OBSERVATIONS

Glucose Instability Is Associated With a High Level of Circulating P-Selectin

We have previously shown that glycemic instability, as measured by the coefficient of variation for fasting plasma glucose (CV-FPG), is an independent predictor of cardiovascular mortality in type 2 diabetes (1,2). The mechanisms, if any, underlying the association between long-term plasma glucose instability and vascular diseases are difficult to explain.

In vitro studies with retinal capillary pericytes have shown that rapid glucose fluctuations in the culture medium induced cellular damage and death by apoptosis (3). Moreover, tubulointerstitial cells exposed to intermittent high glucose concentrations underwent changes in cellular growth, collagen synthesis, and cytokine secretion that were more severe than those observed in cells exposed to stable high-glucose concentrations (4). These data extrapolated in vivo are consistent with the notion that rapid excursions in glycemia may have relevant pathological effects. Recently, it has been demonstrated that some adhesion molecules (P-selectin in particular) and other markers of inflammation are important risk factors for cardiovascular diseases (5–7).

Therefore, the aim of the present study was to evaluate whether long-term glucose instability is associated with increased plasma concentrations of soluble adhesion molecules in type 2 diabetic patients. To test this hypothesis, type 2 diabetic patients attending the Verona Diabetes Center with at least three fasting plasma glucose (FPG) determinations per year over a 6-year period (1995–2000) were identified, and the CV-FPG was calculated. A total of 11 patients with unstable FPG (CV-FPG $\geq$25%) were selected (group A). To reduce the effects of confounders known to increase levels of adhesion molecules, participants had to be nonsmokers with no clinical evidence of cardiovascular diseases (assessed by physical examination, electrocardiogram, ankle-arm index, and intima-media thickness of the carotid artery). As the control, a group of 14 subjects with stable FPG (CV-FPG $\leq$10%), well-matched for sex, age, duration of diabetes, BMI, total cholesterol, HDL-cholesterol, triglycerides, and blood pressure, were selected (group B). The cutoff of CV-FPG was chosen based on the results of our previous study: a CV-FPG <15% was associated with the lowest cardiovascular mortality rate, whereas a CV-FPG >25% predicted the highest cardiovascular mortality rate (1,2).

Subjects with glucose instability had statistically significant higher mean FPG (183 ± 43 vs. 157 ± 17 mg/dl, $P = 0.01$) and were more frequently treated with insulin. Circulating levels of E-selectin, P-selectin, intracellular adhesion molecule (ICAM)-1, and vascular cell adhesion molecule (VCAM)-1 were measured in all patients between November 2000 and December 2000 by enzyme-linked immunoassay (Bender Medsystems), with intra- and interassay CVs <8%.

In the covariance analysis, after adjusting for mean FPG, P-selectin values were significantly higher in group A (371 ± 102 vs. 284 ± 66.7 ng/ml, $P = 0.03$). The other adhesion molecules tended to be higher in group A, but none of them reached statistical significance (ICAM-1 346 ± 102 vs. 299 ± 58.9 ng/ml, $P = 0.07$; VCAM-1 466 ± 175 vs. 381 ± 153 ng/ml, $P = 0.12$; E-selectin 62 ± 31 vs. 52 ± 30.3 ng/ml, $P = 0.8$). In conclusion, in this small selected sample of diabetic subjects, high FPG instability seems to be associated with an endothelial dysfunction that may increase the risk of cardiovascular morbidity and mortality. The present results must be confirmed in a large prospective study.

ELISABETIA BRUN, MD
GIACOMO ZOPPINI, MD
CRISTINA ZAMBONI, MD
ENZO BONORA, MD
MICHELE MUGGEO, MD

From the Division of Endocrinology and Metabolic Diseases, University of Verona, Verona, Italy.

Address correspondence and reprint requests to Prof. Michele Muggeo, Divisione di Endocrinologia e Malattie del Metabolismo, Ospedale Civile Maggiore Borgo Trento, P.le Stefani 1, 37126 Verona, Italy. E-mail: michele.muggeo@mail.azosp.vr.it.

References

1. Muggeo M, Verlato G, Bonora E, Zoppini G, Corbellini M, de Marco R: Long-term instability of fasting plasma glucose, a novel predictor of cardiovascular mortality in elderly patients with non-insulin-dependent diabetes mellitus: the Verona Diabetes Study. *Circulation* 961:1750–1754, 1997
2. Muggeo M, Zoppini G, Bonora E, Brun E, Bonadonna RC, Moghetti P, Verlato G: Fasting plasma glucose variability predicts 10-year survival of type 2 diabetic patients: the Verona Diabetes Study. *Diabetes Care* 23:45–50, 2000
3. Li W, Liu X, Yanoff M, Cohen S, Ye X: Cultured retinal capillary pericytes die by apoptosis after fluctuation from high to low glucose levels: a comparative study with retinal capillary endothelial cells. *Diabetologia* 39:537–547, 1996
4. Jones SC, Saunders HJ, Qi W, Pollock CA: Intermittent high glucose enhances cell growth and collagen synthesis in cultured human tubulointerstitial cells. *Diabetologia* 42:1113–1119, 1999
5. Frishman WH: Biologic markers as predictors of cardiovascular disease. *Am J Med* 104:18S–27S, 1998
6. Oparil S, Oberman A: Nontraditional cardiovascular risk factors. *Am J Med Sci* 317: 193–207, 1999
7. Ridker PM, Buring JE, Rifai N: Soluble P-Selectin and the risk of future cardiovascular events. *Circulation* 103: 491–495, 2001

Maternal Transmission of Type 2 Diabetes Varies by Ethnic Group

Cross-sectional survey of Europeans and South Asians

People with ancestral origins in the Indian subcontinent who migrate to industrialized countries are at high risk of type 2 diabetes. The relative contribution of genetic and environmental factors to this difference is not completely understood.

European origin populations with type 2 diabetes are substantially more likely to report diabetes in their mothers than in their fathers (1). This maternal excess has not been found in African-Americans (1) or Hispanics (2). Clinic-based reports have found no maternal excess in South Indians with type 2 diabetes (3), but we are not aware of any population-based data to confirm this.

Table 1—*Family history of maternal and paternal diabetes by ethnic group and respondent glucose tolerance status*

	Normal	Impaired glucose tolerance	Diabetes
Europeans			
n (% don't know/missing)	649 (27)	129 (29)	46 (33)
Maternal diabetes only	6.3 (4.3, 8.9)	8.7 (3.8, 16.4)	32.3 (16.7, 51.4)
Paternal diabetes only	2.1 (1.0, 3.8)	3.3 (0.7, 9.2)	3.2 (0.1, 16.7)
Difference	4.2 (1.5, 6.3)	5.4 (−2.6, 10.5)	29.0 (6.3, 35.3)
South Asians			
n (% don't know/missing)	386 (14)	140 (14)	158 (18)
Maternal diabetes only	12.1 (8.8, 16.1)	14.2 (8.5, 21.7)	10.9 (6.1, 17.5)
Paternal diabetes only	8.5 (5.7, 12.0)	15.0 (9.1, 22.7)	7.8 (3.8, 13.8)
Difference	3.6 (1.5, 8.5)	−0.8 (−10.9, 9.3)	3.1 (−5.0, 10.4)

Data are *n* (%) or % (95% CI).

We used data from the Newcastle Heart Project (4) to look for an excess of maternal diabetes in U.K. South Asians.

We studied a stratified population sample of 1,509 adults aged 25–74 years, of whom 684 were of South Asian origin (259 Indian, 305 Pakistani, and 120 Bangladeshi), and 825 were of European origin. All subjects underwent an oral glucose tolerance test, and we defined diabetes and impaired glucose tolerance using the 1985 World Health Organization criteria. A family history of diabetes was based on the report of respondents.

Among Europeans, the proportion reporting maternal diabetes was higher in those with type 2 diabetes (Table 1). There was a significant excess of maternal diabetes in respondents with normal glucose tolerance and diabetes. By contrast, the proportion of South Asians reporting maternal diabetes was similar across categories of glucose tolerance. There was a significant excess of maternal diabetes among South Asians with normal glucose tolerance but not among those with diabetes. Among respondents with diabetes, mother-only diabetes was significantly more common in Europeans than in South Asians ($P = 0.003$), whereas father-only diabetes was not significantly different ($P = 0.37$).

Possible explanations for excess maternal diabetes include mitochondrial inheritance, maternal imprinting, or a detrimental effect of diabetes in pregnancy. Alternatively, the finding may reflect inaccurate reporting, better awareness of the health of mothers, longer female life expectancy, or a greater influence of mothers on lifestyle risk factors. The fact that the maternal excess varied across categories of glucose tolerance for Europeans may make these explanations less likely. The most serious limitation of our study (in common with most other such studies) is its reliance on respondents' reports of family histories. Uncertainty about family history is reflected in the large numbers in the missing or "don't know" category, particularly among Europeans. Nevertheless, our findings in Europeans are consistent with previous clinic- and population-based reports. Our results in South Asians, which were derived from a population-based study, add weight to previous reports that maternal excess is not a feature of type 2 diabetes in these ethnic groups.

Any proposed explanation of the imbalances between maternal and paternal diabetes must take account of the ethnic differences we have described. Such an explanation will likely make an important contribution to understanding the high prevalence of type 2 diabetes in South Asians in the U.K. and elsewhere. We need further studies based on biochemical information about family members of people with type 2 diabetes.

COLIN M. FISCHBACHER, MFPHM[1]
RAJ BHOPAL, MD[2]
NIGEL UNWIN, MFPHM[1,3]
MARK WALKER, FRCP[3]
MARTIN WHITE, FFPHM[1]
K.G.M.M. ALBERTI, FRCP[3]

From the [1]Department of Epidemiology and Public Health, University of Newcastle, Newcastle upon Tyne; the [2]Department of Public Health Sciences, University of Edinburgh, Edinburgh, Scotland; and the [3]Department of Diabetes, University of Newcastle, United Kingdom.

Address correspondence and reprint requests to Dr. Colin M. Fischbacher, Lecturer in Public Health Medicine, Department of Epidemiology and Public Health, The Medical School, University of Newcastle, Newcastle upon Tyne, NE2 4HH, U.K. E-mail: c.m.fischbacher@ncl.ac.uk.

Acknowledgments— We acknowledge financial support from the Barclay Trust, the British Diabetic Association, the Newcastle Health Authority, the research and development directorate of the former Northern Regional Health Authority, the U.K. Department of Health, and the British Heart Foundation.

The authors thank all those mentioned in the acknowledgments of their previous study (4) and Louise Hayes for help with data management.

Material in this letter was previously presented at the Society for Social Medicine's 44th Annual Scientific Meeting in Norwich, U.K., in September 2000.

