

## OBSERVATIONS

## Role of Triglyceride Levels in Identifying Insulin Resistance in Nonobese Type 2 Diabetic Japanese Patients With Hypertension

Although it is generally accepted that hypertension is associated with insulin resistance, not all patients with hypertension have insulin resistance or hyperinsulinemia (1–3). This suggests that factors other than high blood pressure cause insulin resistance or hyperinsulinemia in the patients with hypertension. Lind et al. (2) were the first to show that insulin-resistant patients with hypertension had not only a higher BMI but also more disturbances of glucose and lipid metabolism compared with insulin-sensitive patients with hypertension. Agata et al. (3) later demonstrated that disturbances of glucose and lipid metabolism might be related to insulin resistance in BMI-matched patients with hypertension. Irrespective of the existence of diabetes in the majority of hypertensive subjects (4), diabetic subjects were excluded from the evaluation in these 2 reports. We therefore recruited type 2 diabetic patients with essential hypertension ( $n = 31$ ) and normotension ( $n = 81$ ) and compared the clinical characteristics among these patients. They were all nonobese but were treated with an oral hypoglycemic agent (glibenclamide). Hypertension was diagnosed when blood pressure was  $>160/95$  mmHg or when antihypertensive treatment was in progress (5). Insulin action was measured with homeostasis model assessment-insulin resistance index (HOMA-IR) and validated against a minimal model approach (6,7).

Data were expressed as means  $\pm$  SEM. The statistical analysis was performed with the StatView 5 system. Although no significant difference was observed in age, BMI, total cholesterol, HDL-cholesterol, fasting glucose, or HbA<sub>1c</sub> levels between the patients with hypertension and normotension, fasting insulin ( $7.7 \pm 0.7$  vs.  $5.9 \pm 0.2$   $\mu$ U/ml,  $P = 0.002$ ), serum triglycerides ( $142$

$\pm 19$  vs.  $97 \pm 3$  mg/dl,  $P < 0.001$ ) and HOMA-IR levels ( $2.5 \pm 0.3$  vs.  $1.9 \pm 0.1$ ,  $P = 0.008$ ) were significantly higher in subjects with hypertension compared with those with normotension. Our present data for HOMA-IR in the hypertensive group indicate that the subjects with hypertension were divided into 2 distinct populations. Of the 31 patients, 19 (61%) had normal HOMA-IR ( $<2.5$ ) ( $1.6 \pm 0.1$ ,  $P > 0.05$  vs. normotensives) and this group was referred to as the normal insulin sensitivity (N-SI) subset (8). Of 31 patients, 12 (39%) had increased HOMA-IR ( $>2.5$ ) ( $4.0 \pm 0.4$ ,  $P < 0.001$  vs. normotensives) and this group was called the reduced insulin sensitivity subgroup (R-SI). When the subjects with hypertension were divided into N-SI and R-SI subgroups, fasting glucose ( $144 \pm 7$  vs.  $125 \pm 5$  mg/dl,  $P = 0.008$ ), serum triglycerides ( $225 \pm 36$  vs.  $90 \pm 6$  mg/dl,  $P < 0.001$ ), and serum insulin ( $11.4 \pm 1.2$  vs.  $5.4 \pm 0.4$  uU/ml,  $P < 0.001$ ) levels were significantly higher in R-SI subgroups ( $n = 12$ ) than in N-SI subgroups ( $n = 19$ ). There was, however, no significant difference in fasting glucose, serum triglycerides, and insulin levels between N-SI subgroups and normotensives. No significant difference was observed in BMI, HbA<sub>1c</sub>, total cholesterol, and HDL-cholesterol levels among the 3 groups.

The reason our type 2 diabetic patients with hypertension are not all insulin resistant is not known, since type 2 diabetes is another disease that is associated with insulin resistance. One possible explanation may be because of the difference in populations studied. Japanese and African-American type 2 diabetic patients are divided into 2 distinct variants; one being insulin resistant and the other being insulin sensitive (9–11). On the other hand, 92% of type 2 diabetic patients are reported to be insulin resistant among the Caucasian population (12). Another possible explanation for the results may be because of the degree of BMI studied. Our patients with hypertension had a BMI  $<27.0$  kg/m<sup>2</sup> (i.e., nonobese). It is well recognized that being overweight causes insulin resistance in humans (13). Cabezas-Cerrato et al. (14) previously showed that obese type 2 diabetic patients had a similar degree of insulin resistance irrespective of hypertension and suggested that hypertension is not generally associated with any significant increase in insulin resistance. Therefore, the prevalence of insulin resistance among hypertensive diabetic individuals would probably be higher

in a population-based study in which obese groups were included. One might argue that antihypertensive medications might affect insulin action in our present study. However, it seems unlikely, since the antihypertensive drugs used were either ACE inhibitors or calcium channel blockers and because no difference was found in the frequency of the use of the ACE inhibitors (10 of 19 in N-SI and 7 of 12 in R-SI) or calcium channel blockers (9 of 19 in N-SI and 5 of 12 in R-SI) between the 2 groups. From these results, it can be hypothesized that nonobese type 2 diabetic Japanese patients with hypertension can be subdivided into at least 2 subpopulations: one with insulin resistance and higher triglyceride levels and the other with normal insulin sensitivity and a normal lipid profile. In this regard, the previous study by Groop et al. (15), which showed that type 2 diabetic patients with hypertension had not only insulin resistance but also hypertriglyceridemia, supports our hypothesis.

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### References

1. Reaven GM, Lithell H, Landsberg L: Hypertension and associated metabolic abnormalities: the role of insulin resistance and the sympathoadrenal system. *N Engl J Med* 334:374–381, 1996
2. Lind L, Berne C, Lithell H: Prevalence of insulin resistance in essential hypertension. *J Hypertens* 13:1457–1462, 1995
3. Agata J, Miyazaki Y, Takada M, Murakami H, Masuda A, Miura T, Ura N, Shimamoto

- K: Association of insulin resistance and hyperinsulinemia with disturbed lipid metabolism in patients with essential hypertension. *Hypertens Res* 21:57–62, 1998
4. Morales PA, Mitchell BD, Valdes RA, Hazuda HP, Stem MP, Haffner SM: Incidence of NIDDM and impaired glucose tolerance in hypertensive subjects: the San Antonio Heart Study. *Diabetes* 42:154–161, 1993
  5. Subcommittee of WHO/ISH Mild Hypertension Liaison Committee: Summary of 1993 World Health Organization International Society hypertension guidelines for the management of mild hypertension. *BMJ* 307:1541–1546, 1993
  6. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Tracher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 28:412–419, 1985
  7. Fukushima M, Taniguchi A, Sakai M, Doi K, Nagasaka S, Tanaka H, Tokuyama K, Nakai Y: Homeostasis model assessment as a clinical index of insulin resistance: comparison with the minimal model analysis (Letter). *Diabetes Care* 22:1911–1912, 1999
  8. 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: the role of the triglycerides on insulin resistance (Letter). *Diabetes Care* 22:2100–2101, 1999
  9. Taniguchi A, Nakai Y, Fukushima M, Kawamura H, Imura H, Nagata I, Tokuyama K: Pathogenic factors responsible for glucose tolerance in patients with NIDDM. *Diabetes* 41:1540–1546, 1992
  10. Nagasaka S, Tokuyama K, Kusaka I, Hayashi H, Rokkaku K, Nakamura H, Kawakami A, Higashiyama M, Ishikawa San-e, Saito H: Endogenous glucose production and glucose effectiveness in type 2 diabetic subjects derived from stable-labeled minimal model approach. *Diabetes* 48:1054–1060, 1999
  11. Banerji MA, Lebovitz HE: Insulin-sensitive and insulin-resistant variants in NIDDM. *Diabetes* 38:784–792, 1989
  12. Haffner SM, D'Agostino R Jr, Mykkanen L, Tracy R, Howard B, Rewers M, Selby J, Savage PJ, Saad MF: Insulin sensitivity in subjects with type 2 diabetes: relationship to cardiovascular risk factors: the Insulin Resistance Atherosclerosis Study. *Diabetes Care* 22:562–568, 1999
  13. Bonadonna RC, Groop L, Kraemer N, Ferrannini E, Prato SD, DeFronzo RA: Obesity and insulin resistance in humans: a dose-response study. *Metabolism* 39:452–459, 1990
  14. Cabezas-Cerrato J, Darcia-Estevanz DA, Araujo D, Iglesias M: Insulin sensitivity, glucose effectiveness, and beta-cell function in obese males with essential hypertension: investigation of the effects of treatment with a calcium channel blocker (diltiazem) or an angiotensin-converting enzyme inhibitor (quinapril). *Metabolism* 46:173–178, 1997
  15. Groop L, Ekstrand A, Forsblom C, Widen E, Groop P-H, Teppo A-M, Eriksson J: Insulin resistance, hypertension and microalbuminuria in patients with type II (non-insulin-dependent) diabetes mellitus. *Diabetologia* 36:642–647, 1993

## Effect of Physical Training on Insulin Sensitivity in Japanese Type 2 Diabetic Patients

### Role of serum triglyceride levels

Type 2 diabetes is a complex disorder characterized by insulin resistance and/or impaired insulin secretion (1). Along with dietary therapy, exercise has been advocated for the management of type 2 diabetes. Although the potential role of exercise in diabetic subjects is primarily to increase insulin sensitivity, information concerning the mechanism is limited in type 2 diabetic patients (2–4). Exercise has been found to reduce serum triglyceride levels in patients with type IV hyperlipoproteinemia (5). However, to the best of our knowledge, it is not clear whether exercise training-induced reduction in triglyceride levels enhances insulin sensitivity in type 2 diabetic patients. In this context, a major issue is that not only exercise training but also body weight reduction might lower triglyceride levels and improve insulin sensitivity in type 2 diabetic patients. To separate the effects of exercise from those of weight reduction, short-term exercise protocol was performed in weight-stable patients during the study. Therefore, the purpose of this study was to determine whether short-term physical training of type 2 diabetic patients could reduce serum triglyceride levels and insulin resistance without affecting body weight.

Fifteen sedentary Japanese type 2 diabetic patients participated in the study after the provision of informed consent. Their age and BMI were  $59.9 \pm 2.6$  years and  $25.4 \pm 1.7$  kg/m<sup>2</sup>, respectively. HbA<sub>1c</sub> levels were  $9.3 \pm 0.4\%$  (range 6.3–11.3%).

Type 2 diabetes was diagnosed based on the criteria of the World Health Organization (6). None of the subjects had a physical finding suggestive of cardiovascular or renal disease. Eight patients were taking sulfonylureas (glibenclamide), but their medication was not changed during the course of the study. None of them took any medications affecting lipid metabolism. All patients were hospitalized and ingested the following diet under supervision:  $1,488 \pm 43$  kcal/day (range 1,200–1840 kcal/day) (50–58% carbohydrate, 18–20% protein, and 24–30% fat).

