

International Diabetes Federation Meeting, 1997, and Metropolitan Diabetes Society of New York Meeting, November 1997

Approaches to treatment and other topics in type 1 diabetes; Genetic heterogeneity of diabetes

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This is the first of six reports on the International Diabetes Federation (IDF) meeting held in Helsinki, Finland, in July 1997. In addition, some interesting presentations from the meeting of the Metropolitan Diabetes Society of New York held in New York, NY, on November 17, 1997, will be discussed.

Nutritional Versus Immune Therapy and Related Topics in Type 1 Diabetes

A debate on nutritional versus immune therapy of type 1 diabetes occurred at the Metropolitan Diabetes Society meeting. Hans Michael Dosch, Toronto, Canada, spoke in favor of nutritional approaches. He reviewed evidence that immune therapy can benefit patients with type 1 diabetes, beginning with the immune suppression trials of the 1980s and continuing with current, ongoing prevention and intervention trials with nicotinamide (NA) treatment, insulin treatment, and BCG immunization. Mentioning that T-cells with specificity for islet antigens play a crucial role in the disease process and that two-thirds of patients with recent-onset diabetes and one-third of their relatives show T-cell responsiveness to such antigens, Dosch suggested two models for the development of clinical disease. Either the prediabetic state may end "catastrophically," with destruction of most β -cells over several months, or additional phenomena,

such as increases in cytokine levels, may cause diabetes to become clinically manifest. A number of antigens have been shown to produce antibodies in patients with recent-onset type 1 diabetes and are potentially of therapeutic value, but Dosch commented, "Antigen-specific therapies work, but there is a caution. We can mitigate the disease but there is the possibility of accelerating the disease." In contrast, he described nutritional therapy as highly safe and commented, "We do not understand the mechanisms, but there is a strong link between the gut and diabetes."

Interest in nutritional therapy began in 1984 when cow's milk was shown to increase diabetes development in rodents. In a variety of studies, the substitution of casein hydrolysates for cow's milk has been shown to prevent these forms of diabetes. The latest clinical data suggest a 13-fold increase in the frequency of diabetes in people with "high-risk" HLA types who are exposed to cow's milk before age 3 months. The Trial to Reduce Type 1 Diabetes in the Genetically at Risk will examine the effect of weaning either to a casein hydrolysate formula or to milk-containing products for 6 months. Dosch observed that "we will not know for a long time" whether the approach is successful.

Jay Skyler, Miami, FL, began his argument in support of immune therapy with the statement that insulin "is a very specific antigen" in the process whereby an environ-

mental trigger in a genetically susceptible individual initiates insulinitis and leads to type 1 diabetes. The NOD mouse model suggests that antigen-presenting cells activate T_H1 cells, causing cytotoxic T-cells to secrete cytokines or exert direct cytotoxic effects. The cytokine-mediated response is more active in actively secreting cells, which was the initial rationale for administration of insulin to prevent development of diabetes, but insulin has also been shown to be an immune response-altering factor that downregulates cytotoxic macrophages and T-cells. Metabolically inactive forms of insulin, such as the B chain alone and $B^{25}Asp$ insulin, are also effective in decreasing the incidence of type 1 diabetes. Further, antigen presentation across mucosal surfaces such as that of the gastrointestinal tract favors T_H2 cell activation, which decreases the immune response. The precise mechanism by which insulin prevents the development of diabetes is therefore uncertain. Nevertheless, Skyler reviewed a large number of studies of parenteral and oral insulin administration in animals and in humans that show the efficacy of this approach, with several studies showing that T-cells from animals so treated can be used for adoptive transfer of immunity.

In rebuttal, Dosch agreed that antigen-based therapy will be important, but stated that the ultimate goal will be to develop a vaccine rather than rely on antigen administration, which is "not very practical" for the overall population. He pointed out that in primate models complications have been associated with insulin administration, particularly the development of immune encephalitis, so the risk of this approach is unknown at present. Skyler concluded by discussing the ongoing Diabetes Prevention Trial 1, the goal of which is to determine whether insulin administration to nondiabetic relatives of patients with type 1 diabetes can delay or prevent the development

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Abbreviations: CVD, cardiovascular disease; ICA, islet cell antigens; IDF, International Diabetes Federation; IGF1BP, IGF-binding protein; LADA, latent autoimmune diabetes in adults; MODY, maturity-onset diabetes of the young; NA, nicotinamide; STZ, streptozotocin.

of the disease. Relatives with an estimated 5-year risk over 50% will receive insulin injections twice daily, and relatives with a 25–50% estimated risk will receive oral insulin. As of the end of October 1997, 200 relatives of patients with type 1 diabetes had been randomly assigned to the parenteral arm and 106 to the oral arm of the trial. It is estimated that that it will be necessary to screen up to 100,000 relatives. Skyler agreed with Dosch, however, that this is only a preliminary approach, and emphasized that “if we focus on relatives we will miss 90%” of those who develop type 1 diabetes.

A number of presentations at the IDF meeting addressed related topics in type 1 diabetes. Gorbenko et al. (abstract 262) reported that NA prevents the development of islet damage and insulin deficiency in a streptozotocin (STZ)-induced newborn rat model of type 1 diabetes. The IMDIAB Group (abstract 260) reported that with intensive insulin therapy, NA doses of either 25 or 50 mg/kg body weight administered to patients with recent-onset type 1 diabetes were associated with preservation of C-peptide secretion without evidence of adverse effects.