References

1. Karter AJ, Rowell SE, Ackerson LM, Mitchell BD, Ferrara A, Selby JV, Newman B: Excess maternal transmission of type 2 diabetes: the Northern California Kaiser Permanente Diabetes Registry. *Diabetes Care* 22:938–943, 1999
2. Mitchell B, Kammerer C, Reinhart L, Stern M, MacCluer J: Is there an excess in maternal transmission of NIDDM? *Diabetologia* 38:314–317, 1995
3. McCarthy M, Hitman G, Shields D, Morton N, Snehalatha C, Mohan V, Ramachandran A, Viswanathan M: Family studies of non-insulin-dependent diabetes mellitus in South Indians. *Diabetologia* 37:1221–1230, 1994
4. Bhopal R, Unwin N, White M, Yallop J, Walker L, Alberti K, Harland J, Patel S, Ahmad N, Turner C, Watson W, Kulkarni A, Laker M, Tavridou A: Heterogeneity of coronary heart disease risk factors in Indian, Pakistani, Bangladeshi and European origin populations: cross sectional study. *BMJ* 319:215–220, 1999

Epidemiology of Nontraumatic Lower-Extremity Amputation in Area 7, Madrid, Between 1989 and 1999

A population-based study

Diabetes remains the main cause of lower-extremity amputations (LEAs) (1–3). Recently (3), the number of LEAs carried out worldwide has been estimated to be $>162 \times 10^6$ each year, with

a high economic and social cost. More than 50% of these LEAs are performed in subjects with diabetes. Differences in the incidence of LEAs could reflect variations in risk factors, diabetes prevalence, health care systems, and lifestyle, as well as differences in population age-structure, the sources from which the case was identified, the levels of ascertainment, and the definitions of LEAs and the distinctions between major and minor LEAs. Recently, a standard approach to data collection has been described (4,5), and the first article (3) about the epidemiology of LEAs in several countries has been published. In this study, area 7 from Madrid presents the lowest LEA rates. The purpose of this article is to compare the incidence of LEAs in diabetic and nondiabetic subjects—in relation to the provision of improved footcare after the St. Vincent Declaration—by use of a standardized approach, in the area 7 of Madrid between 1989 and 1993, between 1994 and 1996, and between 1997 and 1999.

The National Health Service has divided Madrid into 13 health care areas. The public health care system is serving >99% of the total population from the area 7 and is provided by a single hospital (Hospital Clínico San Carlos) with a single specialized service for vascular patients. According to the census in 1991, the total population for the catchment area was 569,307 (261,529 men and 307,778 women). The population with diabetes was calculated according to the Lejona study (6). In this study, the prevalence of diabetes was estimated by a randomized sample stratified by 5-year age-groups and sex. The data of this study were used to calculate the number of people with diabetes according to age and sex distribution, bearing in mind that the population composition in both Lejona (Vizcaya) and Madrid are comparable. The total diabetic population estimated (diagnosed and unknown) was 37,932 (15,505 men and 22,427 women). From 1991 to 1996, the date of the last census, the catchment area was reduced by ~4,000 inhabitants, which was taken into account in order to calculate incidence rates. According to the Global Lower Extremity Amputation Study (3), amputation was defined as the complete loss of any part of the lower limb. The term "minor amputation" refers to an amputation distal to the tarsometatarsal joint, whereas "major amputation" refers to one through or proximal to the tarsometatarsal joint. All patients who underwent an LEA between 1 January 1989 and 31 December 1999 were identified through operating theater records. Vascular surgery department and endocrinology service discharge records were used as secondary sources. Only patients who had resided in area 7 for at least the last 6 months were included in the study. The social security system in our country covers between 70 and 100% of the cost of pharmacological treatments when prescribed by a social security doctor. Patients suffering from LEAs usually have some pharmacological treatment. Thus, prescribing physicians (family doctors and physicians of the endocrinology service in an outpatient setting) were used as an additional source to identify people who were amputated outside area 7. This source was operative since 1994. Date of birth, sex, address, date and level of the actual amputation, and potential causes of the actual amputation were registered. Estimations of the level of ascertainment were derived by using capture-recapture methods (5). Because three independent sources were available from 1994, the level of ascertainment was calculated during the 1994–1999 period. To have a greater number of cases, men and women were analyzed together.

Incidence of LEA data are presented over three periods. From 1989 to 1993 no intervention was done. Since 1994, a series of improvements in therapeutic measures in diabetes management are available in our area, including a prophylactic foot care teaching and treatment program (7). As the efficacy of these measures have improved across time, we have studied the incidence of LEAs during two 3-year periods (1994–1996 and 1997–1999). From 1989 to 1993, 139 diabetic subjects and 118 nondiabetic subjects underwent 156 and 139 nontraumatic LEAs, respectively, while from 1994 to 1999, 91 diabetic and 43 nondiabetic subjects suffered 111 and 49 nontraumatic LEAs, respectively. Age-adjusted incidence per 10^5 people in risk per year for first LEAs (95% CI) decreased from 1989–1993 to 1997–1999 in diabetic people as follows: major LEAs decreased from 67.1 (60.9–73.3) to 12.3 (14.1–10.5) and from 13.3 (11.6–15.0) to 5.6 (4.9–6.3) for men and women, respectively; minor LEAs decreased from 52.1 (45.0–59.2) to 22.5 (19.7–25.3) and from 10.9 (10.3–11.5) to 7.9 (6.8–9.0) for men and women, respectively. Data for nondiabetic people are as follows: major LEAs decreased from 2.6 (2.2–3.0) to 1.1 (0.4–1.8) and from 1.3 (1.1–1.5) to 0.5 (0.4–0.6) in men and women, respectively; minor LEAs decreased from 2.7 (2.1–3.3) to 0.7 (0.6–0.8) and from 1.5 (1.3–1-7) to 0 for men and women, respectively. Crude and age-adjusted incidence per 10^5 people in risk per year for first and all (major and minor) LEAs for the three periods are displayed in Table 1.

The incidence of LEAs rose steeply with age >40 years and it rose dramatically with age >80 years; it was higher in men than in women. The incidence of major LEAs was greater than that of minor LEAs in nondiabetic people, whereas diabetic subjects more frequently suffered minor LEAs.

For major LEAs, estimations of ascertainment were 100 and 98.7%, and for minor LEAs, they were 83.8 and 76.9%. Given that more than one condition was present per patient, LEAs in diabetic subjects were associated with peripheral vascular disease (PVD) (major/minor: 100/62%), neuropathy (major/minor: 78/92%), and infection (major/minor: 24/84%), whereas LEAs in nondiabetic subjects were associated with PVD (major/minor: 100/98%), neuropathy (major/minor: 22/33%), and infection (major/minor: 6/18%). According to the St. Vincent Declaration, several studies in recent years have found a decrease in the incidence of LEAs in people with diabetes.

However, only a few studies (8–10) have analyzed a 10-year period, as this one has. Furthermore, to our knowledge, no other study has used the capture-recapture method to estimate the level of ascertainment from more than two separate data sources and to measure the incidence of first and all LEAs in a separate way in the whole population (i.e., those with and without diabetes). According to our data, LEA incidence in area 7 of Madrid remains the lowest reported incidence in a Caucasian population of both diabetic and nondiabetic people. Primary prevention is defined as a reduction in the incidence of a disease, e.g., first LEA. Since 1994, following the provision of improved foot care after the diabetes program was available (7), a reduction in first LEA of 57 and 81% (minor and major, respectively) in diabetic men and of 28

Table 1—*Crude and age-adjusted incidence (number per 10^5 people in risk per year) of first and all LEAs*

	1989–1993				1994–1996				1997–1999				Decrease (%)	
	First		All		First		All		First		All		First	All
	Crude	A-A	Crude	A-A	Crude	A-A	Crude	A-A	Crude	A-A	Crude	A-A		
Diabetic subjects														
Major														
Men	69.5	67.1	72.5	70.6	38.2	36.9	42.5	41.4	12.7	12.3	12.7	12.4	81.7	82.5
Women	21.3	13.3	24.5	15.3	12.6	7.9	14.4	9.0	9.0	5.6	9.0	5.6	57.7	63.3
Minor														
Men	54.3	52.1	60.6	58.9	40.4	38.8	59.9	57.8	23.4	22.5	34.0	33.1	56.9	43.9
Women	17.5	10.9	19.0	11.9	14.4	9.0	18.0	11.3	12.6	7.9	18.0	11.3	28.1	5.3
Nondiabetic subjects														
Major														
Men	2.7	2.6	2.8	2.7	1.1	1.1	1.1	1.1	1.1	1.1	1.3	1.3	59.2	48.1
Women	2.0	1.3	2.4	1.5	0.6	0.4	0.6	0.4	0.8	0.5	0.9	0.6	60.0	62.5
Minor														
Men	1.1	1.1	1.4	1.4	0.5	0.5	0.5	0.5	0.7	0.7	0.7	0.7	36.4	50.0
Women	0.8	0.5	0.9	0.6	0.1	0.1	0.1	0.1	0	0	0.1	0.1	100	88.9

A-A, age-adjusted.

and 57% were detected, with a 5- to 6-year increase in age at the first LEA.

These data indicate that efforts to delay and reduce the incidence of first LEA in people with diabetes succeeded. In a similar way, a slightly higher reduction in first LEAs was found in the nondiabetic population also at the end of the study. Several factors may contribute to these data. The decline in LEA rates can be partially explained by favorable trends in some risk factors and a better control in others, including dietary factors as well as a tighter control of hypertension, hypercholesterolemia, and reduced tobacco and alcohol consumption (11). Better control of these risk factors should positively affect the entire population. In addition, the increasing availability of resources for the usual clinical management of these patients, including new drugs and surgical procedures (12) covered by the social security system, should also be operative.

This study also evaluated the conditions associated with LEAs. The proportion of major LEAs associated with PVD was high and similar in both diabetic and nondiabetic population. The proportion of minor LEAs associated with neuropathy and infection in diabetic subjects was clearly greater than that in the nondiabetic population. This could explain the excess of minor LEAs in diabetic people, suggesting that we should try to cope with these problems.

In conclusion, our data show that the incidence of LEAs in area 7 of Madrid remains as the lowest incidence reported in European countries in both diabetic and nondiabetic people. A substantial decrease in LEAs and a later presentation in relation to a series of improvements in diabetic treatment were detected. Despite these figures, the incidence of LEAs remains higher in diabetic subjects than in the nondiabetic population, suggesting that diabetic foot care remains suboptimal in Madrid. Taking into account that diabetic subjects suffer minor LEAs more frequently than nondiabetic people and that it is associated with neuropathy and infection, a more substantial reduction in LEAs in diabetic people should be achieved with an earlier neuropathy diagnosis and an adequate antibiotic management.

Alfonso L. Calle-Pascual, md[1]
Nuria Garcia-Torre, md[1]
Inmaculada Moraga, md[1]
Jose A. Diaz, md[1]
Alejandra Duran, md[1]
Guillermo Moñux, md[2]
Francisco J. Serrano, md[2]
Pedro J. Martín-Alvarez, phd[3]
Aniceto Charro, md[1]
Juan P. Marañes, md[1]

From the [1]Department of Endocrinology Metabolism and Nutrition, Hospital Clínico San Carlos, Madrid; the [2]Department of Vascular Surgery, Hospital Clínico San Carlo, Madrid; and [3]Juan de la Cierva College, Consejo Superior Investigaciones Cientificas, Madrid, Spain.

Address correspondence to A.L. Calle-Pascual, MD, Department of Endocrinology Metabolism and Nutrition 1ªS. Hospital, Clínico San Carlos, C/Martín Lagos s/n, E-28040 Madrid, Spain. E-mail: acalle@hcsc.insalud.es.