The patients engaged in an exercise program that consisted of aerobic and resistance exercise for 12 days. They were instructed to walk at least 7,000 steps per day as an aerobic exercise and perform dumbbell exercise described previously (7). The foot count was monitored by a pedometer (Calorie Counter; Suzuken, Nagoya, Japan). During the course of the study, they walked a mean of  $11,398 \pm 1,435$  steps per day. After an overnight fast, the blood was drawn from an antecubital vein for the determination of glucose, insulin, and lipid profile before and after the exercise training. The estimate of insulin resistance by homeostasis model assessment (HOMA-IR) was calculated with the fasting serum insulin formula ( $\mu\text{U/ml}$ )  $\times$  fasting plasma glucose (mmol/l)/22.5, as described by Matthews et al. (8). Statistical analysis was performed by Student's *t* test, taking a value of  $P < 0.05$  as significant.

After exercise, BMI decreased from  $25.4 \pm 1.7$  to  $24.9 \pm 1.6$  kg/m<sup>2</sup>, but was not statistically significant ( $P = 0.425$ ). Serum triglyceride level significantly fell from  $175 \pm 21$  to  $119 \pm 9$  mg/dl ( $P = 0.013$ ), after exercise. In contrast, no significant changes in total cholesterol or HDL cholesterol concentrations occurred after exercise. Plasma glucose and serum insulin levels significantly decreased from  $163 \pm 11$  to  $128 \pm 9$  mg/dl ( $P = 0.011$ ) and  $7.2 \pm 0.9$  to  $5.2 \pm 0.6$   $\mu\text{U/ml}$  ( $P = 0.042$ ), respectively. The HOMA-IR value was significantly lower after exercise ( $2.97 \pm 0.40$ ) than before exercise ( $1.62 \pm 0.19$ ,  $P = 0.003$ ).

Exercise had long been considered in the treatment regimen for patients with type 2 diabetes. In the present study, we investigated the effect of exercise on insulin sensitivity and glucose levels in Japanese type 2 diabetic patients and found that exercise not only improved insulin sensitivity but also glycemic control. The beneficial effect of exercise on insulin sensitivity and





## Plasma Brain Natriuretic Peptide Levels in Normotensive Type 2 Diabetic Patients Without Cardiac Disease

**B**rain natriuretic peptide (BNP) plays an important role in the regulation of body fluid and blood pressure (1,2). BNP is produced mainly by cardiac tissue and is secreted from the ventricle in humans. It has been reported that plasma concentrations of BNP in patients with congestive heart failure, chronic renal failure, and essential hypertension are greater than normal (3,4). Although abnormally high levels of BNP are sometimes found in patients with diabetes, there have been very few studies on the plasma levels of BNP in diabetic patients without cardiac dysfunction (5). We studied plasma BNP levels in normotensive diabetic patients without cardiac disease.

This study was comprised of 100 type 2 diabetic patients (62 men and 38 women, aged  $57.4 \pm 9.9$  years,  $HbA_{1c}$   $7.6 \pm 1.6\%$ , serum creatinine level  $65 \pm 18$   $\mu\text{mol/l}$ ). Data obtained from 386 healthy volunteers involved plasma levels of BNP observed no more than twice. The first-time BNP value is called BNP1 and the second-time value is called BNP2. BNP1 (273 men and 21 women, aged  $55.8 \pm 3.3$  years) and BNP2 (305 men and 23 women, aged  $54.7 \pm 3.4$ ), with normal glucose tolerance, were available for comparison. The diabetic patients had no history of ischemic heart disease and had normal blood pressure ( $<140/90$  mmHg), normal cardiothoracic ratio (on plain radiography of the chest), and no left ventricular hypertrophy (on electrocardiography). Fourteen of the 100 patients showed proteinuria, and 28 had retinopathy. None of the patients was receiving antihypertensive drugs, including ACE inhibitors, before beginning the study. Plasma BNP levels were measured with an immunoradiometric assay kit (Shionoria BNP kit; Shionogi, Osaka, Japan), following a method previously described (6). The detection limit of the assay was 2.0 pg/ml.

In the regression analysis with age as an explanatory variable, the regression of BNP levels (BNP1 and BNP2) with loga-

rithmic transformation in healthy subjects was not significant. According to the results of the Jonckheere's test, the values of BNP1 ( $10.8 \pm 11.7$  pg/ml,  $P = 0.5117$ ) and BNP2 ( $10.1 \pm 10.2$  pg/ml,  $P = 0.1022$ ) weren't significantly related to age. However, plasma levels of BNP that estimated the regression coefficient of age were positive (coefficient = 0.0125,  $P = 0.0176$ ), and plasma  $HbA_{1c}$  levels were negative (coefficient =  $-0.0691$ ,  $P = 0.0273$ ) in all diabetic subjects. Plasma BNP levels that estimated the regression coefficient of the other clinical findings, such as serum creatinine levels and presence of proteinuria and retinopathy, were not significant. The values of plasma BNP levels ( $14.7 \pm 9.2$  vs.  $11.1 \pm 6.2$  pg/ml,  $P = 0.1520$ ) were not significantly different between patients with proteinuria and those without proteinuria by Wilcoxon's rank-sum test. Also, the relationship of BNP plasma levels to the presence of retinopathy did not differ significantly between patients without retinopathy and with retinopathy ( $14.0 \pm 9.1$  vs.  $14.6 \pm 8.3$  pg/ml,  $P = 0.4678$ ).

Although the plasma BNP levels have not been well documented in diabetic patients, it was only reported that the plasma levels of BNP are significantly correlated with the degree of microalbuminuria in patients with diabetic nephropathy (5). On the contrary, the present findings show that there was no relationship between the plasma BNP levels and the presence of proteinuria, but the plasma levels of BNP were significantly correlated with  $HbA_{1c}$  and age in diabetic patients without cardiac disease. The reason for the negative correlation found between the plasma levels of BNP and  $HbA_{1c}$  remains unclear. One possible explanation is that the presence of hyperglycemia increases plasma osmotic diuresis and may suppress BNP secretion in the regulation of body fluid. It was reported that even in elderly inpatients without overt heart failure, the plasma BNP concentration tended to be greater in association with systolic dysfunction, cardiac hypertrophy, and renal dysfunction, and also with diastolic dysfunction (7). The plasma BNP levels in our diabetic patients tended to be greater in association with age. We speculated that the increase in plasma BNP levels may be attributable to some unknown factors related to diabetic duration. These factors include diabetic cardiomyopathy probably due to microangiopathy and the increased mass of myocardium, which cannot be estimated by ordinary routine examinations (8).

In conclusion, the results of our study suggest that the determination of plasma BNP levels is not an indicator of diabetic complications without cardiac disease, which can be estimated through routine examinations. Therefore, it is important to point out, as a clinical manager, that increased plasma level of BNP is a sign of cardiac dysfunction in diabetic patients and is the main indication for further cardiac examination.

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### References

1. Wilkins MR, Redondo J, Brown LA: The natriuretic-peptide family. *Lancet* 349: 1307-1310, 1997
2. Nakao K, Ogawa Y, Suga S, Imura H: Molecular biology and biochemistry of the natriuretic peptide system. I. Natriuretic peptides. *J Hypertens* 10:907-912, 1992
3. Mukoyama M, Nakao K, Saito Y, Ogawa Y, Hosoda K, Suga S, Shirakami G, Jougasaki M, Imura H: Human brain natriuretic peptide: a novel cardiac hormone. *Lancet* 335: 801-802, 1990
4. Omland T, Aakvaag A, Bonarjee VV, Caidahl K, Lie RT, Nilsen DW, Sundsfjord JA, Dickstein K: Plasma brain natriuretic peptide as an indicator of left ventricular systolic function and long-term survival after acute myocardial infarction: comparison with plasma atrial natriuretic peptide and N-terminal proarterial natriuretic peptide. *Circulation* 93:1963-1969, 1996
5. Yano Y, Katsuki A, Gabazza EC, Ito K, Fujii M, Furuta M, Tuchihasi K, Goto H, Nakatani K, Hori Y, Sumida Y, Adachi Y: Plasma brain natriuretic peptide level in normotensive noninsulin-dependent diabetic patients with microalbuminuria. *J Clin Endocrinol Metab* 84:2353-2356, 1999
6. Yasue H, Yoshimura M, Sumida H, Kikuta K, Kugiyama K, Jougasaki M, Ogawa H, Okumura K, Mukoyama M, Nakao K: Localization and mechanism of secretion of B-type natriuretic peptide in comparison with those of A-type natriuretic peptide in normal subjects and patients with heart failure. *Circulation* 90:195-203, 1994

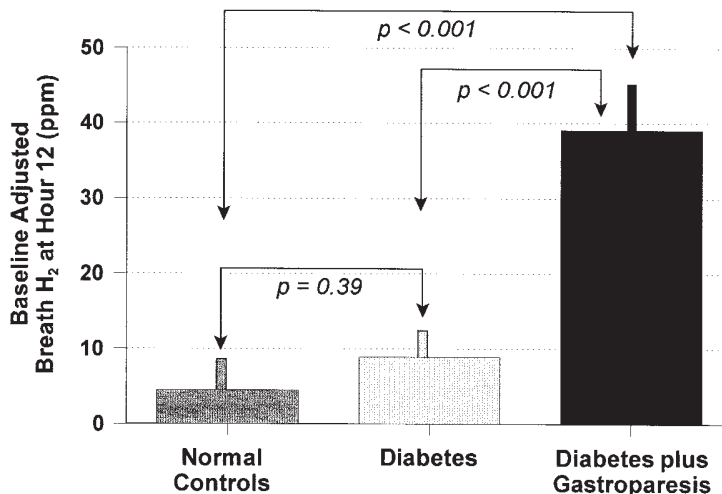
7. Sayama H, Nakamura Y, Saito N, Kinoshita M: Why is the concentration of plasma brain natriuretic peptide in elderly inpatients greater than normal? *Coron Artery Dis* 10:537–540, 1999
8. Fukugawa NK, Young VR: Nutrition. In *Geriatric Medicine*. 2nd ed. Rowe JW, Besdine RW, Eds. Boston, MA, Little, Brown, 1988, p. 99–113

## Breath Hydrogen Testing Identifies Patients With Diabetic Gastroparesis

**D**elayed gastric emptying is a common and debilitating complication of long-standing diabetes (1). Unfortunately, diagnosis of this condition with current scintigraphic techniques is limited by high cost, wide inter- and intraindividual variability, and exposure of the patient to low levels of ionizing radiation. Because the ingestion of lactulose results in the colonic production of lactate, carbon dioxide, and hydrogen ( $H_2$ ) gas, and because colonic  $H_2$  is excreted by the lungs in direct proportion to the amount of lactulose being metabolized in the colon, lactulose-breath hydrogen testing can be used for the quantitative assessment of oral-cecal transit time in both diabetic and nondiabetic subjects (2,3). Ingestion of complex carbohydrates, such as potato starch, also results in breath  $H_2$  excretion (4). We, therefore, hypothesized that breath hydrogen excretion would be abnormally prolonged in patients with previously diagnosed diabetic gastroparesis after ingestion of a potato-lactulose test meal compared with subjects without gastroparesis.