Yang et al. (abstract 229) and Yang et al. (abstract 244) discussed the relationship between consumption of various foods and the incidence of type 1 diabetes in China, where a national dietary survey showed the strongest associations of diabetes to be with sugar and milk intake. (Abstract numbers in parentheses are from Abstracts of the 16th International Diabetes Federation Congress, Helsinki, 20–25 July 1997, *Diabetologia* 40 [Suppl. 1]:A1–A722.) When the capture-recapture method was used to adjust for differences in ascertainment, the incidence rate was relatively low at 0.51/100,000, with a 12-fold difference among 22 centers (0.13/100,000–1.61/100,000), and a 7-fold difference among various ethnic groups (highest: Mongol, 2.12/100,000; lowest: Zhuang, 0.32/100,000). Virtanen et al. (abstract 231), studying siblings of children with type 1 diabetes, found evidence that high consumption of cow's milk during childhood was associated with the presence of disease-specific autoantibodies, with an odds ratio for the highest versus lowest quartile of 2.6. Neither duration of breastfeeding nor age at introduction of supplementary feeding with cow's milk was a risk factor. Gimeno et al. (abstract 235) compared 346 diabetic children in Brazil with paired controls and showed that lack of

exclusive breast feeding was a risk factor (odds ratio 2.13) for type 1 diabetes, with introduction of milk products at an early age increasing the risk somewhat further (odds ratio 2.29). However, Arbel et al. (abstract 237) reported that in a similar study of 199 Israeli diabetic children, breast-feeding actually predisposed to diabetes. Feeding with cow's milk remained a significant risk factor, with an 8% higher rate of disease onset for every month's earlier introduction of this nutrient. Elliott et al. (abstract 233) suggested, based on data from the NOD mouse model of type 1 diabetes, that the promotion of diabetes by cow's milk casein is associated with A1 β -casein, not the A2 variant. The A1 β form is metabolized to β -casomorphine-7 after intestinal digestion, and the diabetogenic effect of A1 casein is neutralized by the opioid antagonist naloxone. In prediabetic NOD mice or islet antibody-positive children, but not control Swiss mice or islet antibody-negative children, synthetic β -casomorphine-7 inhibited macrophage immune response, which suggests a mechanism for the diabetogenic effect of this peptide.

In view of the evidence that cellular immune hyporesponsiveness can be induced by the presentation of soluble protein antigens to mucosal surfaces, Harrison et al. (abstract 256) administered aerosol insulin to NOD mice and showed decreased pancreatic islet pathology and incidence of diabetes. CD8⁺ $\gamma\delta$ T-cells were detected and appeared to regulate islet autoimmunity in this animal model. Anastasi et al. (abstract 259) reported that prophylactic subcutaneous administration of insulin decreased anti-islet antibody expression, islet pathology, and diabetes development in an STZ mouse model, in association with decreased expression of islet molecules, such as the cytoplasmic islet cell antigens (ICAs) and the monosialoganglioside GM2-1, that are targets of the autoimmune response leading to type 1 diabetes. Zhou et al. (abstract 264) administered porcine brain GAD with complete Freund's adjuvant to NOD mice and showed decreased severity of insulinitis, suggesting another strategy to decrease the incidence of autoimmune diabetes. In view of the hypothesis that molecular mimicry between coxsackie virus B4 protein 2C and GAD plays a role in the pathogenesis of type 1 diabetes, it is interesting that Vreugdenhil et al. (abstract 270) showed that similar molecules are expressed by a number of coxsackie B-like enteroviruses, which represent the most prevalent enteroviruses.

GAD and the coxsackie virus protein bind with equal affinity to DR3 and thereby can be presented to the immune system, but neither binds to DR4 or DR1, suggesting an explanation for one association of type 1 diabetes with an HLA type.

Sternesjo and Sandler (abstract 360) noted that the suppressed glucose-stimulated insulin release by islets isolated from 12-week-old prediabetic NOD mice can be reversed by culture of the islets in vitro, away from the autoimmune milieu. They reported that islets isolated from animals as old as 26 weeks with advanced insulinitis also can recover, suggesting the potential to reverse type 1 diabetes in humans, at least at early stages. A number of presentations characterized the “honeymoon phase” of type 1 diabetes. Lalic et al. (abstract 347) showed an association of increased numbers of the CD4⁺ helper-inducer subset of T-cells with decreased duration of clinical remission in recent-onset type 1 diabetes. Yilmaz et al. (abstract 372) studied 60 patients with type 1 diabetes who had complete remission of insulin requirement for at least 15 days. Older male patients with normal BMI and lower baseline insulin requirement had greater likelihood of remission. It is interesting that insulin autoantibody levels were increased at the time of clinical remission. Browne et al. (abstract 1311) studied 33 patients who experienced clinical remission from a total of 189 newly diagnosed type 1 diabetic patients aged 5–35 years in 11 European centers. The mean duration of remission was 7.4 months, and the frequency was greater in males and in those who had required lower insulin doses. Scholin et al. (abstract 1326) studied 62 adult patients developing type 1 diabetes and found that 38 entered remission, defined as maintenance of HbA_{1c} <6.5% with an exogenous insulin requirement of <0.4 U · kg⁻¹ · (24 h)⁻¹ for a minimum of 1 month, with a peak prevalence at 5 months. The duration of remission was 10 months in males but 2 months in females, and normal serum bicarbonate levels at onset were predictive of remission.

Two reports at the IDF meeting discussed autoimmune states that may be related to type 1 diabetes. Goswami et al. (abstract 310) reported that insulin autoantibodies were found in 19% of patients with Graves' disease before carbimazole therapy and in an additional 10% of patients after treatment. This was not associated with changes in glucose tolerance or insulin

secretion. Similar antibody changes have been reported in Japanese patients, but not in Caucasian patients, with Graves' disease. Ciampalini et al. (abstract 316) showed that anticardiolipin autoantibodies were present in 30% of children with type 1 diabetes, although the presence of such antibodies was infrequently sustained on repeat testing and was not associated with clinical evidence of another autoimmune disorder.