References

1. Amos AF, McCarty DJ, Zimmet P: The rising global burden of diabetes and its complications: estimates and projections to the year 2010. *Diabet Med* 14 (Suppl. 5): S7–S85, 1997
2. Mayfield JA, Reiber GE, Sander LJ, Janisse D, Pogach LM: Preventive foot care in people with diabetes. *Diabetes Care* 21: 2161–2177, 1998
3. The Global Lower Extremity Amputation Study Group: Epidemiology of lower extremity amputation in centres in Europe, North America and East Asia. *Br J Surg* 87:328–337, 2000
4. The LEA Study Group: Comparing the incidence of lower extremity amputations across the world: the global lower extremity amputations study. *Diabet Med* 12:14–18, 1995
5. International Working Group for Disease Monitoring and Forecasting: Capture-recapture and multiple-record systems estimation: applications in human diseases. *Am J Epidemiol* 142:1047–1058, 1995
6. Bayo J, Sola C, Garcia F, Latorre PM, Vazquez JA: Prevalencia de la diabetes mellitus no dependiente de la insulina en Lejona (Vizcaya). *Med Clin* 101:609–612, 1993
7. Ebskov LB, Schroeder TV, Holstein P: Ep-

idemiology of leg amputations: the influence of vascular surgery. *Br J Surg* 81: 1600–1604, 1994
8. Ebskov B, Ebskov LB: Major lower limb amputations in diabetic patients: development during 1982–1993. *Diabetologia* 39:1607–1610, 1996
9. Larsson J, Apelqvist J, Agardh CD, Stenstrom A: Decreasing incidence of major amputation in diabetic patients—a consequence of a multidisciplinary foot care team approach? *Diabet Med* 12:770–776, 1995
10. Calle-Pascual, Duran A, Benedi A, Calvo MI, Charro A, Diaz JA, Calle JR, Gil E, Ibarra J, Maranes JP, Cabezas-Cerrato J: Reduction in foot ulcer incidence: relation to compliance with a prophylactic foot-care programme. *Diabetes Care* 24:405–407, 2001
11. The Diabetes and Nutrition Study Group of the Spanish Diabetes Association: Diabetes Nutrition and Complications Trial (DNCT): Food intake and targets of diabetes treatment in a sample of Spanish people with diabetes. *Diabetes Care* 20: 1078–1080, 1997
12. Calle-Pascual AL, Duran A, Diaz A, Monux G, Serrano FJ,: Garcia de la Torre N, Moraga I, Calle JR, Charro A, Maranes JP: Comparison of peripheral arterial reconstruction in diabetic and nondiabetic patients: a prospective clinic-based study *Diabetes Res Clin Pract* 53:129–136, 2001

Increased Risk of Lower-Extremity Amputation Among Caucasian Diabetic Patients on Dialysis

In two recent issues of *Diabetes Care*, high amputation rates among Maori people from New Zealand (1) and Native Americans (2) with diabetes on dialysis were reported. We would like to add some information concerning Caucasians with diabetic foot syndrome and advanced renal disease from a prospective sample of 400 consecutive patients treated at our diabetic foot clinic. Similar to chronic renal insufficiency (defined by serum creatinine concentration 1.5–3.0 mg/dl), chronic renal failure and end-stage renal disease (ESRD) should be regarded as a continuum (3). We analyzed the frequency of these conditions among the entire study population and compared presentation features and outcomes among the concerned patients and among diabetic foot patients without obvious renal impairment.

At the time of presentation for the index foot lesion, 14 patients (4%, group A) were on dialysis, 72 patients (18%, group B) suffered from chronic renal insufficiency (serum creatinine ≥1.5 mg/dl), and 314 patients (78%, group C) had no signs of advanced renal impairment.

There were no significant differences among the groups regarding age, diabetes duration, or quality of metabolic control. The proportions of underlying neuropathy (86, 91, and 79%, respectively) and peripheral vascular disease (71, 56, and 50%, respectively) were higher among patients with renal impairment but not significantly different from the proportions of patients with normal renal function. However, the coincidence of peripheral vascular disease (PVD) and neuropathy was far more common in diabetic patients on dialysis than in patients without renal impairment (71% in group A vs. 36% in group C, $P = 0.015$); therefore, patients on dialysis are at even higher risk for unrecognized nonhealing foot lesions. Coexistence of PVD and mediasclerosis was also far more frequent in the dialysis group than in the other two groups (80 vs. 26 and 34%, $P = 0.01$ and $P = 0.003$).

Total amputation rate was 57% for dialysis patients and was therefore significantly higher than that among patients with chronic renal insufficiency (25%, $P = 0.006$) or patients without obvious renal impairment (16%, $P < 0.001$). This was primarily caused by a sixfold increased risk of major amputation of dialysis patients compared with patients with normal renal function (29 vs. 4.5%, $P < 0.001$). One-year survival was poor among diabetic patients on dialysis with foot lesions compared with their counterparts without renal impairment (71 vs. 94%, $P = 0.007$). However, a higher proportion of patients in the dialysis group were suitable for vascular intervention compared with the other two groups (46 vs. 14 and 13%, $P = 0.02$). After successful revascularization, the risk of consecutive major amputation was similar in all three groups (17, 20, and 16%, respectively). This last finding is in contrast to previous publications (4–7) that indicated an increased risk of amputation even in the presence of patent grafts after revascularization among ESRD patients, caused by nonhealing large foot ulcers and uncontrolled infection. The better outcome in our series of diabetic patients on dialysis with foot lesions might be explained by the structured interdisciplinary inpatient care after surgery, as well as the continued care by the diabetic foot clinic after hospital discharge. This interpretation is supported by the observations of Foster et al. (8), who showed that care by a special diabetic foot clinic reduces episodes of digital gangrene and major amputation in diabetic renal transplant patients.

In conclusion, Caucasian patients with diabetes on dialysis are at high risk for amputation once a foot lesion occurs. However, vascular interventions should not be refused to those patients if structured interdisciplinary inpatient care after surgery and long-term follow up by a special diabetic foot clinic are available. Prevention of foot complications among diabetic patients with ESRD and prognosis improvement in the case of an established foot lesion should be targeted by intense collaboration of dialysis units and specialized diabetic foot centers.

STEPHAN MORBACH, MD[1]
CHRISTOPH QUANTE, MD[1]
HERMANN RUDOLF OCHS, MD[1]
FRANZ GASCHLER, MD[2]
JEAN-MARIO PALLAST, MD[3]
UDO KNEVELS, MD[3]

From the Marienkrankenhaus Departments of [1]Internal Medicine, Diabetic Foot Clinic, and [2]Vascular Surgery, Soest, Germany, and the [3]Limited Care Dialysis Unit, Soest, Germany.

Address correspondence to Stephan Morbach, MD, Department of Internal Medicine, Marienkrankenhaus, Widumgasse 5, 59494 Soest, Germany. E-mail: morbachs@aol.com.

References

1. McGrath NM, Curran BA: Recent commencement of dialysis is a risk factor for lower-extremity amputation in a high-risk diabetic population. *Diabetes Care* 23: 432–433, 2000
2. Rith-Najarian S, Gohdes D: Preventing amputations among patients with diabetes on dialysis. *Diabetes Care* 23:1445–1446, 2000
3. Luke G: Chronic renal failure: a vasculopathic state. *N Engl J Med* 339: 841–843, 1998
4. Edwards JM, Taylor LM, Porter JM: Limb salvage in end-stage renal disease (ESRD). *Arch Surg* 123:1164–1168, 1988
5. Sanchez LA, Goldsmith J, Rivers SP, Pan-

etta TF, Wengerter KR, Veith FJ: Limb salvage surgery in end stage renal disease: is it worthwhile? *J Cardiovasc* Surg 33:344–348, 1992
6. Johnson BL, Glickman MH, Bennik DF, Esses GE: Failure of foot salvage in patients with end-stage renal disease after surgical revascularization. *J Vasc Surg* 22: 280–286, 1995
7. Leers SA, Reifsnyder T, Delmonte R, Caron M: Realistic expectations for pedal bypass grafts in patients with end-stage renal disease. *J Vasc Surg* 28: 976–983: 1998
8. Foster AVM, Snowden S, Grenfell A, Watkins PJ, Edmonds ME: Reduction of gangrene and amputations in diabetic renal transplant patients: the role of a special foot clinic. *Diabet Med* 12:632–635, 1995

Acute Charcot's Arthropathy Despite 11 Years of Normoglycemia After Successful Kidney and Pancreas Transplantation

Charcot's arthropathy is one of the most debilitating orthopedic sequelae of diabetes. Regarding its pathogenesis, the current theory suggests a role of diabetic peripheral and autonomic neuropathy (1).

Kidney-pancreas transplantation is an accepted therapeutic approach in uremic type 1 diabetic patients, providing normalization of glycometabolic control and improvement of secondary diabetic complications, including neuropathy (2–4).

We report a case of a diabetic uremic patient who developed acute Charcot's arthropathy despite 11 years of tight metabolic control after successful kidney and pancreas transplantation. A 41-year-old type 1 diabetic uremic male patient underwent kidney and pancreas transplantation. His immunosuppression regimen consisted of steroids, cyclosporin, and azathioprine. During the entire follow-up period, the patient was insulin-free, in good metabolic control (HbA_{1c} 5.2 ± 0.6%), and had good renal function (creatinine 1.3 ± 0.1 mg/dl). At baseline, neurological examination and electromiography documented symmetrical sensory loss in the distal lower extremities and motor deficits mostly on the right side, with footdrop. A test of autonomic function showed a near normal blood pressure response to standing. During deep breathing, the heart rate was compromised, whereas the response to standing was normal.

After transplantation, an improvement of motor and sensory nerve conduction velocity was observed during the follow-up period, and the median nerve reached normal values at 5 years, whereas sural and peroneal nerves remained severely impaired. Bone mineral density by dual-energy X-ray absorptiometry with Hologic was evaluated regularly, and the results showed stable osteopenia.

Eleven years after transplantation, the patient showed a painless profound swelling of the right ankle joint with locally increased skin temperature, joint effusion, and erythema. X-ray, computerized tomography, magnetic resonance, and Technetium-99 bone scan showed an erosive area at the third distal segment of the tibia, fibula, and astragalus, with involvement of the surrounding soft tissue and joint effusion. The patient was submitted to surgical tibiotalar fusion. During surgery, samples were collected for histological test and microbiological evaluation. Histological examination showed broken detached osteocartilagineus connection without evidence of phlogistic infiltration, and cultures were negative.

This patient, despite long-term functioning kidney and pancreas transplantation, developed Charcot's arthropathy. The clinical diagnosis was confirmed by histological analysis via bone and synovial biopsy—negative cultures ruled out the hypothesis of an infective arthropathy. The development of Charcot's arthropathy in this patient was probably multifactorial. Among the factors that could have influenced the development of this artropathy, steroids did not seem to have played a fundamental role because they were administered for a short period after transplantation and because a progression of osteopenia was not observed. Cyclosporin was described to induce peripheral neuropathy (5). This could counterbalance the positive effects of tight metabolic control on diabetic neuropathy, thus leading to impairment of nerve function. In fact, in this patient, diabetic neuropathy was partially reversed.

We can conclude that in patients already affected by severe diabetic complications, tight metabolic control achieved with pancreas transplantation does not prevent the development of Charcot's arthropathy.

ROSSANA CALDARA, MD[1]
CRISPINO GRISPIGNI, MD[2]
ENNIO LA ROCCA, MD[1]
PAOLA MAFFI, MD[1]
ELENA ORSENIGO, MD[3]
CARLO SOCCI, MD[3]
GIANFRANCO FRASCHINI, MD[2]
VALERIO DI CARLO, MD[3]
GUIDO POZZA, MD[1]
ANTONIO SECCHI, MD[1]

From the Departments of [1]Internal Medicine, [2]Orthopedics, and [3]General Surgery, San Raffaele Scientific Institute, University of Milan, Milan, Italy.

Address correspondence to Antonio Secchi, Associate Professor of Medicine, University of Milan, San Raffaele Scientific Institute, Via Olgettina 60, 20132 Milan, Italy. E-mail: secchi.antonio@hsr.it.