This 24-h pilot study consisted of 10 healthy nondiabetic control subjects, 10 diabetic subjects without gastroparesis (gastric emptying  $T_{1/2} < 90$  min), and 10 diabetic subjects with previously diagnosed gastroparesis (gastric emptying  $T_{1/2} > 90$  min). Radionuclide determination of gastric emptying was performed as previously described (5). Gastric motility agents were withheld 24 h before study, and euglycemia was established and maintained overnight in diabetic subjects with a continuous intravenous insulin infusion. At 6:00 A.M., all subjects ingested a breakfast containing 100 g dry-cooked potato starch and 20 g lactulose. Breath  $H_2$  excretion was monitored at baseline and for 12 h after ingestion of the

**Figure 1**—Mean baseline-adjusted breath  $H_2$  concentrations 12 h after ingestion of 100 g of potato starch and 20 g of lactulose among the 3 study groups.



test meal. All subjects provided written informed consent before the study as approved by the University of New Mexico Human Research Review Committee.

Breath  $H_2$  samples were analyzed in duplicate by gas chromatography using the QuinTron SC Hydrogen Breath Analyzer (QuinTron Instruments, Milwaukee, WI). Study data were analyzed using a repeated measures analysis of variance with posthoc application of Fisher's least significant difference method of multiple pairwise comparisons, using SAS (SAS Institute, Cary, NC).

There was no significant difference in plasma glucose concentrations during the 12-h study period between the diabetic group and the gastroparetic group ( $P = 0.79$ ). Baseline breath  $H_2$  levels were elevated in the diabetic plus gastroparetic group (normal control group =  $3.6 \pm 5.2$ , diabetic group =  $4.1 \pm 5.9$ , diabetic plus gastroparetic group =  $13.1 \pm 12.4$  ppm;  $P = 0.06$  compared with the groups with no gastroparesis). There was no significant difference between the groups in peak breath  $H_2$  concentrations or in time-to-peak breath  $H_2$ . However, the time course of breath  $H_2$  concentrations was significantly different in the gastroparetic group compared with those of the other 2 groups by repeated measures analysis of variance ( $P < 0.01$ ). Figure 1 shows that baseline-adjusted breath  $H_2$  concentrations were significantly elevated in the diabetic plus gastroparetic group compared with the diabetic and normal control groups 12 h after ingestion of the test meal.

Although current standards of practice call for a radionuclide gastric-emptying

study to aid in the diagnosis of gastrointestinal motility disorders in diabetic patients, the development of a functional test of upper gastrointestinal function after the ingestion of a solid meal may prove to be a valuable diagnostic aid for patients affected with this problem (1,6–10). This pilot study suggests that the presence of an elevated baseline-adjusted breath  $H_2$  concentration, 12 h after the ingestion of an easily prepared test meal containing potato starch and lactulose, accurately identifies those patients with previously diagnosed diabetic gastroparesis. Such a test may prove to be a useful outpatient screening test for the identification of those symptomatic patients who should receive a more definitive (but expensive) scintigraphic study and those in whom the presence of gastroparesis can be excluded.

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**Acknowledgments**— This research was supported by the University of New Mexico General Clinical Research Center (NIH NCRR GCRC Grant 5M01-RR00997) and by NIH



National Institute of Diabetes and Digestive and Kidney Diseases Grant 1-K23-DK02680-01.

References

1. Varis K: Diabetic gastroparesis: a review. *J Diabetes Complications* 5:207-217, 1991
2. Sahota SS, Bramley PM, Menzies IS: The fermentation of lactulose by colonic bacteria. *J Gen Microbiol* 128:319-325, 1982
3. Sciarretta G, Furno A, Mazzoni M, Garagnani B, Malaguti P: Lactulose hydrogen breath test in orocecal transit assessment: critical evaluation by means of scintigraphic method. *Dig Dis Sci* 39:1505-1510, 1994
4. Levitt MD, Hirsh P, Fetzer CA, Sheahan M, Levine AS: H<sub>2</sub> excretion after ingestion of complex carbohydrates. *Gastroenterology* 92:383-389, 1987
5. Mettler FA: The gastrointestinal tract. In *Essentials of Nuclear Medicine Imaging*, 4th ed. Mettler FA, Guiberteau MJ, Eds. Philadelphia, WB Saunders, 1998, p. 237-281
6. Loo FD, Palmer DW, Soergel KH, Kalbfleisch JH, Wood CM: Gastric emptying in patients with diabetes mellitus. *Gastroenterology* 86:485-494, 1984
7. Lartigue S, Bizais Y, Des Varannes SB, Murat A, Pouliquen B, Galmiche JP: Inter- and intra-subject variability of solid and liquid gastric emptying parameters: a scintigraphic study in healthy subjects and diabetic patients. *Dig Dis Sci* 39:109-115, 1994
8. Schwarcz E, Palmer M, Aman J, Horowitz M, Stridsberg M, Berne C: Physiologic hyperglycemia slows gastric emptying in normal subjects and patients with insulin-dependent diabetes mellitus. *Gastroenterology* 113:60-66, 1997
9. Malmud LS, Fisher RS, Knight LC, Rock E: Scintigraphic evaluation of gastric emptying. *Semin Nucl Med* 2:116-125, 1982
10. Poitras P, Picard M, Dery R, Giguere A, Picard D, Maorais J, Plourde V, Boivin M: Evaluation of gastric emptying function in clinical practice. *Dig Dis Sci* 42:2183-2189, 1997

## Insulin-Induced Hypoglycemia Induces a Rise in C-Reactive Protein

The regulatory responses to hypoglycemia result in diverse physiological and biochemical changes. In a study to address physiological changes in lipids and blood viscosity after hypoglycemia, we measured C-reactive protein (CRP) before and 4 and 24 h after the hypoglycemic

nadir. We used a sensitive enzyme-linked immunosorbent assay capable of measuring levels between 1 and 8 mg/l with an intra-assay coefficient of variation <7% (1).

We matched 6 male patients with type 1 diabetes (median age 36.5 years, range 28-38; median duration of diabetes 15 years, range 10-17) with 6 nondiabetic male control subjects (median age 31.5 years, range 24-39) recruited from within the hospital staff. All patients had good glycemic control, median HbA<sub>1c</sub> 7% (+4SD), range 6-8.3% (+1.5 to 7.3 SD), laboratory mean 5.4 ± 0.4%. No diabetic patient had a history of antecedent hypoglycemic episodes in the preceding 6 weeks, suffered diabetic complications, smoked, drank >10 U/week, or was on any drug treatment other than insulin.

After an overnight fast, acute hypoglycemia was induced by the intravenous administration of a soluble insulin bolus 0.15 U/kg. Hypoglycemia was confirmed by plasma measurement (Beckman Synchron CX3, Brea, CA), the physiological changes of tachycardia, and typical autonomic symptoms (2). The depth of the hypoglycemic stimulus and the subsequent hemodynamic changes are similar to those described in other studies (3), and they suggest that activation of the autonomic nervous system took place with epinephrine release.

Basal median plasma glucose fell to a nadir of 1.4 ± 0.2 and 1.5 ± 0.3 mmol/l in control subjects and diabetic patients, respectively. In the diabetic patients, the median basal CRP was 0.77 mg/l (range 0.26-2.1); at 4 h, it was 0.84 mg/l (range 0.26-2.06), before rising to 2.31 mg/l (range 1.01-2.79) 24 h after the hypoglycemic episode. Similar rises in the control subjects were observed from a median initial level of 0.32 mg/l (range 0.13-2.52) to 0.96 mg/l (range 0.49-6.38) at 24 h. No significant difference was present by 4 h, but the levels increased by 24 h (*P* < 0.04, *P* < 0.04) in both groups. The data were analyzed using a nonparametric statistical sign test on Minitab statistical software standard version 7.2 (State College, PA).

Studies have demonstrated that increased CRP concentrations are associated with preexisting peripheral vascular disease (4), increased atherosclerotic complications (5), and increased fibrinogen concentrations (5). In patients admitted with unstable angina, those with increased CRP levels have a worse prognosis (6). The reported CRP levels are similar to those found in this study.

Hypoglycemia has been postulated to aggravate diabetic microvascular disease (7), and recurrent hypoglycemic episodes may provoke changes in hemostatic factors and viscosity (7), resulting in reduced perfusion. It is possible that recurrent hypoglycemia in an individual contributes to such hemostatic perturbations by provoking significant subclinical inflammatory response, since an association between inflammation and thrombosis is established (8). Future studies are needed to elucidate the changes in inflammatory markers after hypoglycemia with particular respect to endothelial function and thrombosis.

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References

1. Highton J, Hessan P: A solid phase enzyme immunoassay for C-reactive protein: clinical value and the effect of rheumatoid factor. *J Immunol Methods* 68: 185-192, 1984
2. Hepburn DA: Symptoms of hypoglycaemia. In *Hypoglycaemia and Diabetes: Clinical and Physiological Aspects*, 1st ed. Frier BM, Fisher BM, Eds. London, Edward Arnold, 1993, p. 93-103
3. Frier BM, Fisher BM, Gray CE, Beastall GH: Counter-regulatory hormonal responses to hypoglycaemia in type 1 (insulin-dependent) diabetes: evidence for diminished hypothalamic-pituitary hormonal secretion. *Diabetologia* 31:421-429, 1998
4. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH: Plasma concentrations of C-reactive protein and risk of developing peripheral vascular disease. *Circulation* 97:425-428, 1998
5. Mendall MA, Patel P, Ballam L, Strachan D, Northfield TC: C Reactive protein and its relation to cardiovascular risk factors: a population based cross sectional study. *BMJ* 312:1061-1065, 1996
6. Morrow DA, Rifai N, Antman EM, Weiner DL, McCabe CH, Cannon CP, Braunwald E: C-reactive protein is a potent predictor of mortality independently of and in combination with troponin T in acute coronary syndromes: a TIMI 11A substudy. *thrombolysis in myocardial infarction. J Am Coll Cardiol* 31:1460-1465, 1998

7. Fisher BM, Quin JD, Rumley A, Lennie SE, Small M, MacCuish AC, Lowe GDO: Effects of acute insulin-induced hypoglycaemia on haemostasis, fibrinolysis and haemorheology in insulin dependent diabetic patients and control subjects. *Clin Sci* 80:525–531, 1991
8. Mendall MA: Inflammatory responses and coronary heart disease. *BMJ* 316:953–954, 1998

## Trp64Arg Polymorphism of the $\beta_3$ -Adrenergic Receptor Is Not Associated With Diabetic Nephropathy in Japanese Patients With Type 2 Diabetes

Diabetic nephropathy is a clinical syndrome that is characterized by persistent albuminuria, an increase in blood pressure, a relentless decline in kidney function, and increased cardiovascular morbidity and mortality. Of type 2 diabetic patients, 15–60% develop diabetic nephropathy (1,2). Nevertheless, the precise mechanisms of development and progression of nephropathy are not fully understood. Diabetic nephropathy has been shown to cluster in the families of both in type 1 (3,4) and type 2 (5) diabetic patients to an extent that cannot be explained by shared environmental factors, suggesting that 1 or more genetic factors may be involved.