Genetic and Metabolic Heterogeneity of Diabetes

At a sponsored symposium on aspects of type 1 diabetes, Paul Zimmet gave an interesting presentation on "the distinction between NIDDM and slow IDDM." Type 2 diabetes now occurs at an earlier age among many populations. This phenomenon had previously been seen only in populations with extremely high diabetes prevalence, such as the Pima Indians and Nauruans, but is now relatively common in populations with a high prevalence of obesity and sedentary lifestyle. Furthermore, Zimmet stressed, "Insulin-dependent diabetes is not just juvenile diabetes. Perhaps 40–50% of all insulin-dependent diabetes comes in the over-20-year-old category." He discussed latent autoimmune diabetes in adults (LADA), a condition that initially responds to diet or treatment with oral hypoglycemic agents, but in which 76% of patients have antibodies to GAD and 41% to ICA⁺, there is a high-risk HLA type, and there is progression to insulin dependence over 1–3 years (1). Anti-GAD antibody is a marker of rapid deterioration of type 2 diabetes and is present during pregnancy in 83% of women who subsequently develop type 1 diabetes (2). Interestingly, in the U.K. Prospective Diabetes Study (UKPDS), 10% of patients were positive for anti-GAD and 5% were positive for anti-ICA. This group too was more likely to progress to insulin dependency.

Leif Groop, Malmö, Sweden, discussed similar topics at the IDF meeting in a presentation on the genetic and metabolic heterogeneity of type 2 diabetes. There is strong evidence of family clustering of type 2 diabetes, with a 40% risk to offspring, but environmental and dietary factors as well as genetic factors may explain this. Impaired β -cell function seems to be strongly inherited, but there is somewhat less evidence of inheritance of obesity and insulin resistance. Groop discussed LADA, pointing out that 9% of Finnish patients with type 2 diabetes have GAD antibodies and that 50% of this

group, but only 3% of those without this marker, became insulin dependent at a 10-year follow-up. Patients with LADA do not have the same HLA genotype patterns as seen in those with type 1 diabetes. Mixed IDDM/NIDDM was described by Tuomilehto-Wolf (3) as a condition characterized by a DR4 HLA haplotype and associated with both decreased β -cell function and improved survival because of relative insulin sensitivity and the consequent decrease in cardiovascular disease (CVD) risk. This condition also affects approximately 10% of people with diabetes, who may belong to a group that experienced immune attack on β -cells earlier in life with subsequent development of the type 2 diabetic phenotype. Maturity-onset diabetes of the young (MODY) affects approximately 5% of Finnish diabetic patients and can be caused by several genetic defects, the most common of which is linked to a gene on chromosome 12 that impairs insulin responses to a variety of stimuli. These patients tend to have extremely low triglyceride and high HDL levels and do not have insulin resistance. Macrovascular disease is uncommon in these patients, but microvascular disease occurs as in type 1 diabetes. Finally, mitochondrial diabetes with deafness constitutes 1% of diabetes and is due to a mitochondrial gene defect resulting in abnormal ATP generation. Thus, in Finland, although only approximately 15% of diabetic patients have type 1 diabetes, another 25% have these syndromes of insulin-deficient type 2 diabetes. Only about 60%, then, have insulin-resistant type 2 diabetes. Groop advised listeners to "forget about the old classification. It's really more complicated." Candidate genes are being discovered for type 2 diabetes. These include abnormalities of the β_3 -adrenergic receptor and the adipocyte "uncoupling protein," affecting adipocyte thermogenesis, the hormone leptin and its receptor, and the lipases involved in adipocyte and triglyceride-containing lipoprotein metabolism. It is likely that it will soon be possible to more accurately categorize those patients as well.

Immunological data from several groups suggest that there is overlap between individuals classified as having the two major disease subtypes. Libman et al. (abstract 280) presented data pertaining to LADA from analysis of 53 individuals who developed diabetes after first showing serologic evidence of β -cell autoimmunity. All were first-degree relatives of pediatric type 1 diabetes probands in a prospective

study conducted in Pittsburgh. Of these subjects, 39 started insulin treatment immediately or within 1 year of diagnosis, but 14 did not require insulin until 1–8 years after diagnosis, at a mean age of 43. ICA antibodies were present in 92.3% and 64.2%, and GAD antibodies in 87.2% and 50.0%, of the two groups, giving further support to the existence of a slowly progressing autoimmune form of diabetes related to type 1 diabetes but initially showing characteristics of type 2 diabetes. Fava et al. (abstract 282) reported that GAD65 antibodies were present in 10% of type 2 and 77% of newly diagnosed type 1 diabetic patients. Rattarasarn et al. (abstract 286) reported that 25% of Thai type 2 diabetic patients with secondary failure of oral hypoglycemic agents were positive for GAD antibodies. These patients had lower fasting C-peptide levels and shorter (5 vs. 10 year) duration of treatment with oral agents, further confirming the existence of a slowly progressing form of type 1 diabetes. Landin et al. (abstract 291) used C-peptide measurement and antibody testing to categorize individuals developing diabetes between age 15 and 34. Of those classified clinically as type 1, 40% were negative for ICA and GAD antibodies, and this group had higher C-peptide levels than those who were antibody positive. Of the antibody-negative group, 8% had C-peptide levels >0.5 nmol/l and BMI >25 , suggesting underlying type 2 diabetes. Of clinically classified type 2 diabetic patients, 34% were positive for ICA or GAD65 antibodies. This group showed lower C-peptide levels than the antibody-negative type 2 diabetes group, and 51% required insulin treatment.