References

1. Armstrong DG, Todd WF, Lavery LA, Harkless LB, Bushman TR: The natural history of acute Charcot's arthropathy in a diabetic foot specialty clinic. *Diabet Med* 14:357–367, 1997
2. Caldara R, La Rocca E, Maffi P, Secchi A: Effects of pancreas transplantation on late complications of diabetes and metabolic effects of pancreas and islet transplantation. *J Pediatr Endocrinol Metab* 12:777–787, 1999
3. Landgraf R: Impact of pancreas transplantation on diabetic complications and quality of life. *Diabetologia* 39:1415–1424, 1996
4. Martinenghi S, Comi G, Galardi G, Di Carlo V, Pozza G, Secchi A: Amelioration of nerve conduction velocity following simultaneous kidney/pancreas transplantation is due to the glycaemic control provided by the pancreas. *Diabetologia* 40: 1110–1112, 1997
5. Gijtenbeek JM, van den Bent MJ, Vecht CJ: Ciclosporine neurotoxicity: a review. *J Neurol* 246:339–346, 1999

Homogeneity of Metabolic Control in New South Wales and the Australian Capital Territory, Australia

Socioeconomic disadvantage is associated with higher morbidity and poorer health behavior, despite higher rates of health service utilization (1). In a study of American children with

diabetes, lower socioeconomic status was associated with poorer glycemic control and more hypoglycemic episodes (2). In an audit of 1,190 children with type 1 diabetes aged <15 years, we examined whether socioeconomic disadvantage or urban/rural location affect metabolic control in Australia, which has universal health insurance and subsidies for diabetes supplies.

Case attainment, based on the Diabetes Register, was 67% of all eligible children living in New South Wales (NSW) and the Australian Capital Territory (ACT). There were 649 girls and 541 boys recruited from three multidisciplinary hospital-based pediatric diabetes units, four private city-based pediatric diabetes practices, and 18 rural diabetes outreach clinics. Social disadvantage was assessed using a graded postcode-based social disadvantage risk score (SDRS) derived from nine risk indicators (3). HbA_{1c} was assayed in a central laboratory using the Bio-Rad Variant HPLC Capillary Collection System (Bio-Rad Laboratories, Munich, Germany). Level II Laboratory Certification of Traceability to the Diabetes Control and Complications Trial (DCCT) reference method was obtained from the University of Missouri Secondary Reference Laboratory no. 1.

The median HbA_{1c} was 8.2% (interquartile range 7.6–9.1%). The significant predictors of HbA_{1c} in a multiple regression model were longer duration (b = 0.04, P = 0.0001) and insulin dose per kilogram (b = 0.44, P = 0.0001). After excluding subjects with duration <2 years, a more complex model resulted, with a significant interaction between age and duration: HbA_{1c} = 8.74 + 0.28 (insulin per kilogram) − 0.15 (duration) − 0.05 (age) + 0.01 (duration × age).

Rural children lived in areas of lower social advantage (SDRS −0.4 ± 0.6) than city-based children cared for in either the public sector (0.4 ± 0.7) or the private sector (1.0 ± 0.7) (P < 0.0001). There was no significant difference in the median HbA_{1c} levels among the 25 different centers (range 7.7–8.7%), and SDRS was not significant in the multiple regression model. There was no difference in the metabolic control of children living in rural locations compared with those from urban locations (median HbA_{1c} 8.2% in both).

The incidence of severe hypoglycemia (unconsciousness or seizures) was 36 per 100 patient-years. HbA_{1c}, urban/rural location, or SDRS were not significant predictors of hypoglycemia in a Poisson regression model. No significant differences were found for either metabolic control or rate of hypoglycemia between children cared for in the public or private system, despite significantly greater social advantage in the latter group.

The median HbA_{1c} of 8.2% in NSW/ACT is comparable with results from the multinational Hvidore study (mean HbA_{1c} 8.6%, DCCT equivalent 8.3%) (4). In that study, there was significant variation in metabolic control among 22 centers from 18 countries. In contrast, the audit from NSW/ACT shows remarkable homogeneity in metabolic control among centers, regardless of location, socioeconomic status, or system of care.

PENNY HANDELSMAN, MPH[1]
MARIA E. CRAIG, FRACP[1]
KIM C. DONAGHUE, FRACP[1]
ALBERT CHAN, M APP STAT[1]
BARBARA BLADES, PHD[1]
ROSETTA LAINA, MSC[1]
DARNA BRADFORD, BSC (HONS)[1]
ANGELA MIDDLEHURST, RN[1]
GEOFFREY AMBLER, FRACP, MD[1]
CHARLES F. VERGE, FRACP, PHD[2]
PATRICIA CROCK, FRACP[3]
PATRICK MOORE, FRACP[4]
MARTIN SILINK, FRACP, MD[1]
FOR THE NSW/ACT HbA_{1c} STUDY GROUP

From the [1]The Children's Hospital at Westmead, Sydney, New South Wales; the [2]Sydney Children's Hospital, Randwick, New South Wales; the [3]John Hunter Children's Hospital, Newcastle, New South Wales; and [4]The Canberra Hospital, Canberra, Australia Capital Territory, Australia.

Address correspondence to Dr. Maria Craig, Ray Williams Institute of Paediatric Endocrinology, Diabetes and Metabolism, The Children's Hospital at Westmead, Locked Bag 4001, Westmead, NSW 2145, Australia. E-mail: mariac@chw.edu.au.

Acknowledgments— This study was supported by an educational grant from Novo Nordisk and Bio-Rad Australia.

References

1. Turrell G, Mathers CD: Socioeconomic status and health in Australia. *Med J Aust* 172:434–438, 2000
2. Overstreet S, Holmes CS, Dunlap WP, Frentz J: Sociodemographic risk factors to disease control in children with diabetes. *Diabet Med* 14:153–157, 1997
3. *Unequal in Life*: *The Distribution of Social Disadvantage in Victoria and New South Wales*. Victoria, Australia, The Ignatius Centre for Social Policy and Research, 1999
4. Mortensen HB, Hougaard P: Comparison of metabolic control in a cross-sectional study of 2,873 children and adolescents with IDDM from 18 countries: the Hvidore Study Group on Childhood Diabetes. *Diabetes Care* 20:714–720, 1997

Remnant-Like Particle Cholesterol and Insulin Resistance in Nonobese Nonhypertensive Japanese Glucose-Tolerant Relatives of Type 2 Diabetic Patients

Patients with type 2 diabetes and impaired glucose tolerance (IGT) have increased incidence of coronary heart disease (CHD) (1,2). The risk of CHD appears to be similar in patients with type 2 diabetes and IGT (3,4). Thus, factors other than the level of glycemia seem to accelerate the development of CHD in type 2 diabetes. This idea is supported by the notion that the duration of diabetes and the level of glycemia are risk factors for microvascular disease but not for CHD in type 2 diabetic patients (5,6). Alternatively, it may be hypothesized that the relation between type 2 diabetes and CHD may not be causal and that the events preceding the onset of diabetes (i.e., the prediabetic state) may contribute to CHD.

Whereas insulin resistance is considered to be associated with CHD, Kugiyama et al. (7) showed that fasting remnant lipoprotein levels predict coronary events in patients with CHD. Ai et al. (8) and our group (9) recently disclosed that remnant lipoprotein is associated with insulin resistance in IGT subjects and type 2 diabetic patients. Thus, remnant lipoprotein, in conjunction with insulin resistance, is considered one of the important risk factors for the development of CHD in type 2 diabetes.

Although glucose-tolerant relatives of type 2 diabetic patients are considered to

be insulin resistant (10–12), it is unclear whether remnant lipoprotein is increased in glucose-tolerant relatives of type 2 diabetic patients. Only one study contains data on the lipid profile in nondiabetic relatives of type 2 diabetic patients. Laws et al. (13) documented that nondiabetic relatives of patients with type 2 diabetes had insulin resistance and hypertriglyceridemia. However, they did not study remnant lipoprotein, and the patients studied were all obese. It is well known that the degree of being overweight per se affects insulin resistance and lipid profile (14). In addition, hypertension itself is associated with insulin resistance and lipid abnormalities in humans (15). We therefore recruited nonobese nonhypertensive glucose-tolerant relatives of type 2 diabetic patients who were carefully matched for BMI, blood pressure, and fasting glucose to glucose-tolerant subjects without any family history of type 2 diabetes. This is the first report showing that nonobese nonhypertensive Japanese glucose-tolerant relatives of type 2 diabetic patients have already high remnant lipoprotein and diminished insulin sensitivity before the onset of diabetes.

A total of 45 healthy glucose-tolerant relatives with type 2 diabetes (offspring) participated in the study (34 men and 11 women). They all had a parent who developed type 2 diabetes. The control group consisted of 65 healthy glucose-tolerant subjects without a family history of type 2 diabetes (52 men and 13 women). All subjects studied had a BMI <25 kg/m^2 and a blood pressure measurement $<140/90$ mmHg. They all had normal glucose tolerance on the basis of at least two fasting plasma glucose concentrations <110 mg/dl (16,17).

All subjects studied were Japanese and had ingested at least 150 g carbohydrate for the 3 days preceding the study. They did not perform heavy exercise for at least 1 week before the study. None of the subjects had significant renal, hepatic, or cardiovascular disease or were taking any medication. Blood was drawn the morning after a 12-h fast. Plasma glucose was measured with the glucose oxidase method, and serum insulin was measured using a two-site immunoradiometric assay (Insulin Riabead II; Dainabot, Osaka, Japan). Coefficients of variation were 4% for insulin >25 μU/ml and 7% for insulin <25 μU/ml, respectively. The total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, and remnant-like particle cholesterol (RLP-C) were also measured. The LDL cholesterol level was calculated using the Friedewald formula (18). The RLP-C level was measured by the method reported by Nakajima et al. (19). The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the following formula: fasting serum insulin (μU/ml) × fasting plasma glucose (mmol/l)/22.5, as described by Matthews et al. (20).

The statistical analysis was performed with the StatView 5.0 system (Statview, Berkeley, CA). The differences of the means were determined by Student's *t* test. Data are expressed as means ± SEM.

The clinical characteristics and clinical profile between offspring ($n = 45$) and control subjects ($n = 65$) were compared. No significant difference was observed in age (36.5 ± 1.3 vs. 37.6 ± 1.0 years, $P = 0.281$), systolic (117 ± 2 vs. 114 ± 1 mmHg, $P = 0.158$) and diastolic (68 ± 1 vs. 66 ± 1 mmHg, $P = 0.226$) blood pressure, and fasting glucose (91 ± 1 vs. 89 ± 1 mg/dl, $P = 0.493$) levels between the two groups. The offspring subjects had higher BMI (22.2 ± 0.3 vs. 22.0 ± 0.2 kg/m^2, $P = 0.252$) and total (190 ± 4 vs. 184 ± 2 mg/dl, $P = 0.092$) cholesterol levels than normal control subjects, but the difference was not statistically significant. In contrast, the offspring subjects had significantly higher serum triglyceride (98 ± 9 vs. 76 ± 3 mg/dl, $P = 0.005$), RLP-C (4.0 ± 0.3 vs. 2.8 ± 0.1, $P = 0.006$), serum insulin (5.6 ± 0.3 vs. 4.7 ± 0.1 μU/ml, $P = 0.008$), and HOMA-IR (1.25 ± 0.07 vs. 1.05 ± 0.03, $P = 0.008$) levels than normal control subjects. No significant difference was observed in HDL (60 ± 2 vs. 59 ± 1 mg/dl, $P = 0.359$) and LDL (110 ± 4 vs. 110 ± 2 mg/dl, $P = 0.455$) cholesterol levels between the two groups.

In the present study, we demonstrated that nonobese healthy glucose-tolerant relatives of type 2 diabetic patients had higher HOMA-IR levels compared with normal control subjects. This is compatible with some previous reports that showed insulin insensitivity in relatives of type 2 diabetic patients (10–12). However, which factor contributes to insulin resistance in diabetic offspring subjects has yet to be clarified. BMI, blood pressure, and fasting glucose levels are known to be associated with insulin resistance in humans (14). Hence, we recruited nonobese nonhypertensive glucose-tolerant relatives of type 2 diabetic patients and normal control subjects who had no family history of diabetes, taking into account BMI, blood pressure, and fasting glucose levels. All subjects studied had a BMI <25 kg/m^2, blood pressure $<140/90$ mmHg, and a fasting glucose level <110 mg/dl.