The Trp64Arg mutation of the  $\beta_3$ -adrenergic receptor gene has been considered to be underlying in some forms of obesity and insulin resistance (6). It was recently suggested that Trp64Arg mutation of the  $\beta_3$ -adrenergic receptor gene is associated with diabetic nephropathy in Japanese type 2 diabetic patients (7), whereas the association was not observed in Caucasian (Polish) patients with type 2 diabetes (8). On this basis, we conducted a cross-sectional association study and a 4-year follow-up study to test the contribution of the  $\beta_3$ -adrenergic receptor gene polymorphism as a candidate gene for diabetic nephropathy in Japanese type 2 diabetic patients.

**Table 1—Genotype distribution of  $\beta_3$ -adrenergic receptor gene and frequency of Trp64 and Arg64 alleles in normoalbuminuric, microalbuminuric, and proteinuric type 2 diabetic patients**

	Type 2 diabetic patients		
	Normoalbuminuric	Microalbuminuric	Proteinuric
<i>n</i>	41	47	52
Genotype			
Trp64/Trp64	26 (63.4)	34 (72.3)	40 (76.9)
Trp64/Arg64	13 (31.7)	13 (27.7)	11 (21.2)
Arg64/Arg64	2 (4.9)	0 (0.0)	1 (1.9)
Allele			
Trp64	65 (79.3)	81 (86.2)	91 (87.5)
Arg64	17 (20.7)	13 (13.8)	13 (12.5)

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

In the study, there were 140 patients with type 2 diabetes (88 men and 52 women, aged  $56 \pm 6$  years) from the out-patient diabetes clinic at Kitasato University Hospital: 41 normoalbuminuric patients ( $54.6 \pm 6$  years of age, diabetes duration  $14.1 \pm 5.4$  years), 47 microalbuminuric patients ( $56.7 \pm 7.9$  years of age,  $12.7 \pm 5.9$  years), and 52 proteinuric patients ( $55.8 \pm 6.7$  years of age,  $14.9 \pm 5.2$  years). The patients were chosen on the basis of age (40–69 years old) and known duration of diabetes (>5 years). Normoalbuminuria was defined as having all 3 of the measurements of the urinary albumin-to-creatinine ratio (ACR) <30 mg/g creatinine during the last 6 months, and microalbuminuria as having all 3 of the measurements of ACR >30 mg/g creatinine in protein dipstick–test negative patients. Proteinuria was diagnosed when patients showed persistent proteinuria together with the presence of retinopathy.

Genomic DNA was extracted from peripheral blood leukocytes of each patient. The Trp64Arg mutation in the  $\beta_3$ -adrenergic receptor gene was detected by the polymerase chain reaction–restriction fragment length polymorphism method with BstNI, as reported by Widén et al. (6).

The difference in the measured variables among the 3 groups was tested by the Kruskal-Wallis test, and the genotype distribution and allele frequency among the groups were analyzed by the  $\chi^2$  test. A *P* value <0.05 was considered to be significant. In addition, a backward stepwise multiple regression analysis ( $F > 4.0$  to enter) was performed to assess the influence of independent variables (i.e., sex, age, known duration of diabetes, current BMI, current HbA<sub>1c</sub>, presence of hypertension, and genotype of the  $\beta_3$ -adrenergic receptor gene) on diabetic nephropathy.

The genotype distributions and allele frequency of the  $\beta_3$ -adrenergic receptor gene are summarized in Table 1. There was no difference among the genotype distribution ( $P = 0.857$ ) in the 3 groups. The frequency of mutated allele was even slightly, but not significantly ( $P = 0.130$ ), lower in the microalbuminuric (13.6%) and proteinuric patients (12.5%) as compared with the normoalbuminuric patients (20.7%). A 4-year follow-up revealed that 25% of patients with wild homozygotes and 33.3% of patients with mutated alleles progressed from normoalbuminuria to microalbuminuria ( $P = 0.750$ ). Similarly, 22.2 and 16.7% of microalbuminuric

**Table 2—Progression of nephropathy during 4-year follow-up in type 2 diabetic patients with and without ARG64 allele**

Genotype	Normoalbuminuria to microalbuminuria	Microalbuminuria to proteinuria	Proteinuria to hemodialysis
Wild type			
Trp64/Trp64	4/16 (25.0)	6/27 (22.2)	11/36 (30.6)
Mutated type			
Trp64/Arg64	4/12 (33.3)	2/12 (16.7)	4/12 (33.3)
Arg64/Arg64			

Data are *n* of progressor/total (%).

patients with wild homozygotes and with mutated alleles progressed from microalbuminuria to persistent proteinuria, respectively ( $P = 0.745$ ). The progression rate from persistent proteinuria to end-stage renal failure (i.e., introduction to hemodialysis) was similar in the 2 groups (30.6% in those with wild homozygotes vs. 33.3% in those with mutated alleles, respectively,  $P = 0.917$ ) (Table 2). The result of multiple regression analysis did not suggest a significant contribution of the mutation to nephropathy ( $F = 0.928$ ), whereas the presence of hypertension was the most relevant variable ( $F = 25.340$ ).

We could not confirm the suggested association (7) between the  $\beta_3$ -adrenergic receptor gene polymorphism and nephropathy in Japanese type 2 diabetic patients. Our observations, however, are in accordance with those of Grzeszczak et al. (8), who also failed to find the association in Polish type 2 diabetic patients. Therefore, the difference between the findings of Sakane et al. (7) and the observations of Grzeszczak et al. (8) is not likely to be caused by the type of diabetes or the ethnic origin of the study subjects. In addition, there was no significant difference in the progression of nephropathy between the patients with and without a mutated allele, although we are aware that the follow-up period might be too short and the number of patients studied is too small to draw any definite conclusions. Nevertheless, these results imply that the association between the Trp64Arg mutation in the  $\beta_3$ -adrenergic receptor gene and nephropathy, even if it exists, would be modest, and is not a clinically useful marker for the predisposition to diabetic nephropathy in type 2 diabetic patients.

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**Acknowledgments**— This study was supported by grants from the Ministry of Education and the Ministry of Health and Welfare, Tokyo, Japan.

The authors wish to thank Dr. Yoshitada Yajima, Kitasato University School of Medicine, and Dr. Tsuyoshi Watanabe, Fukushima Medical University School of Medicine, for their useful comments and cooperation.

#### References

1. Nelson RG, Kunzelmann CL, Pettitt DJ, Saad MF, Bennett PH, Knowler WC: Albuminuria in type 2 (non-insulin dependent) diabetes mellitus and impaired glucose tolerance in Pima Indians. *Diabetologia* 32: 870–876, 1989
2. Sasaki A, Horiuchi N, Hasegawa K, Uehara M: Risk factors related to the development of persistent albuminuria among diabetic patients observed in a long-term follow-up. *J Japan Diabetes Soc* 29:1017–1023, 1993
3. Seaquist ER, Goetz FC, Rich S, Barbosa J: Familial clustering of diabetic kidney disease. *N Engl J Med* 320:1160–1165, 1989
4. Borch-Johnsen K, Nøgaard K, Hommel E, Mathiesen ER, Jensen JS, Deckert T, Parving H-H: Is diabetic nephropathy an inherited complication? *Kidney Int* 41:719–722, 1992
5. Pettitt DJ, Saad MF, Bennett PM, Nelson RG, Knowler WC: Familial predisposition to renal disease in two generations of Pima Indians with type 2 (non-insulin dependent) diabetes mellitus. *Diabetologia* 33: 438–443, 1990
6. Widén E, Lehto M, Kanninen T, Walston J, Shuldiner AR, Groop LC: Association of polymorphism in the  $\beta_3$ -adrenergic receptor gene with features of the insulin resistance syndrome in Finns. *N Engl J Med* 333:348–351, 1995
7. Sakane N, Yoshida T, Yoshioka K, Nakamura Y, Umekawa T, Kogure A, Takakura Y, Kondo M: Trp64Arg mutation of  $\beta_3$ -adrenoceptor gene is associated with diabetic nephropathy in type II diabetes mellitus (Letter). *Diabetologia* 41:1533–1534, 1998
8. Grzeszczak W, Saucha W, Zychma MJ, Zukowska-Szczechowaska E, Labuz B, Lacka B, Szydłowska I: Is Trp64Arg polymorphism of  $\beta_3$ -adrenergic receptor a clinically useful marker for the predisposition to diabetic nephropathy in type II diabetic patients? (Letter) *Diabetologia* 42: 632–633, 1999

## Telemedicine in the Management of Pregnancy in Type 1 Diabetic Women

The intensive care and treatment of pregnant women with type 1 diabetes, which aims for normoglycemia during the entire time of pregnancy, is very

important to prevent congenital malformations, macrosomia, and peripartur complications in the children (1). We studied whether the diabetological care of pregnant women can be improved through the use of telemedicine, which offers facilitated communication between clinicians and patients (2,3). For this purpose, we used the prototype of a remote data management system (CareLink; Abbott-MedSense, New Bedford, MA) and are reporting our preliminary results.

Eleven pregnant women with type 1 diabetes (all treated with an intensified insulin therapy, either with multiple daily injections or continuous subcutaneous insulin infusion, mean age  $30.5 \pm 3.4$  years, mean diabetes duration  $13.5 \pm 7.7$  years) were controlled by the CareLink system from the 15th gestational week on. This was in addition to the usual diabetological care, which consists of regular ambulatory visits with consultations and examinations every 2–3 weeks in our diabetes center. The appointments made for the visits depended partially on the achieved metabolic control and on the personal situation of the patients (e.g., distance between home and hospital, mothers with babies or young children).

