first offspring member examined from each family included in statistical analyses

penditure expressed as kcal per LBM as well as lipid oxidation expressed as mg of LBM per min in the fasting state and during the euglycaemic clamp were similar between the study groups. Moreover, serum free fatty acid concentrations and plasma lactate concentrations did not differ between the two groups in the fasting state and during the euglycaemic clamp (Table 2).

HLA-DQB1 genotypes and autoantibody status. Table 3 shows the distribution of HLA-*DQB1* genotypes among the study subjects according to associated risk for Type I diabetes in the Finnish population [39]. We also compared the frequencies of protective HLA-*DQB1* genotypes of the LADA-offspring to those determined in 756 healthy blood donors from different parts of Finland and 649 patients with juvenile-onset Type I diabetes participating in the Childhood Diabetes in Finland (DiMe) Study [39]. The frequency of low-risk HLA-*DQB1* genotypes was higher in the LADA-offspring (45.7%) than among the healthy blood donors and the patients with Type I diabetes (22.1%, $p < 0.001$ and 28.0%, $p = 0.025$, respectively). Because the number of family members differed among the LADA families we also determined the distribution of HLA-*DQB1* genotypes by including only one family member (the first offspring member studied) in statistical analyses. Also in this case the frequency of low-risk HLA-*DQB1* genotypes was higher in the LADA-offspring than among healthy blood donors ($p = 0.033$) (Table 3). The frequency of protective HLA-*DQB1* genotypes was lower in the LADA-offspring (34.3%) than in the healthy blood donors (63.6%, $p < 0.001$) but higher than in the young patients with Type I diabetes (10.0%, $p < 0.001$). Furthermore, the frequency of the high-risk HLA-*DQB1* genotype was lower in the LADA-offspring (2.9%) than in the children with Type I diabetes (24.5%, $p = 0.003$).

The risk conferring HLA-*DQB1* genotypes for Type I diabetes were related to the lowest tertile of insulin secretory capacity measured by the hyperglycaemic clamp in the LADA-offspring ($p = 0.032$). The frequency of risk conferring HLA-*DQB1* genotypes for Type I diabetes in the tertiles of insulin secretory capacity was 91.0% in the first tertile, 58.4% in the second tertile and 40.0% in the third tertile in the LADA-offspring. There was no association between the risk conferring HLA-*DQB1* genotypes and insulin secretory capacity in the control group.

Among the LADA-offspring six (16.7%) were positive for ICA or GAD antibodies or both (mean ICA level 19 ± 2, range 0–66 JDF and mean GAD antibody level 26.6 ± 3.8, range –5 to 123 RU) and among the control group three (15.8%) subjects were positive for ICA or GAD antibodies or both (mean ICA level 14 ± 1, range 0–18 JDF and mean GAD antibody level 3.6 ± 1.3, range –1.6 to 13.1 RU). There were no associations between the autoantibodies and early insulin response or insulin secretory capacity within the study groups.

Discussion

Our study found that non-diabetic offspring of probands with LADA had impaired early phase insulin secretion and lowered insulin secretory capacity but their insulin sensitivity was normal. Furthermore,

these subjects had an increased frequency of HLA-*DQB1*-genotypes conferring a low but still increased risk for Type I diabetes. These results suggest that the defects in insulin secretory capacity seen in LADA are familial and most likely genetically determined. Furthermore our findings confirm the conclusions of previous studies suggesting that LADA has genetic and metabolic features characteristic for Type I diabetes [6–20].

Previous studies on the offspring of Type II diabetic patients explaining the primary defects in glucose metabolism have given controversial results; some have suggested that insulin resistance is the primary cause of Type II diabetes [40–41] whereas others have emphasised the role of an insulin secretory defect [42–43]. One of the drawbacks of the previous studies has been the heterogeneity of Type II diabetes. We have recently shown, by studying offspring of ICA and GAD antibody negative Type II diabetic patients with deficient insulin secretion and insulin resistant phenotype, that defects in insulin secretion and insulin action are inherited and represent probably the primary defects in the pathogenesis of Type II diabetes [4]. In that study we reported impaired early insulin secretion response but normal insulin secretory capacity and insulin sensitivity in offspring of patients with deficient insulin secretion phenotype of Type II diabetes. This study examines metabolic characteristics of the offspring of patients who were initially clinically diagnosed as having Type II diabetes but who had antibodies to GAD or ICA or both. In this study the offspring of LADA probands had an even more pronounced defect in their early phase insulin secretion than the offspring of patients having insulin deficient but antibody negative phenotype of Type II diabetes [4], and in addition, they had reduced insulin secretory capacity in the hyperglycaemic clamp ($p = 0.048$ between the groups). Although plasma C peptide concentrations were somewhat lower in the offspring of LADA patients than in the control group during the hyperglycaemic clamp the difference was not statistically significant. Furthermore, the hepatic insulin extraction in the fasting state and during the hyperglycaemic clamp did not differ between the groups. These results indicate that insulin secretion is impaired in the offspring of LADA patients and that maximum insulin secretory capacity might be less affected than is the early insulin secretion.