More immunological data were presented by Bhatia et al. (abstract 768), who showed that another form of diabetes, non-insulin-dependent diabetes in the young, which is frequent among North Indian Asians and differs clinically from MODY, has a GAD antibody prevalence of 57%. Kanungo et al. (abstract 642) found a 39% prevalence of antibodies to tyrosine pyrophosphatase, a type of ICA, in 218 type 2 diabetic patients from Cuttack in Eastern India. Such antibodies were particularly prevalent among those aged 21–30 and 51–60 years, although GAD65 antibody prevalence was no higher than in controls. Chan et al. (abstract 644) reported that 16% of adult Hong Kong Chinese diabetic patients had experienced onset of disease before age 35. Of 140 Chinese diabetic

patients with disease onset before 35 years and aged less than 40, 10% had a typical presentation with diabetic ketoacidosis, >3+ ketonuria, or continuous insulin treatment within 1 year of diagnosis. Post-glucagon plasma C peptide was <0.6 nmol/l in 50% of patients, but GAD antibodies were present in only 12%, confirming the rarity of classical type 1 diabetes and the heterogeneity of presentation in this population. The patients who were negative for GAD antibodies had more obesity, higher blood pressure and triglycerides, and lower HDL cholesterol. Joffe et al. (abstract 672) and Kalk (abstract 777) presented data showing that type 2 diabetes in blacks in South Africa is associated with lower insulin secretion and lesser degrees of insulin resistance than are seen in Caucasians, although data on antibody status were not available. The age-adjusted prevalence approaches 7% of the population, but whether this is a further example of LADA or an alternative form of insulin deficiency is not evident.

Hypoglycemia

At the sponsored symposium on aspects of type 1 diabetes, Oddmund Sovik, Bergen, Norway, presented a retrospective population-based study of the 246 deaths that occurred among diabetic patients <40 years old in a region of Norway from 1981–1990 (4). Mortality was 3 times greater than would be expected in an age-matched cohort of nondiabetic subjects. Of these deaths, 52 were unrelated to diabetes, 57 were due to CVD, 50 were from diabetic ketoacidosis, 25 were attributed to nephropathy, and 16 were of individuals found “dead in an undisturbed bed” and previously in good health, without evidence of diabetic complications. Of the last group, 15 were insulin-dependent, 9 were on a multiple-dose insulin regimen, and 12 had had problems with hypoglycemia (10 with nocturnal hypoglycemia), suggesting that many of these 16 people died following an episode of severe hypoglycemia. An additional 9 of the 246 deaths were documented as being due to hypoglycemia, for a total of more than 10% of deaths in the group. Previous studies suggested a lower frequency of hypoglycemic death, under 5% (5). Possible causes of the increase in frequency of this syndrome may include more intensive insulin treatment or, less likely, the introduction of human insulin or U-100 insulin (6). Sovik recommended that patients on intensive insulin treatment receive careful

education, focusing on late post-exercise hypoglycemia and other potential causes of the syndrome, and that those experiencing increased hypoglycemia frequency, particularly those sleeping alone and those with hypoglycemia unawareness, be cautiously treated. He noted a possible relationship of these episodes to moderate alcohol use.

A somewhat more optimistic presentation at the symposium, by Knut Borch-Johnsen, Gentofte, Denmark, discussed a 3-year study done by the Danish Diabetes Association with the TRYG-Baltica insurance company, in which more than 7,500 diabetic patients, the majority with type 1 diabetes, were studied to identify the frequency of accidents among people with diabetes. This group was compared with two control groups, and the average number of accidents among the diabetic subjects was 0.7 per 1,000 person-years, compared to 4.5 and 5.5 in the two nondiabetic groups. This suggests that individuals with diabetes should at least be offered accident insurance at standard premiums, and the authors suggested that life insurance and health insurance premiums might also need reevaluation under current treatment approaches.

Hopkins et al. (abstract 100) described symptoms, counter-regulatory hormone responses, and cognitive function during hypoglycemia before and after initiation of insulin treatment in seven patients with poorly controlled type 2 diabetes. Counter-regulatory hormone responses were initially greater than those in nondiabetic control subjects and decreased with improved glycemia. The blood glucose level at which hypoglycemic symptoms developed fell from 3.6 to 3.0 mmol/l, while cognitive impairment occurred at 3.1 vs. 2.8 mmol/l before and after glycemic improvement, suggesting that there is an increased risk of asymptomatic hypoglycemia with insulin treatment. Fanelliet al. (abstract 923) performed stepwise hyperinsulinemic hypoglycemic clamps on eight nondiabetic volunteers after no, one, two, or three insulin-induced hypoglycemic episodes on the previous day. After a single episode, a lower blood glucose was required to elicit counterregulatory hormonal responses, but symptoms and cognitive dysfunction were not affected. After two and, to a greater extent, three episodes, autonomic and neuroglycopenic symptoms and cognitive dysfunction required lower glucose levels, suggesting a rapidly developing hierarchy of loss of responses.