The telemedicine system we used enabled the patients to easily transmit their blood glucose values from the memory of the glucose meter (storage capacity 125 values), by means of a modified modem via phone line, directly to a personal computer in our diabetes center. There the data were stored and evaluated by means of appropriate software. The patients performed blood glucose self-monitoring at least 4–6 times a day and usually transmitted their glucose values once a week. We aimed for fasting and preprandial blood glucose values between 60 and 90 mg/dl and postprandial 2-h blood glucose values  $\leq 120$  mg/dl (4). The patients were advised to make additional transmissions when values outside these targets occurred frequently. In this case, we provided advice over the telephone to make appropriate corrections of the insulin doses. Besides different graphs showing the development of the blood glucose values over time, mean values (calculated monthly) and standard deviations of all glucose values and of the fasting glucose values separately were taken for evaluation.

A control group was formed of 10 pregnant women with type 1 diabetes with



comparable age, diabetes duration, self-monitoring practice, and insulin regimen, who received standard diabetological care during the same time period as the Care-Link group, but without the addition of telemedicine. Since these patients used different glucose meters, their glucose values could not be automatically evaluated by the applied computer software. The patients of both groups also kept a conventional diabetes diary.

The mean time between 2 visits was 3.3 weeks for the CareLink group and 2.9 weeks for the control group. HbA<sub>1c</sub> (assessed by high-pressure liquid chromatography, normal range 4.3–6.1%) was improved in the CareLink group (mean duration of care 22.5 ± 5.9 weeks) from 6.1 ± 1.0 to 5.4 ± 0.3% and in the control group (mean duration of care 26.8 ± 4.5 weeks) from 6.2 ± 0.8 to 5.7 ± 0.6% (the difference between the groups in the 2-sided independent-samples Student's *t* test was not significant). The mean blood glucose (all values) in the CareLink group was reduced from 141 ± 90 to 110 ± 18 mg/dl, the mean fasting glucose from 111 ± 17 to 101 ± 23 mg/dl (*P* < 0.05, 2-sided paired-samples Student's *t* test). The variation of blood glucose was markedly reduced, too: the standard deviation in the individual patients fell from 51.6 to 44.4 mg/dl (*P* < 0.01) for the mean blood glucose and from 41.4 to 31.0 mg/dl for the mean fasting glucose. There was no significant difference in the number of instances of severe hypoglycemia in both groups.

From our experience, we conclude that the described remote data management system for glucose monitoring is easy to use and helpful for tight and efficient care of pregnant diabetic women, even when the number of personal ambulatory visits in the diabetes center is decreased. Thus, this tool of telemedicine is suitable especially for women who have difficulties adhering to the regular visits at a diabetes center.

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**References**

1. Langer O: Is normoglycemia the correct threshold to prevent complications in the

pregnant diabetic patient? *Diabetes Rev* 4: 2–10, 1996

2. Balas EA, Jaffrey F, Kuperman GJ, Boren SA, Brown GD, Pinciroli F, Mitchell JA: Electronic communication with patients: evaluation of distance medicine technology. *JAMA* 278:152–159, 1997

3. Ruggiero C, Sacile R, Giacomini M: Home telecare. *J Telemed Telecare* 5:11–17, 1999

4. Kühl C: New approaches for the treatment of pregnant diabetic women. *Diabetes Rev* 3:621–631, 1995

## Gly82Ser Polymorphism of the Receptor of Advanced Glycation End Product Gene Is Not Associated With Coronary Heart Disease in Finnish Nondiabetic Subjects or in Patients With Type 2 Diabetes

Long-lasting exposure to hyperglycemia leads to the accumulation of advanced glycation end products (AGEs), which could lead to diabetic complications (1). The effects of AGEs are mediated via the cellular receptor of AGE (RAGE), and variants in the RAGE gene could potentially enhance the development of coronary heart disease (CHD). Recently, Hudson et al. (2) detected 4 functional amino acid variants in the RAGE gene. The relatively common Gly82Ser polymorphism in exon 3 was not associated with myocardial infarction in type 2 diabetic patients of different ethnic origin. In another study, Liu and Xiang (3) found no association of the Gly82Ser polymorphism with diabetic microangiopathy in Chinese type 2 diabetic patients.

We screened the Gly82Ser polymorphism of the RAGE gene among 308 unrelated Finnish nondiabetic subjects with CHD (221 men and 87 women, aged 60 ± 1 years), 206 unrelated type 2 diabetic patients with CHD (141 men and 65 women, aged 64 ± 1 years), and in 82 randomly selected healthy men (aged 54 ± 1 years). Patients with CHD had to have stenosis >50% in at least 2 coronary vessels in a coronary angiogram. Genotyping was performed according to the method of Hudson et al. (2). There were no differences in genotype frequencies (12 vs. 15 vs. 7%

Gly82Ser), (no Ser82Ser homozygotes) or Ser allele frequencies in codon 82 of the RAGE gene (6 vs. 7 vs. 4%) among the study groups. The Ser allele frequency (6%) was as frequent as that in Caucasian patients (6%) (2), but lower than that in Chinese type 2 diabetic patients (23%) (3). In the present study, the Ser allele was not associated with any of the CHD risk factors (data not shown).

Our study indicates that the Gly82Ser polymorphism in exon 3 of the RAGE gene is not associated with CHD in Finnish nondiabetic subjects or in patients with type 2 diabetes.

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**References**

1. Vlassara H: Recent progress in advanced glycation end products and diabetic complications. *Diabetes* 46 (Suppl. 2):19–25, 1997

2. Hudson BI, Stickland MH, Grant PJ: Identification of polymorphisms in the receptor for advanced glycation end products (RAGE) gene: prevalence in type 2 diabetes and ethnic groups. *Diabetes* 47:1155–1157, 1998

3. Liu L, Xiang K: RAGE Gly82Ser polymorphism in diabetic microangiopathy (Letter). *Diabetes Care* 22:646, 1999

## Relationship Between Plasma Adrenomedullin Levels and Metabolic Control, Risk Factors, and Diabetic Microangiopathy in Patients With Type 2 Diabetes

Well-controlled diabetes prevents the occurrence of microvascular complications and decreases the formation of macrovascular complications (1,2).

**Table 1—Plasma ADM levels and general features of the treatment and control group**

Group	Treatment	n	Sex		Age	BMI (kg/m <sup>2</sup> )	Disease duration	Adrenomedullin (pg/ml)
			F	M				
I	Diet	14	9	5	46.9 ± 9.0	29.2	2.9	63.59 ± 11.71
II	Diet + OAD	11	8	3	47.4 ± 6.6	27.5	6.7	64.94 ± 9.11
III	Diet + OAD + HT	21	16	5	55.6 ± 9.2	28.5	6.6	64.55 ± 9.53
IV	Diet + insulin	18	12	6	55.7 ± 8.1	28.4	10.5	65.43 ± 10.89
Control	—	20	8	12	50.2 ± 6.0	27.6	—	55.90 ± 11.12

Data are n and means ± SEM, unless otherwise indicated. HT, hypertension.

Studies concerning the pathogenesis, complications, and metabolic control of diabetes are still continuing. Adrenomedullin (ADM) is a vasorelaxing peptide produced from endothelium and smooth muscle cells. ADM is known to decrease the levels of insulin and delay the insulin response to oral glucose (3,4). In this study, we investigated plasma ADM levels in patients with type 2 diabetes and healthy subjects.

Enrolled in the study were 64 type 2 diabetic patients (19 men and 45 women) aged 52 ± 9 years. The mean disease duration was 6.9 ± 5 years and the mean BMI was 28.5 ± 4.5 kg/m<sup>2</sup>. These patients were grouped according to age, sex, type of treatment, presence of microangiopathy, fasting blood glucose levels, HbA<sub>1c</sub> levels, hypertension, hyperlipidemia, BMI, and disease duration. Patients with liver disease, renal failure, or congestive heart failure were excluded from the study. The control group consisted of 20 healthy subjects (12 women and 8 men), aged 50 ± 6 years with a mean average BMI of 27.6 ± 4.8 kg/m<sup>2</sup>.

Fasting venous blood samples were immediately transferred into a chilled polypropylene tube containing EDTA (1 mg/ml blood) and aprotinin (500 kU/ml blood). Plasma ADM was measured by radioimmunoassay using the kit supplied by Phoenix Pharmaceutical (Mountain View, CA). Glucose was measured by the hexokinase method (Olympus AU 600; Olympus Diagnostica GmbH, Hamburg, Germany). HbA<sub>1c</sub> was measured by micro-column chromatographic spectrophotometry (Poli Industria Chimica, Milan, Italy).

Mean plasma ADM levels were found to be 64.6 ± 10.1 pg/ml in patients with type 2 diabetes. In the control group, it was 55.9 ± 11.1 pg/ml. The difference was statistically significant ( $P = 0.0036$ ). The 64 patients with type 2 diabetes were divided into 4 groups according to the type of treatment as follows: group I ( $n = 14$ ) was on a diet; group II ( $n = 11$ ) was on

a diet and oral antidiabetic drugs (OAD), e.g., gliclazide, glipizide, glibornuride, metformin, and glimepirid acarbose; group III ( $n = 21$ ) hypertensive diabetic patients were on a diet and OAD; and group IV ( $n = 18$ ) consisted of patients on a diet and insulin treatment. The plasma ADM levels in the 4 groups were 63.5 ± 11.7, 64.9 ± 9.1, 64.5 ± 9.5, and 65.4 ± 10.8 pg/ml, respectively. The differences were statistically significant when compared with the control group (all  $P$  values <0.05). However, the difference between the 4 groups was not significant (all  $P$  values >0.05). Table 1 shows the general characteristics and ADM levels of the control group and patients with type 2 diabetes.

When the effects of microangiopathy on ADM levels were investigated, the mean ADM level was found to be 65.3 ± 10.6 pg/ml in patients with only nephropathy ( $n = 14$ ), 62.4 ± 9.3 pg/ml in patients with only neuropathy ( $n = 7$ ), 62.2 ± 11.7 in patients with only retinopathy ( $n = 3$ ), 67.1 ± 10.1 pg/ml in patients with nephropathy and neuropathy ( $n = 6$ ), 71.1 ± 10.9 pg/ml in patients with nephropathy and retinopathy ( $n = 4$ ), 62.5 ± 10.8 pg/ml in patients with neuropathy and retinopathy ( $n = 4$ ),

and 64.9 ± 9.5 pg/ml in patients with neuropathy, retinopathy, and nephropathy ( $n = 10$ ). The mean ADM level was found to be 63.2 ± 10.2 pg/ml in patients having no diabetic microvascular complications ( $n = 16$ ) (Table 2). There were statistically significant differences in all groups with diabetic microangiopathy when compared with the control group (all  $P$  values <0.05). However, the difference between the groups with and without microangiopathy was not statistically significant (all  $P$  values >0.05). There were also no significant differences in patients with or without nephropathy, neuropathy, or retinopathy (all  $P$  values >0.05) (Table 3). Plasma ADM levels were found to be 65.4 ± 7.1 pg/ml in patients with HbA<sub>1c</sub> levels ≤8% ( $n = 21$ ) and 64.9 ± 11.6 pg/ml in patients with levels >8% ( $n = 43$ ) ( $P > 0.05$ ). When fasting blood glucose levels were compared, plasma ADM levels were found to be 61.5 ± 11.3 pg/ml in patients with fasting blood glucose levels ≤140 mg/dl ( $n = 14$ ) and 65.5 ± 9.7 pg/ml in patients with fasting blood glucose levels >140 mg/dl ( $n = 50$ ) ( $P > 0.05$ ). When hypertensive diabetic patients ( $n = 28$ ) were compared with normotensive

**Table 2—Mean plasma ADM levels of patients with diabetic microangiopathy compared with the control group**

Diabetic microangiopathy	n	ADM (pg/ml)
Nephropathy	14	65.37
Neuropathy	7	62.41
Retinopathy	3	62.23
Nephropathy + neuropathy	6	67.13
Nephropathy + retinopathy	4	71.10
Neuropathy + retinopathy	4	62.55
Nephropathy + neuropathy + retinopathy	10	64.94
Without complication	16	63.28
Control	20	55.90

Data are n, unless otherwise indicated. All  $P$  values are <0.05.