Interestingly, our offspring subjects not only had higher HOMA-IR, but also had higher serum triglycerides and RLP-C levels compared with normal control subjects. However, there was no significant difference in age, BMI, total cholesterol, HDL cholesterol, and LDL cholesterol levels between the two groups. These findings, therefore, suggest that triglyceride or RLP-C, but not BMI, is associated with insulin resistance in nonobese nonhypertensive glucose-tolerant relatives of type 2 diabetic patients.

The mechanisms by which serum triglyceride or RLP-C level is associated with insulin resistance in nonobese nonhypertensive glucose-tolerant relatives of type 2 diabetic patients are presently not known. Although insulin resistance has been suggested to be an underlying defect leading to the development of endogenous hypertriglyceridemia (21), there are some data suggesting that elevated triglyceride levels are preceding factors for the development of insulin resistance (22,23). In families with multiple cases of hypertriglyceridemia, increased serum triglycerides levels serve as a risk marker for subsequent development of type 2 diabetes (22). Mingrone et al. (23) reported on two sisters with extreme hypertriglyceridemia and diabetes in whom the normalization of serum triglycerides by operation improved insulin resistance and glucose tolerance. We recently disclosed that Japanese type 2 diabetic patients with insulin resistance had significantly higher triglyceride levels than those with normal insulin sensitivity (9,24,25). We later demonstrated that physical exercise lowers triglyceride, fasting glucose, and HOMA-IR levels in Japanese type 2 diabetic patients without affecting BMI (26). Furthermore, we showed that bezafibrate not only reduces serum triglyceride levels but also improves insulin sensitivity and glycemic control in type 2 diabetic patients (27). Paolisso et al. (28) recently demonstrated that simvastatin and atorvastatin, a lipid-lowering drug, reduce serum triglycerides, insulin resistance, and HbA_{1c} in type

2 diabetic patients. An in vitro study (29) showed that incubating IM-9 lymphocytes with VLDL downregulates the cell's insulin receptor. Insulin binding to erythrocytes in the blood of patients with hypertriglyceridemia is reported to be low (30).

Regardless of the mechanism, our present study showing that both high RLP-C and insulin resistance exist in glucose-tolerant relatives of type 2 diabetic patients might suggest that the events preceding the onset of diabetes contribute to the evolution of CHD. Alternatively, our present study might support the view that the duration of diabetes and the level of glycemia, the risk factors for microvascular disease, are not risk factors for CHD in type 2 diabetic patients (5,6).

In summary, nonobese nonhypertensive Japanese glucose-tolerant relatives of type 2 diabetic patients are characterized by impairments in insulin sensitivity, high serum triglyceride levels, and high RLP-C levels. Further study should be undertaken to clarify whether diabetic offspring in association with high RLP-C and insulin resistance is a risk factor for coronary atherosclerosis.

Acknowledgments— We thank the Department of Biochemistry, Kansai-Denryoku Hospital, Shionogi Biomedical Laboratory, and Ootsuka Pharmaceutical Company, Japan, for their help in our research.

MITSUO FUKUSHIMA, MD[1]
ATARU TANIGUCHI, MD[2]
YOSHIKATSU NAKAI, MD[3]
MASAHIKO SAKAI, MD[2]
KENTARO DOI, MD[4]
KAZUKO NIN, MD[5]
TSUYOSHI OGUMA, MD[2]
SHOICHIRO NAGASAKA, MD[6]
KUMPEI TOKUYAMA, PHD[7]
YUTAKA SEINO, MD[1]

From the [1]Department of Metabolism and Clinical Nutrition, Graduate School of Medicine, Kyoto University, Kyoto; the [2]Division of Diabetes, Kansai-Denryoku Hospital, Osaka; the [3]College of Medical Technology, Kyoto University, Kyoto; [4]Graduate School of Medicine, Kyoto University, Kyoto; [5]Nagoya University School of Health Sciences, Aichi; the [6]Division of Endocrinology and Metabolism, Jichi Medical School, Tochigi; and [7]Laboratory of Biochemistry of Exercise and Nutrition, Tsukuba University, Tsukuba, Japan.

Address correspondence to Ataru Taniguchi, M.D., First Department of Internal Medicine, Kansai-Denryoku Hospital, 2-1-7 Fukushima, Fukushima-ku, Osaka-city, Osaka 553-0003 Japan. E-mail: k-58403@kepco.co.jp.

References

1. Bierman EL: Atherogenesis in diabetes. *Arterioscler Thromb* 12:647–656, 1992
2. Piedrola G, Novo E, Serrano-Gotarredona J, Escobar-Morreale HF, Villa E, Luna JD, Garcia-Robles R: Insulin resistance in patients with a recent diagnosis of coronary artery disease. *J Hypertens* 14:1477–1482, 1996
3. Fuller JH, Shipley MJ, Rose G, Jarrett RJ, Keen H: Coronary heart disease risk and impaired glucose tolerance. *Lancet* 1:1373–1376, 1980
4. Yano K, Kagan A, McGee D, Rhoads GG: Glucose intolerance and nine-year mortality in Japanese men in Hawaii. *Am J Med* 72:71–80, 1982
5. Diabetes Drafting Group: Prevalence of small vessel and large vessel disease in diabetic persons from 14 centers: the World Health Organization multinational study of vascular disease in diabetics. *Diabetologia* 28:615–640, 1985
6. Herman JB, Medalie JH, Goldbourt U: Differences in cardiovascular morbidity and mortality between previously known and newly diagnosed adult diabetics. *Diabetologia* 13:229–234, 1977
7. Kugiyama K, Doi H, Takazoe K, Kawano H, Soejima H, Mizuno Y, Tsunoda R, Sakamoto T, Nakano T, Nakajima K, Ogawa H, Sugiyama S, Yoshimura M, Yasue H: Remnant lipoprotein levels in fasting serum predict coronary events in patients with coronary artery disease. *Circulation* 99:2858–2860, 1999
8. Ai M, Tanaka A, Ogita K, Sekine M, Numano F, Numano F, Reaven GM: Relationship between hyperinsulinemia and remnant lipoprotein concentrations in patients with impaired glucose tolerance. *J Clin Endocrinol Metab* 85:3557–3560, 2000
9. Taniguchi A, Fukushima M, Sakai M, Miwa K, Makita T, Nagata I, Nagasaka S, Doi K, Okumura T, Fukuda A, Kishimoto H, Fukuda T, Nakaishi S, Tokuyama K, Nakai Y: Remnant-like particle cholesterol, triglycerides, and insulin resistance in nonobese Japanese type 2 diabetic patients. *Diabetes Care* 23:1766–1769, 2000
10. Eriksson J, Kallunki AF, Saloranta EC, Widen E, Schalin C, Groop L: Early metabolic defects in persons at increasing risk for non-insulin-dependent diabetes mellitus. *N Engl J Med* 321:337–343, 1989
11. Osei K, Cottrell KDA, Orabella MM: Insulin sensitivity, glucose effectiveness, and body fat distribution pattern in nondiabetic offspring of patients with NIDDM. *Diabetes Care* 14:890–896, 1991
12. Ramachandran A, Snehalatha AC, Mohan V, Bhattacharyya PK, Viswanathan M: Decreased insulin sensitivity in offspring whose parents both have type 2 diabetes. *Diabet Med* 7:331–334, 1990
13. Laws A, Stefanick ML, Reaven GM: Insulin resistance and hypertriglyceridemia in nondiabetic relatives of patients with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 69:343–347, 1989
14. Reaven GM: Role of insulin resistance in human disease. *Diabetes* 37:1595–1607, 1998
15. Lind L, Berne C, Lithell H: Prevalence of insulin resistance in essential hypertension. *J Hypertens* 13:1457–1462, 1995
16. The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 21 (Suppl. 1):S5–S19, 1998
17. Abbasi F, McLaughlin T, Lamendola C, Yeni-Komshian H, Tanaka A, Wang T, Nakajima K, Reaven GM: Fasting remnant lipoprotein cholesterol and triglyceride concentrations are elevated in nondiabetic, insulin-resistant, female volunteers. *J Clin Endocrinol Metab* 84:3903–3906, 1999
18. Friedewald WT, Levy RI, Frederickson DS: Estimation of the concentration of low-density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. *Clin Chem* 18:499–508, 1972
19. Nakajima K, Saito T, Tamura A, Suzuki M, Nakano T, Adachi M, Tanaka A, Tada T, Nakamura H, Campos E, Havel RJ: Cholesterol in remnant-like lipoproteins in human serum monoclonal anti-apo B-100 and anti apo-A-I immunoaffinity mixed gels. *Clin Chim Acta* 223:53–71, 1993
20. Matthews D, Hosker J, Rudenski A, Naylor B, Treacher D, Turner R: Homeostasis model assessment: insulin resistance and B-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
21. Reaven GM, Lerner RL, Stern MP, Farquhar JW: Role of insulin in endogenous hypertriglyceridemia. *J Clin Invest* 46: 1756–1767, 1967.
22. Sane T, Taskinen MR: Does familiar hypertriglyceridemia predispose to NIDDM? *Diabetes Care* 16:1494–1501, 1993
23. Mingrone G, Henriksen FL, Greco AV, Krogh LN, Capristo E, Gastaldelli A, Castagneto M, Ferrannini E, Gasbarrini G, Beck-Nielsen H: Triglyceride-induced diabetes associated with familiar lipoprotein lipase deficiency. *Diabetes* 48:1258–1263, 1999
24. Taniguchi A, Fukushima M, Sakai M, Kataoka K, Miwa K, Nagata I, Doi K, Tokuyama K, Nakai Y: Insulin-sensitive and insulin-resistant variants in nonobese Japanese type 2 diabetic patients (Letter). *Diabetes Care* 22:2100–2101, 1999

25. Taniguchi A, Fukushima M, Sakai M, Kataoka K, Nagata I, Doi K, Arakawa H, Nagasaka S, Tokuyama K, Nakai Y: The role of the body mass index and triglyceride levels in indentifying insulin-sensitive and insulin-resistant varaints in Japanese non-insulin-dependent diabetic patients. *Metabolism* 49:1001–1005, 2000
26. Taniguchi A, Fukushima M, Sakai M, Nagasaka S, Doi K, Nagata I, Matsushita K, Ooyama Y, Kawamoto A, Nakasone M, Tokuyama K, Nakai Y: Effect of physical training on insulin sensitivity in Japanese type 2 diabetic patients (Letter). *Diabetes Care* 23:857–858, 2000
27. Taniguchi A, Fukushima M, Sakai Y, Tokuyama K, Nagata I, Fukunaga A, Kishimoto H, Doi K, Yamashita Y, Matsuura T, Kitatani N, Okumura T, Nagasaka S, Nakaishi S, Nakai Y: Effects of bezafibrate on insulin sensitivity and insulin secretion in non-obese Japanese type 2 diabetic patients. *Metabolism* 50:477–480, 2001
28. Paolisso G, Barbagallo M, Petrella G, et al: Effects of simvastatin and atorvastatin administration on insulin resistance and respiratory quotient in aged dyslipidemic non-insulin dependent diabetic patients. *Atherosclerosis* 150: 121–127. 2000
29. Steiner G: Insulin and hypertriglyceridemia. *Diabetes*. Waldhausl WK, Ed. Amsterdam, the Netherlands, Excerpta Medica, 1979, p. 590–593
30. Bieger WP, Michel G, Barwich D, Biehl K, Wirth A: Diminished insulin receptors on monocytes and erythrocytes in hypertriglyceridemia. *Metabolism* 33:982–987, 1984

Glucodynamics and Pharmacokinetics of 70/30 vs. 50/50 NPH/Regular Insulin Mixtures After Subcutaneous Injection

Optimal insulin therapy for many patients with type 1 and type 2 diabetes requires the use of combinations of short- and intermediate-acting insulin preparations. A significant number of patients experience difficulties with mixing intermediate- and short-acting insulin preparations because of an inability to mix insulin preparations in correct ratios (1–2) or confusion between the different types of insulins (3–5). The fixed ratio of 70% NPH to 30% regular (70/30) insulin preparation does not fulfill the needs of many of these patients because of significant postprandial blood glucose excursions. A premix insulin preparation, such as 50% NPH to 50% regular (50/50) insulin, could benefit those individuals with postprandial hyperglycemia. To investigate differences in pharmacodynamic and pharmacokinetic properties between 50/50 and 70/30 premix insulins, we performed a randomized single-dose two-period crossover study using a 12-h euglycemic clamp technique (7) in healthy subjects after double-blinded subcutaneous injections of each insulin preparation.