There is only one study that has thoroughly examined metabolic characteristics of diabetic patients with LADA [44]. That study included LADA patients who had a slow progressive insulin secretory deficiency requiring insulin therapy after 2 years from the diagnosis of Type II diabetes [44]. In that study, LADA patients with a mean duration of diabetes of 10 years had lower rates of whole body glucose uptake than did the healthy control subjects. It is, however, not possible to investigate reliably early metabolic defects of glucose metabolism in subjects with diabetes and long-lasting hyperglycaemia. Studies on offspring of diabetic patients are more reliable in this respect. In this study non-diabetic offspring of LADA patients had similar rates of whole body glucose uptake (i.e. insulin sensitivity), body fat composition and distribution, maximum oxygen uptake, energy expenditure, lipid oxidation and glucose oxidation and non-oxidation than did the control subjects. These results indicate that the reduced insulin sensitivity previously reported in LADA patients is likely to be a secondary defect induced by glucotoxicity of long-lasting hyperglycaemia [45].

The most important genes that influence susceptibility to Type I diabetes are located within the HLA region on human chromosome 6. The number of HLA loci conferring susceptibility to Type I diabetes is not known but the HLA-*DQ* locus contains the strongest known risk markers for Type I diabetes [46]. In our study we assessed the HLA-*DQB1* genotypes by a four-allele method that has been shown to be useful in the assessment of risk for Type I diabetes in the Finnish population [38–39]. Notably we found that the offspring of LADA probands had an increased frequency of HLA-*DQB1* genotypes shown to be associated with a low, although still increased risk for Type I diabetes in the Finnish population. Although an increased frequency of these low-risk HLA-*DQB1* genotypes was observed even when including only one family member in the statistical analyses, this finding must be interpreted with caution because the number of subjects was small. These low-risk HLA-*DQB1* genotypes could predispose to a limited autoantigen exposure to helper T cells which in turn promotes activation of cytotoxic T cells and leads to a slowly progressive insulitis [47]. This hypothesis is supported by the observation that there is an association between the degree of risk conferred by the host's HLA-*DQB1* genotypes and age at diagnosis of Type I diabetes, as children presenting with Type I diabetes before the age of 5 years have an increased proportion of high-risk HLA-*DQB1* genotypes [48]. In this study the risk conferring HLA-*DQB1* genotypes for Type I diabetes were related to the lowest tertile of insulin secretory capacity in the offspring of LADA probands suggesting that the presence of these genotypes is associated with the reduction of beta-cell mass.

Notably, the autoantibody status in itself was not associated with impaired insulin secretory capacity in either study group. Although the control group had unexpectedly high frequency of ICA and GAD antibodies, the levels of these antibodies were low and furthermore, all three control subjects with antibody positivity had a protective HLA-*DQB1* genotype compared with the six antibody positive LADA-offspring from whom only one had a protective HLA-*DQB1* genotype. This finding suggests

that low-grade GAD antibody positivity in subjects with protective genes to autoimmune diabetes is a transient phenomenon and could be a marker of non-specific self-healing beta-cell damage induced by environmental factor(s) [49]. Furthermore, it should be noted that the actual levels of ICA and GAD antibodies in the LADA probands were rather low and transiently positive GAD antibodies were not uncommon during the long follow-up [11, 16]. These low-grade antibodies predicted, however, the impairment of insulin secretory capacity in the LADA probands [11, 16] and furthermore, a subtle impairment of insulin secretory capacity could be detected in their non-diabetic offspring. Notably, in this study defects in insulin secretory capacity were most clearly associated with parental history of ICA but not with GAD antibodies which could indicate that LADA is ultimately a heterogeneous disorder showing distinct pathogenetic mechanisms and immunological heterogeneity [50]. It should also be noted that both ICA and GAD antibodies have been shown to be potential predictors for the development of insulin deficiency in LADA patients [17, 51].

Our study shows clearly that the primary defect in glucose metabolism in LADA lies in insulin secretion and not in insulin action. Although the offspring of LADA probands in our study had genetic characteristics typical to Type I diabetes, it is also, however, not excluded that gene defects leading to an insulin deficient phenotype of Type II diabetes could be involved in the aetiopathogenesis of LADA. This view is supported by several studies that have shown impaired early insulin response to intravenous glucose not only in the relatives or offspring or both of Type I diabetic patients [52–53] but also in those of Type II diabetic patients [42–43]. The lack of association between autoimmune markers and decreased insulin secretory capacity in our study, however, provides a possible explanation for the pathogenesis of LADA. Firstly, an inherited (non-autoimmune) defect in insulin secretory capacity could lead to a slow destruction of beta-cells and to the formation of antibodies as a secondary event [50]. Secondly, low-risk HLA-*DQB1* genotypes could predispose to a slow progressive insulitis where detectable autoimmune markers could be a rather late phenomenon [47].

We conclude that the defective insulin secretion in LADA is familial and involves genetic characteristics typical for Type I diabetes. Moreover, our results provide evidence that the offspring of LADA patients should be considered as at high risk for diabetes.

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