Fritsche et al. (abstract 926) measured β -adrenergic sensitivity with an isoproterenol test after euglycemic and hypoglycemic hyperinsulinemic clamps. Rather than exhibiting an impaired response, cardiovascular and adipose tissue sensitivity to catecholamines increased after the hypoglycemic episode, suggesting that hypoglycemia unawareness is not produced by a decrease in peripheral tissue sensitivity to catecholamines. King et al. (abstracts 928 and 945) found increased subjective sense of fatigue during bicycle exercise in 10 type 1 diabetic patients on the day following a 1-h nocturnal episode of moderate hypoglycemia, although the exercise capacity and plasma glucose, potassium, and catecholamine concentrations were not affected. Subjective well-being, but not cerebral function tests, were similarly affected. Matyka et al. (abstract 930) found, with blood sampling every 15 minutes, that 70% of children with type 1 diabetes were hypoglycemic on at least one of two nights and showed evidence of increased awakening. Hejlesen et al. (abstract 932) presented evidence that the Somogyi effect of insulin-induced hypoglycemia leading to hyperglycemia does occur, based on analysis of data from 20 inpatient studies of type 1 diabetic patients. The daily total dose of intermediate-acting insulin was found to correlate with the degree of hyperglycemia, which typically began 6–8 h following hypoglycemia and lasted for 16–18 h with a blood glucose levels of 4–10 mmol/l above expected. Bajaj et al. (abstract 936) showed that teaching patients “blood glucose awareness” increased the epinephrine response to hypoglycemia, suggesting that such education may have a role in reducing the risk of hypoglycemia.

New Approaches to Glucose Monitoring

Sendlhofer et al. (abstract 1414) gave an interesting analysis of the accuracy of different home blood glucose meters. The overall correlation coefficient across all glucose concentrations was 0.96 or higher for the six meters tested. In the range of 3.89–9.99 mmol/l, all meters were similarly accurate, but at lower and higher glucose levels all were at least somewhat less accurate, and two meters gave readings that were more than 20% different from a reference method 30 and 32% of the time. Karamanos et al. (abstract 1307) reported on 78 type 1 diabetic patients performing home glucose monitoring two to five times

daily. There was only a modestly strong correlation between HbA_{1c} and mean blood glucose levels during the prior 2 months in the overall group, with $r = 0.5953$. However, when patients were separated into those with higher and lower mean daily glycemic excursions, averaging 5.0 and 1.1 mmol/l, the respective correlations were $r = 0.5750$ and $r = 0.7142$. Similarly, Sato et al. (abstract 2497) reported that the mean of home glucose measurements did not correlate with HbA_{1c} levels in five "unstable" diabetic patients.

In light of the above reports, it may then be important to develop glucose testing methodologies that allow more frequent assessment of glycemia. At a sponsored workshop on continuous non-invasive and minimal-invasive glucose monitoring, Lutz Heinemann, Düsseldorf, Germany, spoke on the measurement of glucose concentration by determination of light scattering. Near-infrared light penetrates several centimeters into the skin and is altered predominantly by absorption and scattering effects. It is relatively easy to measure glucose concentrations in clear solutions by analysis of absorption spectra. However, the heterogeneous light scattering in tissues, along with the relatively low concentrations of glucose and the presence of interfering substances, as well as the temperature dependence of these processes, has made systems based on this unsuccessful in determining tissue glucose concentrations. In contrast, the light-scattering approach analyzes the characteristics of a light beam passing through the interstitial fluid and then entering scattering particles of different refractive index, such as fat droplets. Since increasing the glucose concentration increases the refractive index of the interstitial fluid, the degree of light scattering decreases. Heinemann stated that glucose appears to be the major substance altering light scattering, so this approach may have promise. In clinical studies, using a combination light source, light sensor, and thermometer placed over the abdomen, there was close correlation, corrected for body temperature, between increasing glucose concentrations and decreasing degree of scattering, with good inter- and intra-subject variability.

Udo Hoss, Ulm, Germany, spoke on microdialysis techniques for blood glucose measurement, which are based on analysis of the molecular differences between two compartments separated by a semipermeable membrane. A probe is inserted subcu-

taneously, and a perfusion solution is continuously pumped across a measurement point to an analyzer at a flow rate of 0.3 μ l/min. The probe resembles a venipuncture needle and is 1 mm in diameter (similar in size to a 14-gauge syringe needle) with inflow and outflow ports and a semipermeable membrane. The larger the available membrane surface area, the lower the required flow rate. The analysis process can be either continuous or intermittent. H. Rinne, Germany, spoke on trials of the device, which is being produced under the brand name Komo Bodyguard System. The perfusate, a glucose oxidase solution that is stable for up to 1 year, a pump, and a waste reservoir are contained in a unit 10 cm by 6 cm by 1.5 cm in size and worn by the user on a belt. It resembles a subcutaneous insulin infusion pump. This unit measures glucose levels every minute, although with a 5- to 20-min delay because of the length of the tubing and slow flow rate. The unit has programmable alarms for hypo- and hyperglycemia and for abnormal rate of change in blood glucose, and it stores glucose information for 8 h. Information about glucose levels is sent telemetrically to a larger base unit that stores information for 30 days and contains a capillary blood glucose measurement device used for daily calibration of the microdialysis measurement. In clinical studies, measurements from the microdialysis device have shown close correlation with capillary glucose measurements, and episodes of hyperglycemia and hypoglycemia that would not be diagnosed with routine capillary glucose monitoring have been noted. Sternberg et al. (abstract 26) presented additional data pertaining to this meter. Two type 1 diabetic patients had a total of 8 days of monitoring, with good correlation between glucose reference values and microdialysis glucose readings. Glucose values <54 mg/dl occurred 21 times, but were recognized only 12 times by capillary glucose testing. Additionally, hyperglycemic values >250 mg/dl occurred 12 times but patients detected only 7 episodes, despite the fact that most of the hyper- and hypoglycemic episodes occurred in daytime.