The study was approved by the Institutional Review Board of the University of Pittsburgh. Written informed consent was obtained before initiation of screening procedures. All subjects had a fasting blood glucose ≤108 mg/dl at screening, did not ingest alcohol or caffeine-containing beverages for 48 h before receiving study medication, and refrained from exercise for 24 h before the study. In a double-blind crossover study, single subcutaneous doses (0.2 units/kg) of 50/50 or 70/30 insulin were administered to 12 healthy male subjects. Glucose, insulin, and C-peptide levels were measured at baseline, every 10 min for 1 h, and every 15 min for 11 h after injection. Baseline euglycemia was maintained with a variable-rate glucose infusion (GIR). Serum insulin concentrations were analyzed using an enzyme-linked immunosorbent assay (ELISA) with a reference interval of 1.5–15.6 mIU/l, a detection limit of <0.5 mIU/ml, and cross-reactivity to C-peptide and proinsulin <0.3%. Serum C-peptide concentrations were analyzed by ELISA (reference interval 0.7–1.02 nmol/l, precision 8%). Glucose determinations were carried out immediately on site using a Yellow Spring Instruments 2300 STAT glucose analyzer. The 50/50 insulin premix (50% NPH in human insulin isophane suspension + 50% regular human insulin injection [rDNA], 100 units/ml, 3 ml PenFill cartridge) and the 70/30 insulin premix (Novolin 70/30, 3 ml PenFill cartridge) were supplied by Novo Nordisk Pharmaceutical Industries (Clayton, NC). The insulin premixes were administered using a NovoPen 3.0 with a NovoFine 30G needle (Novo Nordisk, Bagsvaerd, Denmark).

Glucodynamic parameters included the area under the curve (AUC) as follows: $AUC_{GIR(0-4)}$, $AUC_{GIR(4-8)}$, $AUC_{GIR(8-12)}$, maximum GIR (GIR_{MAX}), and time to GIR_{MAX} [$T_{GIR(MAX)}$]. The following pharmacokinetic measures were calculated from free insulin concentration–time curves adjusted for C-peptide: $AUC_{INS(0-4)}$, $AUC_{INS(4-8)}$, $AUC_{INS(8-12)}$, and maximum insulin concentration [C_{MAX} and $T_{INS(MAX)}$]. Adjusted insulin was derived according to the following formula: adjusted insulin = actual insulin − (baseline insulin / baseline C-peptide) × actual C-peptide. Analysis of variance was used to test the sequence, subject (within sequence), period, and treatment effect on AUC and C_{MAX} for glucose, insulin, and GIR_{MAX}. The analysis was performed on raw or logarithmic transformed data, depending on the distribution of the data.

A total of 12 subjects completed the study (age 29.7 ± 9.1 years, weight 78.2 ± 11.6 kg, BMI 25.1 ± 1.6 kg/m^2, and 100% Caucasian). Mean basal glucose values of 90.8 ± 4.9 mmol/l and 90.5 ± 5.8 mmol/l during 50/50 and 70/30 periods of the study were maintained for 12 h after the insulin bolus, with coefficients of variability of 5.4 and 6.4%, respectively. $LogAUC_{GIR(0-4)}$ was 6.6 ± 0.6 and 6.1 ± 0.6 with 50/50 and 70/30 injections, respectively (P = 0.0087). No significant differences were observed for $AUC_{GIR(4-8)}$, $AUC_{GIR(8-12)}$, $AUC_{GIR(0-12)}$, $T_{GIR(MAX)}$, or GIR_{MAX} between the two insulin preparations. $LogAUC_{INS(0-4)}$ was 10.2 ± 0.5 after administration of 50/50 insulin and 9.6 ± 0.5 after an injection of 70/30 insulin (P = 0.018). No significant differences were observed in $AUC_{INS(4-8)}$, $AUC_{INS(8-12)}$, and $T_{INS(MAX)}$ between the two premixes.

The major differences observed in the pharmacokinetic and glucodynamic properties of 50/50 and 70/30 insulin premixes occurred during the first 4 h after injection, with significantly greater $AUC_{GIR(0-4)}$, $AUC_{INS(0-4)}$, and C_{MAX} after 50/50 vs. 70/30 insulin. Differences in pharmacokinetics and glucodynamics between NPH and regular insulins have been described previously (9–12). However, there are few published studies available that directly compare different concentrations of premix insulin preparations. In one open-label study directly comparing single injections of 50/50 and 70/30 insulin (6), significant differences between AUC for GIR and insulin concentrations adjusted for C-peptide were ob-

served during the first 4 h after a higher dose of insulin (0.3 units/kg) than was used in this trial. In this previous trial, the investigators were not blinded to the insulin being used, which could have contributed to higher GIR with the 50/50 preparation because of anticipation of peak insulin action. In another study, postprandial hyperglycemic responses to a standard breakfast were measured in elderly individuals with insulin-requiring type 2 diabetes after a single injection of 50/50 or 70/30 insulin mixtures (0.33 units/kg) (8). No differences were observed in nadir and peak glucose concentrations, time to nadir and peak glucoses, or plasma insulin concentrations during the first 4 h after insulin injection. The absence of C-peptide measurements, the use of uncorrected insulin measures, and the low insulin dose used during the study period relative to the usual insulin requirements of the subjects (55.3 ± 24.0 units/day) may have contributed to the absence of an observed difference in the two insulin premix preparations.

No significant differences were observed in any glucodynamic or pharmacokinetic parameters during hours 4–12 after the insulin injections. This suggests that the risk for either late hypo- or hyperglycemia is unaffected by the type of insulin premix used. An overlap in action profiles of regular and NPH insulins during hours 3 and 5 and a relatively flat NPH action profile during hours 6–12 could explain these observations (12–13).

In summary, there are statistically significant differences in glucodynamic and pharmacokinetic parameters between 50/50 and 70/30 insulin mixtures during the first 4 h after a single injection in healthy volunteers. This suggests that 50/50 insulin premix preparations may offer the advantage of reducing postprandial glycemic excursions in patients with insulin-requiring diabetes.

Esther I. Krug, md
Louise DeRiso, crnp
Mary Beth Tedesco, crnp
Harsha Rao, md
Mary T. Korytkowski, md

From the University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania.

Address correspondence to Mary T. Korytkowski, MD, Falk, Room 581, University of Pittsburgh Medical Center, 3601 Fifth Avenue, Pittsburgh, PA 15213. E-mail: korytkowski@msx.dept-med.pitt.edu.

Acknowledgments— This work was supported by a research grant from Novo Nordisk and funds received from the National Institutes of Health/National Center for Research Resources/General Clinical Research Center Grant no. 5M01-RR-00056.

M.T.K. serves on an advisory panel for Novo Nordisk.

References

1. Puxty JAN, Hunter DH, Burr WA: Accuracy of insulin injection in elderly patients. *Br Med J* 287:1762, 1983
2. Bell D, Clements RS, Perentesis G, Roddam R, Wagenknecht L: Dose accuracy of self-mixed vs. premixed insulin. *Arch Int Med* 151:2265–2269, 1991
3. Coscelli C, Calabrese G, Fedele D, Pisu E, Calderini C: Use of premixed insulin among the elderly: reduction of errors in patient preparation of mixtures. *Diabetes Care* 15:1628–1630, 1992
4. Bell D, Bode B, Clements RS, Jerron J, Hollander PA: Premixed vs. self-mixed insulin in the treatment of type 2 diabetes mellitus: a randomized trial. *Today's Ther Trends* 9:63–73, 1991
5. Klauser R, Schernthaner G, Prager R: Mixtures of human intermediate and human regular insulin in type 1 diabetic patients: evaluation of free insulin levels and insulin action on glucose metabolism after combined and separate subcutaneous administration. *Diabetes Res Clin Pract* 5: 185–190, 1988
6. Woodworth JR, Howey DC, Bowsher RR: Comparative pharmacokinetics and glucodynamics of two human insulin mixtures (70/30 and 50/50 insulin mixtures). *Diabetes Care* 17:366–371, 1994
7. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method of quantifying insulin secretion and resistance. *Am J Physiol* 237:E214–E223, 1979
8. Brodows R, Chessor R: A comparison of premixed insulin preparations in elderly patients: efficacy of 70/30 and 50/50 human insulin mixtures. *Diabetes Care* 18: 855–857, 1995
9. Nijs HGT, Radder JK, Frolich M, Krans HMJ: In vivo relationship between insulin clearance and action in healthy subjects and IDDM patients. *Diabetes* 39:333–339, 1990
10. Bilo HJG, Heine RJ, Sikkenk AC, van der Meer J, van der Veen EA: Absorption kinetics and action profiles of intermediate-acting human insulins. *Diabetes Res* 4:39–43, 1987
11. Starke AAR, Heinemann L, Hohmann A, Berger M: The action profiles of human NPH insulin preparations. *Diabet Med* 6:239–244, 1989
12. Gardner DF, Arakaki RF, Podet EJ, Nell LJ, Thomas JW, Field JB: The pharmacokinetics of subcutaneous regular insulin in type 1 diabetic patients: assessment using a glucose clamp technique. *J Clin Endocrinol Metab* 63:689–694, 1986
13. Woodworth JR, Howey DC, Bowsher RR: Establishment of time-action profiles for regular and NPH insulin using pharmacodynamic modeling. *Diabetes Care* 17:64–69, 1994

Presence of Autoantibodies to Carbonic Anhidrase II and Lactoferrin in Type 1 Diabetes

Proposal of the concept of autoimmune exocrinopathy and endocrinopathy of the pancreas

Impairment of exocrine as well as endocrine function of the pancreas has been reported in type 1 diabetes (1). Histologically, lymphocytic infiltration of the exocrine gland has been reported in about half of Japanese type 1 diabetic patients (2). Fibrosis and atrophy of the exocrine gland have also been reported.

On the other hand, the concept of autoimmune pancreatitis has been recently proposed (3). This new clinical entity is characterized by swelling of the pancreas with extensive fibrosis and lymphocyte infiltration in the exocrine pancreas (3–5). It has been suggested that carbonic anhidrase II, an antigen of the duct cells of the pancreas, or lactoferrin in the pancreatic acinus may be the candidates for the target antigen (6).We have reported the first case of association of autoimmune pancreatitis and type 1 diabetes as autoimmune exocrinopathy and endocrinopathy of the pancreas (7).

To test the hypothesis that autoimmunity against the exocrine pancreas is involved in the pathological process of type 1 diabetes, we investigated the presence of autoantibodies against carbonic anhidrase II and lactoferrin in type 1 diabetes.