**Table 3—Plasma ADM levels of patients with and without nephropathy, neuropathy, or retinopathy**

Diabetic microangiopathy	n	ADM (pg/ml)
With nephropathy	34	66.23 ± 9.99
Without nephropathy	30	66.10 ± 11.06
With neuropathy	27	64.41 ± 10.63
Without neuropathy	37	64.41 ± 11.29
With retinopathy	21	65.27 ± 10.10
Without retinopathy	43	64.32 ± 11.24

Data are n and means ± SEM. All P values are >0.05.

patients (n = 36), ADM levels were 64.3 ± 9.0 and 65.0 ± 11.1 pg/ml, respectively, with no statistically significant difference (P > 0.05). When BMI values were compared, they were 59.8 ± 12.4 pg/ml in patients with >28 kg/m<sup>2</sup> (n = 29) and 64.0 ± 10.0 pg/ml in patients with ≤28 kg/m<sup>2</sup> (n = 35) with no statistically significant difference (P > 0.05). There were also no statistically significant differences between men and women (P > 0.05). Patients having a diabetes duration of <5 years (n = 25) were compared with those having a duration of ≥5 years (n = 39). Duration of diabetes did not influence the ADM levels significantly (P > 0.05). The effects of age (patients <50 years of age, n = 27 and >50 years of age, n = 37) had no significant influence on ADM levels (P > 0.05). Patients having hypercholesterolemia (>200 mg/dl) or hypertriglyceridemia (>160 mg/dl) were compared with patients having normal lipid levels, and no statistically significant difference existed (all P values >0.05). Patients having risk factors for atherosclerosis such as hypertension, smoking, hyperlipidemia, low HDL levels, increased age, sex, and family history of diabetes were divided into 2 groups. Group A consisted of patients having 1 or 2 risk factors and group B consisted of patients with ≥3 risk factors for atherosclerosis. The plasma ADM levels were 64.3 ± 10.3 pg/ml in group A and 65.0 ± 10.1 pg/ml in group B with no statistically significant difference (P > 0.05).

It was reported that plasma ADM levels, in patients with type 2 diabetes having poor metabolic control, were increased when compared with control subjects (5). In a Spanish study, there was no statistically significant difference in hypoglycemic or hyperglycemic patients and no correlation between plasma ADM and HbA<sub>1c</sub> levels was found (4). It may be commented that well- or poorly controlled diabetes did

not change the plasma levels of ADM. However, a local increase in ADM levels in the vascular endothelium may not greatly affect the plasma levels. Because ADM is thought to be produced by many of the tissues, local vasodilatory effects are more prominent than the systemic effects (6).

ADM may also affect and play a role in the neovascularization process, which occurs after retinal ischemia. ADM controls proliferation, differentiation, and migration of cell functions and stimulates organ development, normal epithelial turnover, and tissue development (5,7). ADM may take part in the pathogenesis of nephropathy. The use of ACE inhibitors in diabetic patients with microalbuminuria may be the reason for the insignificant ADM levels, since these drugs improve endothelial function and elevated ADM levels may be normalized.

In conclusion, plasma ADM levels were significantly elevated in patients with type 2 diabetes when compared with the control group. However, plasma ADM levels were not affected by poor metabolic control, type of microangiopathy, or other risk factors that can cause endothelial injury. Elevated plasma ADM levels in patients with type 2 diabetes may be related to endothelial damage or leukocyte activation, which is thought to be important in the formation of diabetic micro- and macrovascular complications. Because the source of circulating ADM could not be shown exactly, it could have only a local vasodilatory role (6,8,9). That is why an increase in plasma levels of ADM may not be correlated with the risk factors.

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## References

1. D'Antonio JA, Ellis D, Doft BH, Becker DJ, Drash AL, Kuller LH, Orchard TJ: Diabetes complications and glycemic control: the Pittsburgh Prospective Insulin-Dependent Diabetes Cohort Study Status Report after 5 yr of IDDM. *Diabetes Care* 12:694–700, 1989
2. The Diabetes Control and Complications Trial Research Group: The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. *N Engl J Med* 329:977–986, 1993
3. Martinez A, Weaver C, Lopez J, Bhatena SJ, Elsasser TH, Miller MJ, Moody TW, Unsworth EJ, Cuttitta F: Regulation of insulin secretion and blood glucose metabolism by adrenomedullin. *Endocrinology* 137:2626–2632, 1996
4. Garcia-Unzueta MT, Berrazueta JR, Montalban C, Amado JA, Pesquera C: Plasma adrenomedullin levels in type 1 diabetes. *Diabetes Care* 21:999–1003, 1998
5. Hayashi M, Shimosawa T, Isaka M, Yamada S, Fujita R, Fujita T: Plasma adrenomedullin in diabetes. *Lancet* 350: 1449–1450, 1997
6. Sugo S, Minamino N, Kangawa K, Miyamoto K, Kitamura K, Sakata J, Eto T, Matsuo H: Endothelial cells actively synthesize and secrete adrenomedullin. *Biochem Biophys Res Commun* 201:1160–1166, 1994
7. Kobayashi S, Shikasho T, Nishimura J, Kureishi Y, Kanaide H: Adrenomedullin stimulates the cell cycle progression and the expression of c-fos messenger RNA in vascular smooth muscle cells in primary culture. *Circulation* 621–644, 1995
8. Kitamura K, Kangawa K, Kawamoto M, Ichiki Y, Nakamura S, Matsuo H, Eto T: Adrenomedullin: a novel hypotensive peptide isolated from human pheochromocytoma. *Biochem Biophys Res Commun* 192: 553–560, 1993
9. Kitamura K, Ichiki Y, Tanaka M, Kawamoto M, Emura J, Sakakibara S, Kangawa K, Matsuo H, Eto T: Immunoreactive

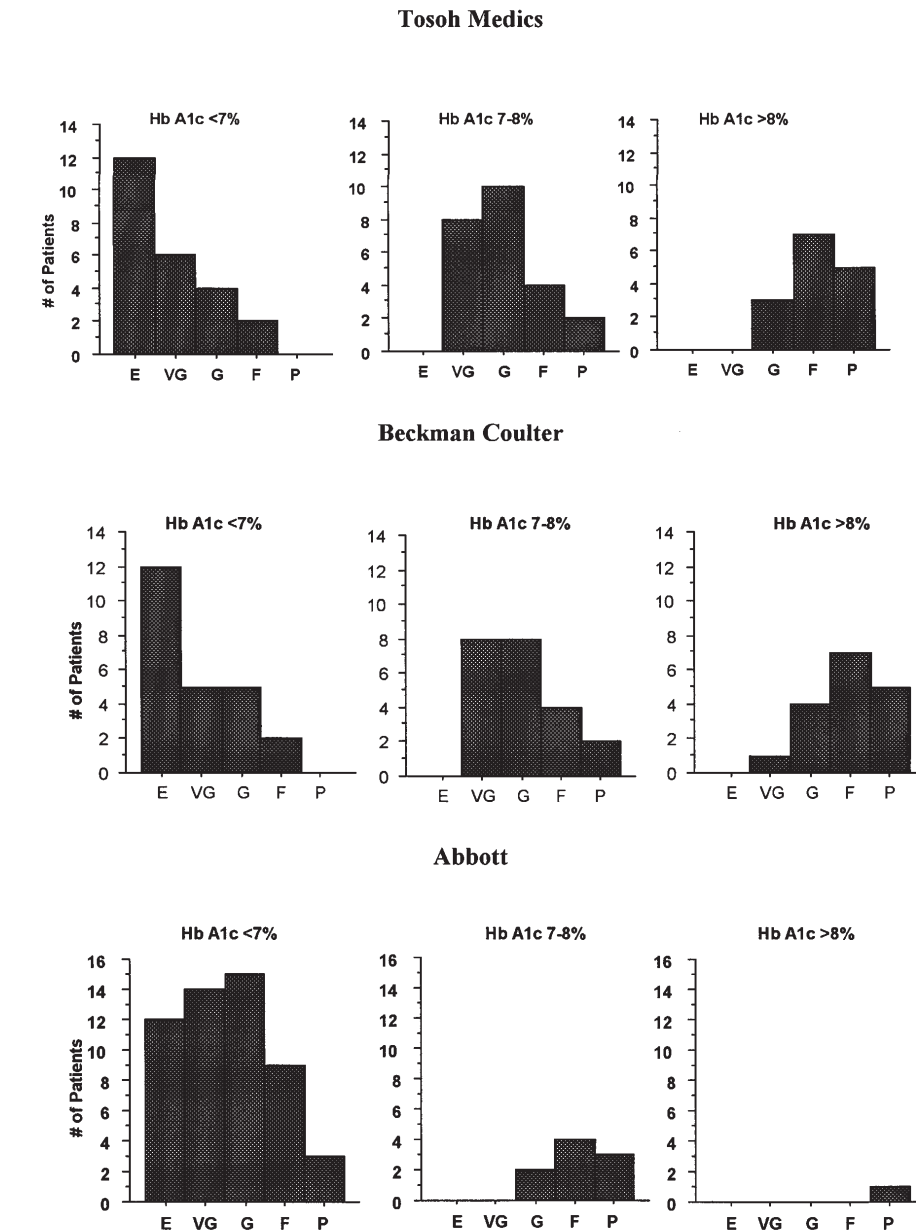


adrenomedullin in human plasma. *FEBS Lett* 341:288–290, 1994

## Cautionary Note Regarding HbA<sub>1c</sub> Methods Predicting the Clinical Status of Diabetic Patients

Our laboratories (Elmhurst Memorial Hospital and Evanston Northwestern Healthcare) were independently involved in discussions with physicians that quickly progressed to clinical complaints about the lack of correlation between HbA<sub>1c</sub> results and the clinical status of the patient. Physicians complained that the current laboratory method (Abbott's affinity ion capture method correlated to HbA<sub>1c</sub>) was giving low values compared with the daily glucose values being reported by the patients. The assessment of clinical status was performed, in part, by review of daily self-monitored glucose values logged by the patient and brought to the office visit. Values for HbA<sub>1c</sub> <7.0% indicate successful glycemic control based on the results of the Diabetes Control and Complications Trial (DCCT) (2). This study demonstrated that intensive treatment of patients with type 1 diabetes reduces the risk of the development or the progression of retinopathy, nephropathy, and neuropathy (50–75% reduction). HbA<sub>1c</sub> values between 7.0 and 8.0% are acceptable, but the patient is advised to watch his or her regimen closely and try to lower the values even further. Values >8.0% indicate the need for a change in treatment and/or behavior.