Approaches to Insulin Treatment

Several speakers at the symposium on aspects of type 1 diabetes discussed approaches to insulin treatment. Juha Tuominen, Helsinki, Finland, discussed approaches to controlling insulin absorption rates from the subcutaneous tissues. There is variability in absorption at different

injection sites (7) and with exercise (8), massage of the injected site, ambient temperature (9), depth of injection (particularly if the injection is accidentally given intramuscularly), and cigarette use. Blood flow in the subcutaneous tissue appears to link these differing factors. Bernard Zinman, Toronto, Canada, pointed out that in nondiabetic subjects who receive regular insulin with a variable insulin infusion to maintain euglycemia, the within-person coefficient of variability of time to peak dose is 51%. The coefficient of variability of the area under the curve of insulin absorbed is 44%, that of the peak insulin level is 39%, and that of the biological action (based on the amount of glucose infused to maintain euglycemia) is 35%.

The above data reveal why new routes of insulin administration and new forms of insulin, such as lispro, are of great importance. Rolf Hilgenfeld, Jena, Germany, further discussed the rationale for treatment with new insulin analogs. The initial suggestion that there may be value in use of insulin analogs that introduce charge repulsion between individual molecules so that they won't display the spontaneous formation into dimers and then hexamers that characterizes native insulin was made by Brange et al. (10). One of the initially studied variants of this sort, B¹⁰Asp insulin, had increased affinity for the IGF-1 receptor, however, leading to the possibility of unwanted adverse effects and showing the need for caution in these studies. Another analog, lispro, has a 300-fold decrease in dimerization and rapid onset of activity after subcutaneous administration, although studies such as those described above to determine whether the variability of a given dosage is less than that of regular insulin have not been performed.

The opposite strategy is among those being used for developing long-acting insulin analogs: design molecules that are more stable in the hexameric conformation. Ralf Rosskamp, Frankfurt, Germany, discussed the long-acting analog HOE 901, which has a longer duration of action than NPH insulin without the distinct peak of action seen with NPH insulin. In phase 2 studies, HOE 901 has peak action at 6–16 h and a duration of action of 24 h after injection, and so can be administered once daily. Moreover, there is some evidence suggesting improved metabolic control with this agent rather than NPH insulin.

Manfred Dreyer, Hamburg, Germany, reviewed aspects of insulin analog devel-

opment at the IDF meeting. He mentioned that the long-acting insulin HOE 901 spontaneously aggregates into a crystalline structure after it is injected and is exposed to physiological pH. Novo Nordisk is developing another long-acting insulin with a fatty acid attached to the B chain, leading to increased albumin binding and less tendency to exhibit peaks of action. The potential of long-term risk of these preparations is not fully known, but it appears that these changes in the carboxyl terminus of the B chain do not alter receptor binding and so do not activate IGF-1 receptors, a potential concern with the earlier rapid-acting ^{B10}Asp insulin.

Radziuk et al. (abstract 1374) compared NPH insulin with [N^ε-palmitoyl Lys(B29)] human insulin, an acylated analog. The analog showed somewhat more stable effects at 6–9 h than NPH insulin, with both acting over a period of at least 12 h. Several studies analyzed different basal insulin approaches. Ebeling et al. (abstract 1382) showed that improved glycemic control during treatment with lispro insulin requires optimization of basal insulin, so an average of 2.7 injections of NPH insulin per patient were used to achieve improved glycemic control in 84 patients with type 1 diabetes. Llewelyn et al. (abstract 1383) compared use of ultralente and NPH insulin as basal regimens in 167 type 1 diabetic patients treated with lispro. Fasting glucose levels were significantly lower with NPH insulin (8.7 vs. 9.6 mmol/l), but glucose levels were higher before the evening meal with NPH insulin (9.1 vs. 8.2 mmol/l). Patients treated with NPH insulin had more hypoglycemic episodes between midnight and 6:00 A.M. but fewer episodes between 6:00 P.M. and midnight during the 3-month treatment period (1.2 vs. 0.8 and 1.6 vs. 2.9), without difference in the overall rate of mild or severe episodes. The basal regimen did not affect overall glycemic control.

Heinemann et al. (abstract 1306) interviewed 202 insulin-treated patients with diabetes to assess the typical interval between regular insulin injection and food intake. About two-thirds of patients eat within 15 min of injection, and only one-fourth typically wait 30 min or longer. This would suggest that rapid-acting insulin preparations should be of benefit. Heinemann et al. (abstract 29) compared the variability of the metabolic effect of regular insulin with that of the rapid acting insulin-analog ^{B28}Asp in nondiabetic subjects studied on four occasions. Regular insulin had

an intra-individual variability of metabolic activity of 13–32%, with ^{B28}Asp showing somewhat less variability, particularly in the time to maximal insulin concentration. A number of presentations dealt with the effect of lispro. Ahmed and Home (abstract 30) showed less frequent nocturnal hypoglycemia in C-peptide-negative type 1 diabetic patients treated with preprandial boluses of lispro than in those treated with regular insulin, although at the expense of higher mean glucose levels from midnight to 4:00 A.M. Holcombe et al. (abstract 1352) compared lispro and regular insulin in an open-label randomized study of 481 adolescents with type 1 diabetes. After breakfast and dinner, 2-h postprandial glucose values were 9.7 and 10.6 mmol/l for lispro and 8.6 and 9.3 mmol/l for regular insulin. Again, hypoglycemia occurred less frequently from midnight to 6:00 A.M. with lispro than with regular insulin. In what will probably be the largest study of hypoglycemia among lispro users, Brunelle et al. (abstract 1386) presented results of a meta-analysis of eight clinical trials of a total of 2,327 patients using lispro and 2,339 patients using regular human insulin. At least one severe hypoglycemic episode occurred during lispro therapy in 72 (3.1%) patients and during regular human insulin treatment in 102 (4.4%) patients, a significant difference ($P=0.024$). HbA_{1c} levels were similar in the two groups. Lispro may also offer benefit during continuous subcutaneous insulin infusion pump therapy. Hanaire et al. (abstract 31) reported that pump patients using lispro had a fall in HbA_{1c} by 0.62%, in comparison to a 0.09% decrease in patients remaining on regular insulin. There was less glycemic variability and no increase in the frequency of hypoglycemia.