Eighteen patients with type 1 diabetes (2 men and 16 women, aged 45.4 ±19.5 years, range 23–72) and 18 patients with type 2 diabetes (7 men and 11 women, aged 59.1 ± 10.9 years) as control sub-

jects were recruited for the study after informed consent was obtained. Diabetes was diagnosed according to the new criteria of the American Diabetes Association. Diagnosis of type 1A (immune-mediated) diabetes was based on the presence of at least one of the two immunological markers: anti-GAD antibody (measured by radioimmunoassay [GAD Ab Cosmic; RSR, Cardif, U.K.]) or anti–IA-2 (tyrosine phosphatase–like protein) antibody (measured by radioimmunoassay [RSR]). Diagnosis of type 1B (idiopathic) diabetes was based on insulin deficiency and the absence of these immunological markers. Among 18 type 1 diabetic patients, 12 had type 1A diabetes and 6 had type 1B diabetes. One patient had chronic thyroiditis and one had Graves' disease. There was no history of other autoimmune diseases in the other patients. No patient had a history of pancreatitis.

Serum levels of autoantibodies against carbonic anhidrase II (ACA) and against human lactoferrin (ALF) were measured using the solid-phase enzyme-linked immunosorbent assay, as previously described (8).

Of 18 patients with type 1 diabetes, ACA was detected in 9 patients and ALF was detected in 8 patients. A total of 12 (67%) patients tested positive for either of the two antibodies. In contrast, neither ACA nor ALF were detected in any patients with type 2 diabetes. The prevalences of both ACA and ALF in type 1 diabetic patients were significantly higher than those of type 2 diabetic patients ($P < 0.01$, χ^2 test). Our results suggest the involvement of autoimmunity against the exocrine as well as endocrine pancreas in the pathogenesis of type 1 diabetes. These conditions could be referred to as autoimmune exocrinopathy and endocrinopathy of the pancreas.

In addition, it is noteworthy that either of these antibodies were detected in three of six type 1B diabetic patients. These observations suggest that ALF and ACA may be novel immunological markers for type 1 diabetes.

TAKAO TANIGUCHI, MD, PHD[1]
KAZUICHI OKAZAKI, MD, PHD[2]
MOTOZUMI OKAMOTO, MD, PHD[1]
SHUJI SEKO, MD, PHD[1]
KAZUSHIGE UCHIDA, MD[2]
YUTAKA SEINO, MD, PHD[3]

From the [1]Department of Internal Medicine, Ohtsu Red Cross Hospital, Shiga, Japan; the [2]Department of Endoscopic Medicine and Gastroenterology, Kyoto University Faculty of Medicine, Kyoto, Japan; and the [3]Department of Metabolism and Clinical Nutrition, Kyoto University Faculty of Medicine, Kyoto, Japan.

Address correspondence to Takao Taniguchi, MD, PhD, Department of Internal Medicine, Ohtsu Red Cross Hospital, 1-1-35, Nagara, Ohtsu, Shiga, Japan.

References

1. Lankisch PG, Manthey G, Otto J, Koop H, Talaulicar M, Willms B, Creutzfeldt W: Exocrine pancreatic function in insulin-dependent diabetes mellitus. *Digestion* 25: 211–216, 1982
2. Waguri M, Hanafusa T, Itoh N, Miyagawa J, Imagawa A, Kuwajima M, Kono N, Matsuzawa Y: Histopathologic study of the pancreas shows a characteristic lmphocytic infiltration in Japanese patients with IDDM. *Endocrine J* 44: 23–33, 1997
3. Yoshida K, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N: Chronic pancreatitis caused by an autoimmune abnormality: proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 40: 1561–1568, 1995
4. Taniguchi T, Seko S, Azuma K, Tamegai M, Nishida O, Inoue F, Okamoto M, Mizumoto T, Kobayashi H: Autoimmune pancreatitis detected as a mass in the tail of the pancreas. *J Gastroenterol Hepatol* 15: 461–464, 2000
5. Taniguchi T, Seko S, Azuma K, Asagoe K, Tamegai M, Nishida O, Inoue F, Okamoto M, Mizumoto T, Kobayashi H: Autoimmune pancreatitis detected as a mass in the head of the pancreas with contiguous fibrosis around the superior mesenteric artery. *Dis Dis Sci* 46:187–191, 2001
6. Okazaki K, Uchida K, Ohana M, Nakase H, Uose S, Inai M, Matsushima Y, Katakura K, Ohmori K, Chiba T: Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology* 118:573–581, 2000
7. Taniguchi T, Seko S, Okamoto M, Hamasaki A, Ueno H, Inoue F, Nishida O, Miyake N, Mizumoto T: Association of autoimmune pancreatitis and type 1 diabetes: autoimmune exocrinopathy and endocrinopathy of the pancreas (Letter). *Diabetes Care* 23:1592–1594, 2000
8. Kino-Ohsaki J, Nishimori I, Morita M, Okazaki K, Yamamoto Y, Onishi S, Hollingsworth MA: Serum antibodies to carbonic anhydrase I and II in patients with idiopathic chronic pancreatitis and Sjögren's syndrome. *Gastrenterology* 110: 1579–1586, 1996

Subcutaneous Continuous Glucose Monitoring

Feasibility of a new microdialysis-based glucose sensor system

Devices for continuous glucose monitoring (1,2) should display the data in real time rather than retrospectively. This is possible by means of the microdialysis technique (3). Therefore, we examined the feasibility of continuous glucose monitoring with functional models of a new microdialysis-based subcutaneous continuous glucose monitoring (SCGM) system (Roche Diagnostics, Mannheim, Germany) for up to 72 h. This system was calibrated by a one-point calibration shortly after the beginning of the recording period.

The SCGM system consists of a microdialysis catheter and a portable extracorporal electrochemical glucose sensor. It displays a new glucose value every minute. The system was tested in 23 ambulatory inpatients with insulin-treated type 1 and type 2 diabetes. For comparison, up to 75 capillary blood glucose samples were taken in each patient. The SCGM signal was corrected for the time that is needed for fluid transportation from the catheter to the extracorporal sensor (31 min) and calibrated by a linear one-point calibration model using the first capillary blood glucose value after an initial equilibration period (mean 4.7 h).

The SCGM system was well tolerated in all patients over the 3-day study. It did not limit the patients' daily activities. No adverse event occurred, except for a mild skin irritation caused by dressing tape. The sensor signal of the SCGM system showed no system-inborn drift. The percent difference between the SCGM system and the capillary blood glucose was comparable over the observed blood glucose range, with an intra-individual mean absolute difference of 14.8 ± 9.9% (mean ± SD). A representative recording is shown in Fig. 1.

Furthermore, the ability of two glucose spot-measurement schemes to detect hypoglycemic episodes was analyzed in a standardized manner. Glucose spot measurements were retrospectively derived on the basis of continuous glucose

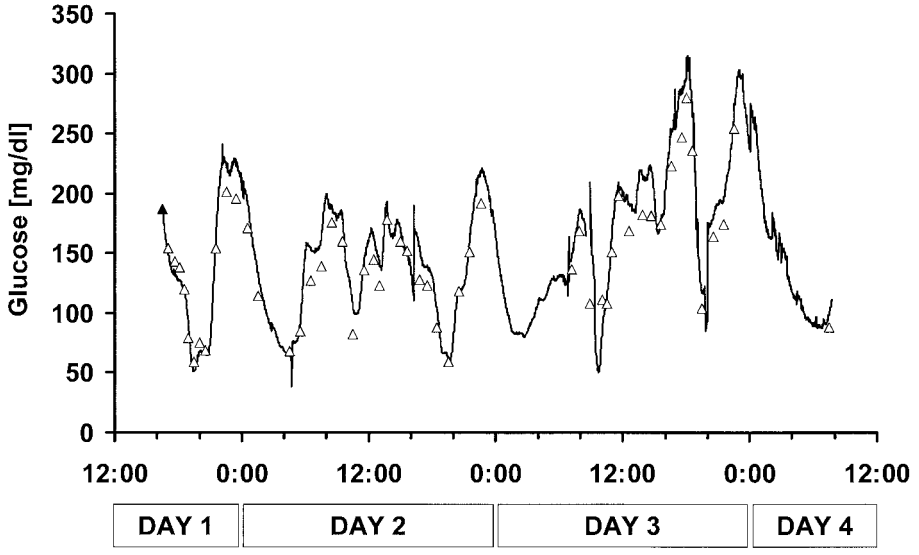

Figure 1—*Continuous glucose recording (—) in a patient with type 1 diabetes by means of the SCGM system. One capillary blood glucose value was used for one-point calibration (▲), and succeeding capillary blood glucose values (△) were used for comparison.*

profiles according to two different measurement schemes: four times per day (three times preprandially and bedtime) and seven times per day (adding three times 2-h postprandially). Hypoglycemia was arbitrarily defined as glucose <70 mg/dl.

Spot measurement of glucose, performed four or seven times per day, would not detect 71% (22 of 31) or 58% (18 of 31), respectively, of all hypoglycemic episodes registered by continuous glucose monitoring.

Continuous glucose monitoring by this new SCGM system in diabetic patients was feasible and safe for at least 3 days. In contrast to intracorporally placed glucose electrodes (4), the SCGM system showed no time-dependent decline in glucose-sensing sensitivity. Therefore, calibration of the system by just one capillary blood glucose measurement at the beginning of the monitoring period was sufficient.

This study further supports the observation that hypoglycemic episodes in insulin-treated patients are, to a large extent, not detected by spot measurements, even if they are performed seven-times per day (3,5).

In conclusion, the SCGM system offers real-time continuous glucose monitoring with sufficient precision in diabetic patients over 72 h. Detecting asymptomatic hypoglycemic episodes by continuous glucose monitoring will facilitate the management of patients with diabetes.

KARSTEN JUNGHEIM, MD[1]
KLAAS-JAN WIENTJES, MD[2]
LUTZ HEINEMANN, PHD[3]
VOLKER LODWIG, PHD[4]
THEODOR KOSCHINSKY, MD, PHD[1]
ADELBERT J. SCHOONEN, PHD[2]
FOR THE GLUCOSE MONITORING STUDY GROUP

From the [1]German Diabetes Research Institute at the Heinrich-Heine University of Duesseldorf, Duesseldorf, Germany; the [2]Department of Pharmaceutical Technology and Biopharmacy, University Center of Pharmacy, University of Groningen, Groningen, the Netherlands; the [3]Profil Institute for Metabolic Research, Neuss, Germany; and [4]Roche Diagnostics, Mannheim, Germany.

Address correspondence to Karsten Jungheim, MD, German Diabetes Research Institute, Auf'm Hennekamp 65, D-40225 Duesseldorf, Germany. E-mail: jungheim@ddfi.uni-duesseldorf.de.

L.H. and T.K. are members of the advisory board on SCGM systems to Roche Diagnostics, and V.L. is employed full-time at Roche Diagnostics.

Acknowledgments— This study was supported by Roche Diagnostics, Mannheim, Germany.

K.J., K-J.W., L.H., T.K., and A.J.S. have received unrestricted research grant support from Roche Diagnostics, Mannheim, Germany.

The authors thank Elisabeth Moll for excellent technical assistance.

APPENDIX

GLUCOSE MONITORING STUDY GROUP

Jens Sandahl Christiansen, Hans Orskov (Aarhus, Denmark); Michael Berger, Max Spraul, Theodor Koschinsky (Duesseldorf, Germany); Lutz Heinemann (Neuss, Germany); Volker Lodwig (Mannheim, Germany); Cornelia Haug (Ulm, Germany); Adelbert J. Schoonen (Groningen, Netherlands); Urban Ungerstedt (Stockholm, Sweden).