Both laboratories investigated the performance of the Abbott method but found no explanation for the discrepancy between the HbA<sub>1c</sub> values and the physician's assessment of the patient status. Therefore, a collaborative retrospective study was designed to evaluate the correlation of patient status with 3 commercially available methods for measuring HbA<sub>1c</sub>. The total patient population consisted of 63 diabetic outpatients (35 type 1 and 28 type 2) treated by physicians at Elmhurst Memorial Hospital. To be enrolled in the study, patients had to have a complete record of their daily glucose values determined by home glucose monitors over the last 60 days. The methods evaluated were a high-performance liquid chromatography

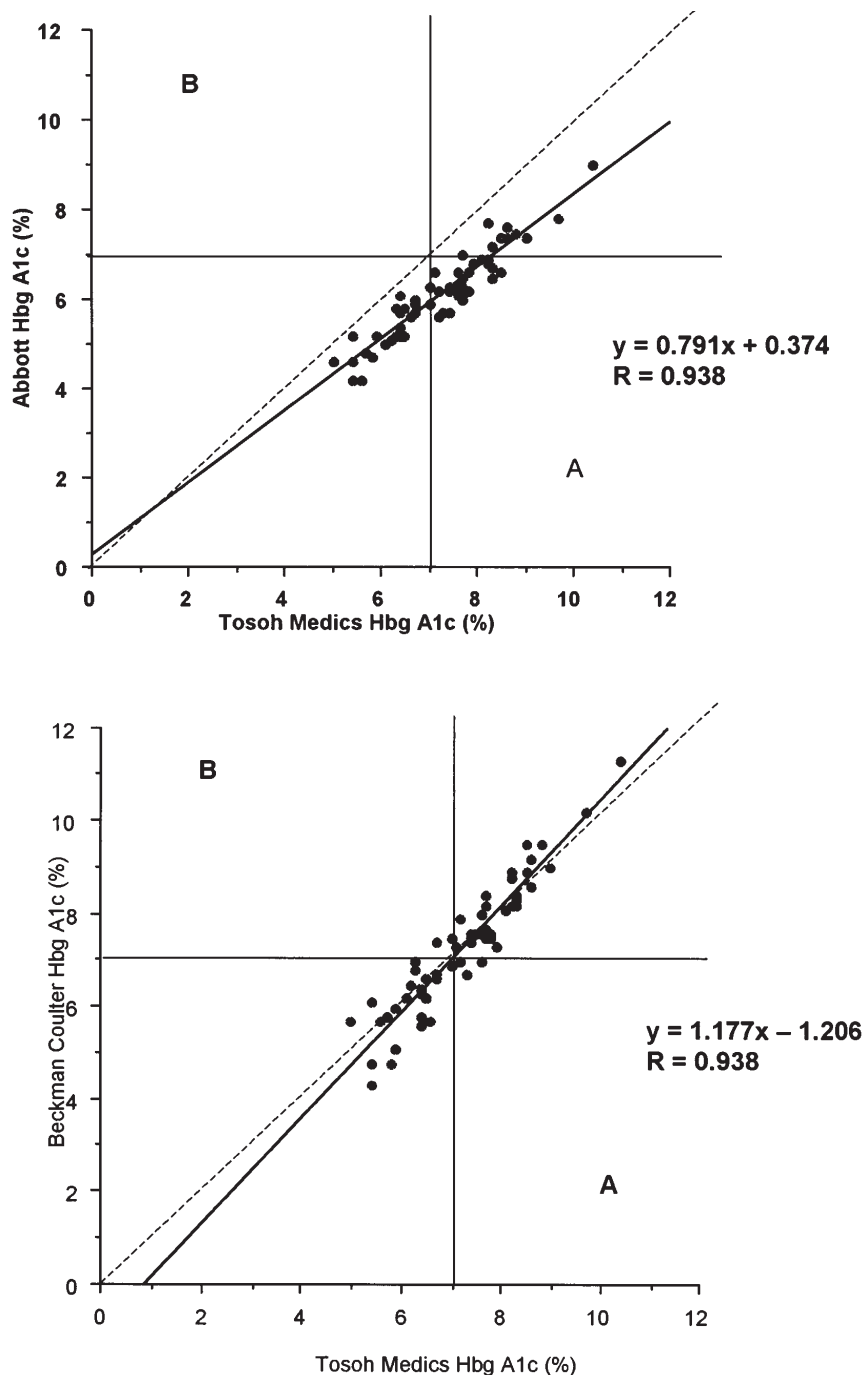


**Figure 1**—The number of patients in each category of glycemic control for each HbA<sub>1c</sub> method separated into HbA<sub>1c</sub> values of <7, 7–8, and >8%. Categories of glycemic control are as follows: excellent (E), very good (VG), good (G), fair (F), and poor (P), based on mean fasting morning glucose values of the previous 60 days.

(HPLC) method by Tosoh Medics (South San Francisco, CA), the Beckman Coulter Synchron turbidimetric immunoinhibition assay (Beckman, Brea, CA), and the Abbott IMX affinity ion capture method (Abbott Diagnostics, Abbott Park, IL). All HbA<sub>1c</sub> methods were performed according to the manufacturer's written specifications.

The mean morning fasting daily serum glucose level was used to assign each patient a specific glycemic control category.

Using their experiences in treating diabetic patients, 2 endocrinologists created the categories. The categories were as follows: poor (7 patients, mean morning fasting glucose >200 mg/dl, 11.1 mmol/l), fair (13 patients, 160–200 mg/dl, 8.9–11.1 mmol/l), good (17 patients, 135–160 mg/dl, 7.5–8.8 mmol/l), very good (14 patients, 110–135 mg/dl, 6.2–7.4 mmol/l), and excellent (12 patients, 80–110 mg/dl, 4.4–6.1 mmol/l). We assessed the validity



**Figure 2**—Linear regression plots of Tosoh Medics versus Beckman (A) and Tosoh Medics versus Abbott (B). Single vertical and horizontal lines mark an HbA<sub>1c</sub> of 7.0%, the target value used in current practice guidelines. The dashed line is the line of perfect regression. The method of least-square regression was used to generate plots.

of using mean morning fasting glucose values from the previous 60 days as compared with the mean of all daily glucose values used in the DCCT trial (3). The results were remarkably similar as shown by the following data: 1) The target glucose means were very similar at the same HbA<sub>1c</sub> levels (at HbA<sub>1c</sub> = 7.0%, DCCT trial glucose = 8.3

mmol/l, current study = 8.3 mmol/l). 2) The ranges of glucose means were similar (HbA<sub>1c</sub> = 7.0%, DCCT trial range = 3.6–13.3 mmol/l, current study = 3.6–12.8 mmol/l). 3) The linear regression statistics were comparable (DCCT trial:  $r = 0.80$ , slope = 36.0; current study:  $r = 0.75$ , slope = 31.0,  $P < 0.005$ ). The similarity of the

study data and the original DCCT trial data indicates that using fasting morning glucose values to predict the expected HbA<sub>1c</sub> level is acceptable. The fasting morning glucose value has the advantage of being easier to use because calculating the mean of all of the glucose results brought to the office visit is often impractical.

Figure 1 shows the number of patients for each method sorted by HbA<sub>1c</sub> values of <7.0, 7.0–8.0, and >8.0%. The distribution of patients across the 3 HbA<sub>1c</sub> groups is almost identical for the Tosoh Medics and Beckman Coulter methods, whereas most patients (53) have values of <7.0% with the Abbott method. Overall, 20 of the 63 patients were categorized as having poor or fair control based on mean fasting morning glucose values. In this group of 20, the HbA<sub>1c</sub> values suggested that improved treatment was needed for 18 patients by both the Tosoh Medics and Beckman Coulter methods and for 8 patients by the Abbott method.

Figure 2A and 2B shows the linear regression graphs for Tosoh Medics versus Beckman Coulter and Tosoh Medics versus Abbott. Each graph is divided into 4 quadrants by a single vertical and horizontal line marking an HbA<sub>1c</sub> value of 7.0%. Data points that fall in quadrant A represent patients for whom the method on the x-axis suggests that improvement is needed, whereas the method on the y-axis suggests that treatment is appropriate. The opposite is true for quadrant B. When the Tosoh Medics versus Beckman Coulter data are compared, a single patient falls into quadrant B and 3 patients fall into quadrant A. For the Tosoh Medics versus Abbott data, 22 patients fall into quadrant A and none are in quadrant B. More significant to the clinical status and treatment of the diabetic patients are any data points >8% for 1 method and <7% with the other method. There were no occurrences in Fig. 2A; however, Fig. 2B showed 6 data points where the Tosoh Medics method had values >8% and the corresponding Abbott value was <7%.

Implementation of the American Diabetes Association recommendations concerning the utilization of HbA<sub>1c</sub> assays in patient management requires that the assays used perform in a manner similar to the DCCT method (HPLC). The Beckman Coulter method, in this study, demonstrates comparability with the Tosoh Medics method in assessing the clinical status of the diabetic patient. The Abbott





cases...[they] recalibrated the tonometer signal assuming equal mean and diastolic pressures between aortic and radial pressure" (4). That is to say, there were such big differences between the mean blood pressure measured in the aorta and that estimated noninvasively using radial artery tonometry with noninvasive brachial artery calibration, that those authors scrapped the noninvasive calibration and relied instead on invasive data from the aorta to calibrate their signals.

It is baffling how researchers can rely on intra-aortic blood pressure data to calibrate blood pressure waveform signals obtained noninvasively from tonometry of the radial artery, and then claim to be able to use such radial artery signals to predict central aortic blood pressure (5).

In addition, those authors have written "there is no argument that individual patient TF [transfer function] differences exist, and for some estimation parameters, such as augmentation index...these differences likely preclude reliable use of a GTF" (6), which rather throws into doubt the reliability of the whole approach used by Brooks et al. to assess central aortic augmentation.

Other researchers have also independently raised substantial concerns about the reliability of the GTF approach for estimating the augmentation index in the aorta (7–9).