Jean-Louis Selam, Paris, France, discussed alternative approaches to insulin administration. In the Diabetes Control and Complications Trial, glucose levels were slightly lower with continuous subcutaneous insulin infusion than with multiple daily injection treatment, but with an increased frequency of severe hypoglycemia and of ketoacidosis. Predictive factors for discontinuation of continuous subcutaneous insulin infusion are higher HbA_{1c}, lower home glucose monitoring frequency, and lower self-esteem. Continuous subcutaneous insulin infusion is indicated for patients with increased HbA_{1c} levels who have good social support. Intraperitoneal insulin has the advantages of portal absorption, leading to faster and more repro-

ducible action than subcutaneous insulin, with lower peripheral-to-portal insulin ratios, and allowing use of pump or, eventually, islet implantation technology. A Veteran's Hospital study of implanted pumps in patients with type 2 diabetes showed a reduced frequency of severe hypoglycemia (11). Fifty-six centers, mainly in France, have been involved in implanted programmable pump treatment. Problems include pump failure from battery failure, catheter obstruction, or insulin precipitation. Because the cost is 3-fold greater than with other treatment approaches, the implanted pump approach is most applicable to compliant intensively treated patients with recurrent hypoglycemia.

Another approach to insulin administration is nasal insulin. Nasal insulin is not absorbed without promoters, and is only 10% absorbed with these. Five controlled trials of various combinations of insulin and promoters show that local irritation is present with preparations that show good absorption and that those that are not irritating tend to act unpredictably. The duration of action of these products is about 1 h. Pulmonary insulin has also been investigated using nasal spray systems to deliver precisely sized droplets acting for several hours, but there is concern about the potential for toxicity.

Another treatment approach is pancreas transplantation. For the 7,505 pancreas transplantations that have been performed, the 5-year failure rate is 50%, and there are risks associated with immunosuppression, so at present pancreas transplantation is most appropriate for patients requiring renal transplantation. Islet transplantation avoids surgery, but measures to avoid the immunosuppression requirement are not yet available. Since 1974, 270 islet transplantations have been performed, with approximately 10% of the patients becoming insulin independent at 1 year and some evidence of improved glycemic control in half of treated patients.

Islet Transplantation

Nacher et al. (abstract 476) studied a variety of protocols of insulin treatment in animal islet transplantation models. For successful transplantation, it was crucial to maintain euglycemia, with the optimal approach being to begin this treatment before transplantation and maintain normal glucose levels for 2 weeks afterwards. Presumably this approach should be adopted in clinical practice.

In a series of remarkable presentations, Bretzel et al. (abstract 480), Hering et al. (abstract 482), and Meyer et al. (abstract 484) gave results of a series of successful islet transplantations. The authors compared eight simultaneous islet-kidney transplantation procedures with eight renal transplantations (abstract 480). Islets were implanted via percutaneous portal vein catheterization under local anesthesia. All patients and renal grafts survived, and seven islet grafts remained functional at 1 year, although only two of eight islet graft recipients had achieved insulin independence, at 10 and 12 months after islet implantation. Mean HbA_{1c} levels were 6.4% with islet transplantation but 7.9% with renal transplantation alone, with a mean daily insulin requirement of 10 U with islet transplantation but 48 U with renal transplantation alone. No patient receiving an islet transplant experienced hypoglycemic episodes during 1 year of follow-up. In a separate study (abstract 482), 14 patients received islet transplantation after renal transplantation. Patients required a mean of 1.5 donor pancreases (8,230 islets/kg body wt). All patients showed evidence of islet function at 2 weeks after transplantation, and 7 showed ongoing function after an average period of 2 years. Two patients became insulin-independent, and all had reduced insulin requirement, HbA_{1c} values of 6.3–8.2%, and a complete cessation of hypoglycemic episodes.

The effect of islet transplantation on hypoglycemia awareness and counterregulation was analyzed in a study in which islet transplantation alone was performed in three nonuremic, non-kidney transplanted patients with long-standing type 1 diabetes who were prone to severe hypoglycemia (abstract 484). Prior to transplantation, the glucagon and epinephrine responses to hypoglycemia were severely impaired, the cortisol response was markedly delayed, and awareness of autonomic warning symptoms was absent in all patients. One month after transplantation, the insulin requirement was reduced by about 50% in all patients, epinephrine and cortisol responsiveness improved, and all patients noticed autonomic warning symptoms well before severe neuroglycopenia occurred. No improvement of the glucagon response was observed.