References

1. Bode BW, Gross TM, Thornton KR, Mastrototaro JJ: Continuous glucose monitoring used to adjust diabetes therapy improves glycosylated hemoglobin: a pilot study. *Diabetes Res Clin Pract* 46:183–190, 1999
2. Maran A, Crepaldi C, Tiengo A, Grassi G, Vitali E, Pagano G, Bistoni S, Calabrese G, Santeusanio F, Leonetti F, Ribaudo M, Di Mario U, Annuzzi G, Genovese S, Riccardi G, Previti M, Cucinotta D, Giorgino R, Poscia A, Varalli M: Continuous subcutaneous glucose monitoring in diabetic patients: a multicenter evaluation (Abstract). *Diabetes Res Clin Pract* 50:S333, 2000
3. Bolinder J, Hagström-Toft E, Ungerstedt U, Arner P: Self-monitoring of blood glucose in type 1 diabetic patients: comparison with continuous microdialysis measurements of glucose in subcutaneous adipose tissue during ordinary life conditions. *Diabetes Care* 20:64–70, 1997
4. Gerritsen M: Problems associated with subcutaneously implanted glucose sensors (Editorial). *Diabetes Care* 23:143–145, 2000
5. Gross TM, Mastrototaro JJ, Fredrickson LP: Detection of unseen hypoglycemia using continuous glucose monitoring (Abstract). *Diabetologia* 43:A5, 2000

Serum Concentrations of Soluble Vascular Cell Adhesion Molecule-1 and E-Selectin Are Elevated in Insulin-Resistant Patients With Type 2 Diabetes

It is well known that soluble intercellular adhesion molecule-1 (sICAM-1), soluble vascular cell adhesion molecule-1 (sVCAM-1), and soluble E-selectin (sE-selectin) levels are elevated in patients

with type 2 diabetes (1–3). Previous studies suggest that hyperglycemia, hyperinsulinemia, or insulin resistance may be responsible for the elevation of adhesion molecules (4–6). However, to our knowledge, the relation between soluble adhesion molecules and directly measured insulin sensitivity has not been studied in patients with type 2 diabetes. To investigate the direct association between insulin resistance and the elevation of soluble adhesion molecules, we performed a case-control study. Serum concentrations of sICAM-1, sVCAM-1, and sE-selectin were compared in insulin-sensitive and insulin-resistant patients who were matched for age, sex, BMI, duration of diabetes, and glycemic control (HbA_{1c}). Insulin sensitivity was assessed by the K index of short insulin tolerance test (K_{ITT}) (7). Briefly, a bolus of regular insulin (0.1 unit/kg) was infused, and blood samples were collected at 3, 6, 9, 12, and 15 min after infusion. K_{ITT} was calculated using the following formula: $K_{ITT} = 0.693/t_{1/2}$ (8). Patients whose K_{ITT} values were <2.00% per min were diagnosed as having insulin resistance, and those with K_{ITT} values >3.00% per min were defined as insulin-sensitive. Serum concentrations of sICAM-1, sVCAM-1, and sE-selectin were measured by enzyme-linked immunosorbent assay kits (R&D Systems, Minneapolis, MN). Data were compared with Student's *t* test or contingency table analysis.

A total of 37 insulin-sensitive patients (24 men and 13 women, mean ± SEM of K_{ITT} = 3.82 ± 0.12% per min) and 37 insulin-resistant patients (24 men and 13 women, mean K_{ITT} = 1.47 ± 0.06% per min) were compared. The age (61 ± 2 vs. 62 ± 2 years, $P = 0.51$, respectively), BMI (23.2 ± 0.5 vs. 24.3 ± 0.7 kg/m^2, $P = 0.22$, respectively), duration of diabetes (6 ± 1 vs. 7 ± 1 years, $P = 0.59$, respectively), and HbA_{1c} levels (8.9 ± 0.2 vs. 9.1 ± 0.3%, $P = 0.66$, respectively) were comparable between the two groups. The percentage of insulin therapy (21.6 vs. 37.8%, $P = 0.20$, respectively), presence of retinopathy (18.9 vs. 10.8%, $P = 0.52$, respectively), and presence of coronary artery disease (8.1 vs. 18.9%, $P = 0.31$, respectively) did not differ between the two groups. Concerning the lipid profile, insulin-sensitive patients had significantly lower serum triglyceride levels than insulin-resistant patients (1.0 ± 0.1 vs. 1.5 ± 0.1 mmol/l, $P < 0.01$, respectively). sICAM-1 levels were comparable between the two groups (166.5 ± 11.9 vs. 186.2 ± 1 0.5 ng/ml, $P = 0.22$, respectively). In contrast, levels of sVCAM-1 and sE-selectin in insulin-sensitive patients were significantly lower than those of insulin-resistant patients (sVCAM-1 683 ± 23 vs. 813 ± 44 ng/ml, $P = 0.01$; and sE-selectin 48.9 ± 4.0 vs. 81.5 ± 5.7 ng/ml, $P < 0.01$, respectively). Because HbA_{1c} levels in insulin-sensitive and -resistant patients were comparable, the significant elevation of sVCAM-1 and sE-selectin in insulin-resistant patients may be independent of the plasma glucose level. Thus, in type 2 diabetic patients, insulin resistance is associated with an elevation in sVCAM-1 and sE-selectin. Increased levels of sVCAM-1 and sE-selectin may partly explain the predisposition of insulin-resistant type 2 diabetic patients to atherosclerotic vascular disease.

KAZUNARI MATSUMOTO, MD[1]
YASUNORI SERA, MD[1]
YASUYO ABE, MD[2]
YUKITAKA UEKI, MD[3]
SEIBEI MIYAKE, MD[1]

From the Departments of [1]Diabetes and Metabolism, [2]Endocrinology, and [3]Rheumatology, Sasebo Chuo Hospital, Nagasaki, Japan.

Address correspondence to Kazunari Matsumoto, MD, Department of Diabetes and Metabolism, Sasebo Chuo Hospital, 15 Yamato-cho, Sasebo, Nagasaki 857-1195, Japan. E-mail: dmkmatsu@hakujyujikai.or.jp.

References

1. Fasching P, Waldhausl W, Wagner OF: Elevated circulating adhesion molecules in NIDDM; potential mediators in diabetic macroangiopathy. *Diabetologia* 39: 1242–1244, 1996
2. Carter AM, Grant PJ: Vascular homeostasis, adhesion molecules, and macrovascular disease in non-insulin-dependent diabetes mellitus. *Diabet Med* 14:423–432, 1997
3. Albertini JP, Valensi P, Lormeau B, Aurousseau MH, Ferriere F, Attali JR, Gattegno L: Elevated concentrations of soluble E-selectin and vascular cell adhesion molecule-1 in NIDDM: effect of intensive insulin treatment. *Diabetes Care* 21:1008–1013, 1998
4. Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, Remuzzi A, Zoja C, Remuzzi G: Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 101: 1905–1915, 1998
5. Chen NG, Holmes M, Reaven GM: Relationship between insulin resistance, soluble adhesion molecules, and mononuclear cell binding in healthy volunteers. *J Clin Endocrinol Metab* 84:3485–3489, 1999
6. Matsumoto K, Miyake S, Yano M, Ueki Y, Tominaga Y: High serum concentrations of soluble E-selectin in patients with impaired glucose tolerance with hyperinsulinemia. *Atherosclerosis* 152:415–420, 2000
7. Matsumoto K, Miyake S, Yano M, Ueki Y, Yamaguchi Y, Akazawa S, Tominaga Y: Insulin resistance and arteriosclerosis obliterans in patients with NIDDM. *Diabetes Care* 20:1738–1743, 1997
8. Bonora E, Moghetti P, Zancanaro C, Cigolini M, Querena M, Cacciatori V, Corgnati A, Muggeo M: Estimates of in vivo insulin action in man: comparison of insulin tolerance tests with euglycemic and hyperglycemic clamp studies. *J Clin Endocrinol Metab* 68:374–378, 1989

COMMENTS AND RESPONSES

Response to Malone et al.

The article on early retinopathy by Malone et al. (1) suggested that all newly diagnosed people with type 1 diabetes should have annual retinal examinations through a dilated pupil. The data to support this suggestion were taken from the Diabetes Control and Complications Trial (DCCT). The DCCT recruited subjects ≥13 years of age who were at least Tanner II stage for pubertal development. Therefore, the data do not apply to prepubertal children. Unfortunately, no such qualification was noted in their article or the accompanying editorial (2). Retinal changes are almost never present in children before puberty. I am concerned that Malone's article will result in even more children than at present having regular ophthalmologic assessments that are entirely unnecessary. The American Diabetes Association recommends that children ≥10 years of age only need to have retinal examinations after 3–5 years, a time when most will have entered puberty (3). Perhaps a qualification should be noted in a future issue of *Diabetes Care*.

ROBERT M. EHRLICH, MD, FRCP

From Markham Stouffville Hospital, Markham, Ontario, Canada.

Address correspondence to Robert M. Ehrlich, 381 Church St., Markham, Ontario L3P 7P3, Canada. E-mail: rerlich@sympatico.ca.

References

1. Malone JI, Morrison AD, Pavan PR, Cuthbertson DD: Prevalence and significance of retinopathy in subjects with type 1 diabetes of less than 5 years' duration screened for the Diabetes Control and Complications Trial. *Diabetes Care* 24: 522–526, 2001
2. Palmberg P: Screening for diabetic retinopathy (Editorial). *Diabetes Care* 24: 419–420, 2001
3. American Diabetes Association: Diabetic retinopathy (Position Statement). *Diabetes Care* 24 (Suppl. 1):S73–S76, 2001

Response to Ehrlich

Dr. Ehrlich's (1) point is well taken. The Diabetes Control and Complications Trial data did not include subjects under the age of 13 years. Our study (2) was not intended to deal with the subject of retinal exams for prepubertal children. Most physicians who care for children with diabetes agree with Dr. Ehrlich, who stated "retinal changes are almost never present in children before puberty." This claim seems to overlook the Wisconsin Epidemiologic Study of Diabetic Retinopathy (3), a population-based study that reported that 4 of 26 children (15.4%) aged 0–9 years and 23 of 42 children (54.8%) aged 10–12 years, or 27 of 68 children (40%) aged 0–12 years, had retinopathy on retinal color photographs. This widely accepted study suggests that retinopathy does occur before puberty. If knowledge of the presence of microvascular disease in a patient is not important before it becomes vision-threatening, as suggested by Dr. Palmberg (4), then exams before puberty may be unnecessary. One should not believe, however, that microvascular disease does not exist in prepubertal children who have diabetes.

JOHN I. MALONE, MD

From the Diabetes Center, Health Sciences Center, University of South Florida, Tampa, Florida.

Address correspondence and reprint requests to John I. Malone, MD, Diabetes Center, Health Sciences Center, University of South Florida, 12901 Bruce B. Downs Blvd., MDC 45, Tampa, FL 33612. E-mail: jmalone@hsc.usf.edu.

References

1. Erlich RM: Response to Malone et al. (Letter). *Diabetes Care* 24:1698, 2001
2. Malone JI, Morrison AD, Pavan PR, Cuthbertson DD: Prevalence and significance of retinopathy in subjects with type 1 diabetes of less than 5 years' duration screened for the Diabetes Control and Complications Trial. *Diabetes Care* 24:522–526, 2001
3. Kline R, Klein BEK, Moss SE, Davis MD, DeMets DL: The Wisconsin Epidemiologic Study of Diabetic Retinopathy. IX. Four-year incidence and progression of diabetic retinopathy when age at diagnosis is less than 30 years. *Arch Ophthalmology* 107:237–243, 1989
4. Palmberg P: Screening for diabetic retinopathy (Editorial). *Diabetes Care* 24:419–420, 2001