IGF Treatment

In the symposium on new treatments for hyperglycemia, David Dunger, Oxford, U.K., discussed the role of IGF-1. First iso-

lated in the 1960s as a portion of serum insulin-like activity, IGF-1 has a receptor that is functionally and structurally similar to the insulin receptor. IGF-1 increases glucose uptake, inhibits lipolysis, increases glycogen synthesis, and inhibits protein catabolism, but with only about one-tenth the glucose-lowering action of insulin and with greater growth-promoting effects. There are few IGF-1 receptors in liver and adipocytes; the major effect is on muscle. Most IGF-1 circulates bound to IGF-binding protein (IGFBP)-3, one of 10 circulating binding proteins that are responsible for regulating bioavailability. IGFBP-1 is regulated inversely by insulin, shows the best correlation with free IGF-1 in plasma, and may be the major binding protein for glucose homeostasis. In type 1 diabetes, IGF-1 levels are decreased, particularly at the time of maximal height velocity during puberty. This may be related to insulin's role in modulating the hepatic growth hormone receptor, and hence regulating IGF-1 synthesis, but also to its inhibition of IGFBP-1 synthesis. After IGF-1 is administered subcutaneously, it remains in the circulation for approximately 20 h because of the presence of IGFBPs. Early studies used high doses of IGF-1 and showed adverse effects such as jaw pain and edema. In a 1-month controlled trial of IGF-1 treatment, HbA_{1c} levels improved. There may be improvement in glycemic control and in insulin sensitivity with treatment in type 2 diabetes as well.

Acerini et al. (abstract 1299) presented results of administration of IGF-1 in doses of 0, 20, or 40 $\mu\text{g} \cdot \text{kg}^{-1} \cdot \text{day}^{-1}$ to 53 adolescents with type 1 diabetes on multiple insulin dose regimens. HbA_{1c} was 0.6% lower with the higher dose than with placebo, without change in insulin requirement, BMI, glomerular filtration rate, albuminuria, retinal appearance, or hypoglycemia frequency. Carroll et al. (abstract 1487) reported results of administration of IGF-1 at a dose of 50 $\mu\text{g}/\text{kg}$ twice daily to six adults with type 1 diabetes. After 4 days, free insulin levels decreased from 8.38 to 4.98 mU/l, mean overnight growth hormone decreased from 12.6 to 3.8 mU/l, and total cholesterol and triglycerides decreased from 4.68 to 4.25 and from 1.27 to 0.95 mmol/l.

On a cautionary note, Chantelau and Eggert (abstract 1970), reported observations of 13 patients with increased background diabetic retinopathy following improved glycemic control, with increased IGF-1 documented in three of the patients, suggesting that IGF-1 may play a role in this

clinical phenomenon. IGF-1 may also play a role in the treatment of type 2 diabetes. Hansen et al. (abstract 1363) administered IGF-1 at a dose of 40 $\mu\text{g}/\text{kg}$ twice daily to monkeys with spontaneous type 2 diabetes. Insulin doses were decreased 32% without change in blood glucose or body weight. Moses et al. (abstract 1484) reported that IGF-1 administration to patients with type 2 diabetes in doses up to 80 $\mu\text{g}/\text{kg}$ twice daily decreased HbA_{1c}, fasting triglycerides, and visceral fat determined by computed tomography. Adverse effects included edema and jaw pain.

Amylin

In the symposium on new treatments for hyperglycemia, Rudolf Prager, Vienna, Austria discussed the role of amylin. The first study of pancreatic islet hyalinosis was by Austrian pathologists in 1901. The hormone responsible, originally named islet amyloid polypeptide but now called amylin, was discovered in 1987 and found to be co-secreted with insulin, to be deficient in type 1 diabetes, and to be relatively decreased in type 2 diabetes, although it is secreted at high levels in association with hyperinsulinemia in insulin-resistant states. Amylin does not affect insulin sensitivity at physiological levels. Gebre-Medhin et al. (abstract 94) presented studies designed to assess the physiological role of amylin with the use of mice in which the amylin gene has been disrupted. Insulin responsiveness to oral glucose was increased, and there was decreased response to noxious stimuli, which is related to two major sites of expression of amylin, the pancreatic β -cells and dorsal root ganglion neurons. Kahn et al. (abstract 95) reported that amylin release is decreased in patients with impaired glucose tolerance and in those with type 2 diabetes, which argues against the hypothesis that increased amylin release explains the propensity for amyloid deposition in type 2 diabetes. Janson et al. (abstract 583), noting the histological finding of loss of cortical neurons with deposition of amyloid caused by β protein deposits in Alzheimer's disease, measured islet amyloid in patients with this illness. Islet amyloid was increased over that in control patients despite a lower BMI, implying an underlying defect in protein trafficking in both Alzheimer's disease and type 2 diabetes.

Prager discussed the use of the amylin analog pramlintide, which is more soluble, allowing subcutaneous injection. It does not affect glucose after intravenous injection.

tion but does lower glucose levels after oral glucose administration because of its action as a potent gastric-emptying inhibitor, as well as by suppressing glucagon secretion. Conversely, the amylin deficiency of type 1 diabetes may be associated with more rapid absorption of dietary glucose, although hyperglycemia interferes with this. Pramlintide administration may therefore be of benefit in decreasing postprandial glycemia in diabetes, and studies have shown it to be effective both in type 1 and in type 2 diabetes. Nyholm et al. (abstract 174) studied 13 male type 1 diabetic patients with and without administration of 30 µg of pramlintide or placebo four times daily for 4 weeks. Mean plasma glucose levels were 8.4 mmol/l with pramlintide vs. 10.2 mmol/l with placebo, with lower levels of glucagon and glycerol and higher alanine levels also noted. Suppression of glucagon secretion may contribute to the reduction in postprandial hyperglycemia observed during pramlintide treatment. Thompson et al. (abstract 1397) reported a study with a similar protocol involving 203 patients with type 2 diabetes requiring insulin. HbA_{1c} fell 0.51–0.58% with pramlintide doses of 120 to 240 µg/day vs. 0.27% with placebo, and fructosamine fell 18–24 vs. 4 µmol/l without increased incidence of

hypoglycemia. Redalieu et al. (abstract 1399) reported that pramlintide is well absorbed when mixed in the same syringe with NPH and/or regular insulin.

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