In this respect, despite the obvious commercial claims that are being made about the approach (10), to date there has been no published independent peer-reviewed study of a proper validation of the BPA system (11,12). A proper validation study would have had peripheral waveform data collected noninvasively and calibrated noninvasively with sphygmomanometric brachial artery blood pressure readings. These calibrated peripheral waveform data would then have been passed through the GTF, and the computed central aortic blood pressure values and waveform shapes would have been compared with those measured simultaneously in the aorta using a catheter-tipped pressure transducer (12).

The closest to such a study that can be found in the literature involves the use of a brachial artery cuff oscillometric method (instead of a sphygmomanometer) for calibration of the radial artery waveform signals. In this study involving 20 patients, there was a mean difference of 11 mmHg between measured and esti-

mated aortic systolic blood pressure, and a mean difference of 8 mmHg between measured and estimated aortic diastolic blood pressure (13).

According to the Association for the Advancement of Medical Instrumentation, in guidelines endorsed by the U.S. Food and Drug Administration, when the accuracy of any new blood pressure measurement method is compared with that of an intra-arterial catheter, the maximal mean allowable difference both for systolic and diastolic readings is 5 mmHg (14). Therefore, far from the BPA system being validated, it appears that it does not actually comply with these guidelines.

Given all of the above, Brooks et al. should exercise caution in making claims about the validity of the approach they have adopted, which at present remains completely unproven with noninvasive sphygmomanometric calibration, especially in diabetic individuals.

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**References**

1. Brooks B, Molyneaux L, Yue DK: Augmentation of central arterial pressure in type 1 diabetes. *Diabetes Care* 22:1722–1727, 1999
2. Karamanoglu M, O'Rourke MF, Avolio AP, Kelly RP: An analysis of the relationship between central aortic and peripheral upper limb pressure waves in man. *Eur Heart J* 14:160–167, 1993
3. Chen C-H, Nevo E, Fetis B, Pak PH, Yin FCP, Maughan WL, Kass DA: Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure. *Circulation* 95:1827–1836, 1997
4. Fetis B, Nevo E, Chen C-H, Kass DA: Parametric model derivation of transfer function for noninvasive estimation of aortic pressure by radial tonometry. *IEEE Trans Biomed Eng* 46:698–706, 1999
5. Lehmann ED: Estimation of central aortic pressure waveform by mathematical transformation of radial tonometry pressure data (Letter). *Circulation* 98:186, 1998
6. Kass DA, Chen C-H, Nevo E, Fetis B, Pak PH, Maughan WL, Yin FCP: Estimation of central aortic pressure waveform by math-

- emtical transformation of radial tonometry pressure data (Letter). *Circulation* 98:186–187, 1998
7. Cameron J: Estimation of arterial mechanics in clinical practice and as a research technique. *Clin Exp Pharmacol Physiol* 26:285–294, 1999
8. Cameron JD, McGrath BP, Dart AM: Use of radial artery applanation tonometry and a generalized transfer function to determine aortic pressure augmentation in subjects with treated hypertension. *J Am Coll Cardiol* 32:1214–1220, 1998
9. Cameron JD, McGrath BP, Dart AM: Use of radial artery applanation tonometry (Letter). *J Am Coll Cardiol* 34:952, 1999
10. O'Rourke MF: Method for ascertaining the pressure pulse and related parameters in the ascending aorta from the contour of the pressure pulse in the peripheral arteries. U.S. Patent No. 5265011, 1993
11. Lehmann ED: Non-invasive measurements of aortic stiffness: methodological considerations. *Pathol Biol* 47:716–730, 1999
12. Lehmann ED: Regarding the accuracy of generalized transfer functions for estimating central aortic blood pressure (Letter). *J Hypertens* 17:1225–1226, 1999
13. Takazawa K, O'Rourke MF, Fujita M, Tanaka N, Takeda K, Kurosu F, Ibukiya C: Estimation of ascending aortic pressure from radial arterial pressure using a generalised transfer function. *Z Kardiol* 85 (Suppl. 3):137–139, 1996
14. Association for the Advancement of Medical Instrumentation: *Electronic or Automated Sphygmomanometers*. Arlington, VA, Association for the Advancement of Medical Instrumentation, 1992

**Response to Lehmann**

**D**r. Lehmann's negative views on the derivation of central arterial pressure waveform by the mathematical transformation of radial tonometry pressure data are well known, as evidenced by the several debates he has entered into in the correspondence columns of various journals. These issues have been addressed by Kass et al. in one letter by Lehmann (1) and Wilkinson et al. in another letter (2). The debate largely concerns the accuracies of indirect methods for measuring arterial pressure (3). There is no argument that the most accurate measurement of aortic pressure is by direct catheterization. However, this is obviously not possible for studying a large group of individuals. Therefore, we will address only the question of whether this method is applicable to various disease



izations for patients with diabetes, inpatient care by specialists may be an extremely cost-effective measure if LOS is reduced by as little as 1 day (2).

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References

1. Buchanan L, Paterson K: Hospital management of diabetic ketoacidosis in the U.K. (Letter). *Diabetes Care* 23:871, 2000
2. National Association of Physician Recruiters: Physician salary surveys: Physician Compensation Survey—in practice three years plus. <http://www.napr.org/salary/salary2.html>
3. U.S. Center for Health Statistics: 1997 National Hospital Discharge Survey (Public-Use Data Tape), Washington, DC, U.S. Department of Health and Human Services, 1999

## Hemophilus Vaccine Associated With Increased Risk of Diabetes

### Causality likely

Graves et al. (1) recently published a study that cited our research several times and questioned the ability of the hemophilus vaccine to cause type 1 diabetes. We recently published our findings from a prospective clinical trial on the hemophilus influenza B vaccine (2). The data indicate that the hemophilus vaccine is likely to cause type 1 diabetes and the risk of the vaccine exceeds the benefit multifold. The study by Graves et al. did find the hemophilus vaccine associated an odds ratio of 1.18 with a mean follow-up of 6.2 years, and we found the vaccine associated with a relative risk of 1.19 at age 5.

The differences between our finding and that of Graves et al. can be explained by several factors. We measured an increase of 58 cases of type 1 diabetes per 100,000 patients when the vaccinated cases were

studied from immunization at 3 months until the age of 10. The additional cases did not begin to occur until ~3.5 years postimmunization. In contrast, the authors rely on a single autoantibody to predict the development of type 1 diabetes, and it is well known that a single autoantibody has very low specificity for predicting the development of type 1 diabetes. Our analysis involved studying more than 100,000 vaccinated children and an equal number of control subjects. The authors, in contrast, studied only 25 individuals with an autoantibody and 292 control subjects. Their study group has found only 5 antibody-positive children who developed diabetes, whereas our study involved 886 cases of type 1 diabetes. In summary, their study is too small, their follow-up too short, and their markers too nonspecific to obtain the findings we made. However, even with all of these limitations, the authors found the hemophilus vaccine associated with an odds ratio of 1.18 (72/62, Graves et al. Table 1), which was similar to the relative risk of 1.19 (166/140) that we found in the hemophilus vaccinated children by age 5.

We would like to clarify several points pertaining to our research to which Graves et al. elude. We propose that immunization starting in the first month of life will lead to a decreased risk of type 1 diabetes when compared with immunization starting after 2 months of life (3,4). We are not proposing that immunization be delayed until 2 or 5 years of age but instead be administered earlier. There is now a large amount of data to support an association between immunization starting after 2 months and an increased risk of type 1 diabetes (3,5). The data include the hemophilus, pertussis, measles, mumps, rubella, hepatitis B, and Bacillus Calmette-Guérin vaccines. We are aware that vaccine manufacturers and public health officials do not want to alarm the public. However, we believe that the public has the right to know that data indicate that the increased risk of diabetes associated with the hemophilus and other vaccines appears to exceed the benefit of these vaccines (2), and efforts to deny this cause many children to forgo the needed financial compensation to which they are entitled.

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References

1. Graves PM, Barriga KJ, Norris JM, Hoffman MR, Liping Y, Eisenbarth GS, Rewers M: Lack of association between early childhood immunizations and  $\beta$ -cell autoimmunity. *Diabetes Care* 22:1694–1697, 1999
2. Classen JB, Classen DC: Association between type 1 diabetes and hib vaccine: causal relation is likely (Letter). *BMJ* 319:1133, 1999
3. Classen DC, Classen JB: The timing of pediatric immunization and the risk of insulin-dependent diabetes mellitus. *Infect Dis Clin Pract* 6:449–454, 1997
4. Classen JB, Classen DC: Immunization in the first month of life may explain decline in incidence of IDDM in the Netherlands. *Autoimmunity* 31:43–45, 1999
5. Classen JB, Classen DC: Immunisation and type 1 diabetes mellitus: is there a link? *Drug Saf* 21:423–425, 1999

## Hemophilus Vaccine and Diabetes

In our recent case-control study of the association between immunizations and  $\beta$ -cell autoimmunity (1), the odds ratio for exposure to *Hemophilus* vaccine before the age of 9 months was 1.64, not 1.18 as calculated by Classen and Classen (2). However, it was not statistically significant, since the 95% CI of 0.62–4.5 included 1. In reporting their analysis of data from a Finnish randomized trial of different *Hemophilus* vaccine schedules (3), Classen and Classen do not mention that a separate published analysis of the data has concluded that there is no evidence of a significant increase in incidence of type 1 diabetes in children given a 4-dose schedule starting at age 2 months compared with 1 dose at age 2 years (4). There is no evidence from this Finnish trial or any other study in humans that vaccination with *Hemophilus* at birth would reduce the risk of diabetes.

Graves et al. agree that a larger sample size would be desirable to increase the power of the study, but feel that the importance of a possible harmful or protective effect of immunizations precluded waiting for more cases to accrue in the Diabetes Autoimmunity Study in the Young.



Although Classen and Classen imply that the cases in our study had transient autoantibodies, in fact the case definition required persistent autoimmunity and the majority of cases had multiple autoantibodies, which are predictive of a high risk of developing type 1 diabetes (5). Nevertheless, a larger case-control study to further investigate this issue in children who have type 1 diabetes is in progress.

Prospective studies of the effect of immunizations on diabetes incidence, such as that performed in Finland (4), will be extremely difficult because of the large number of participants and the very long follow-up time required. Case-control studies are a more productive way of investigating this important topic. Studies to date have not produced any evidence that change in the childhood immunization schedule would prevent  $\beta$ -cell autoimmunity or lower the risk of type 1 diabetes.

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References

1. Graves PM, Barriga KJ, Norris JM, Hoffman MR, Yu L, Eisenbarth GS, Rewers M: Lack of association between early childhood immunizations and type 1 diabetes. *Diabetes Care* 22:1694-1697, 1999
2. Classen JB, Classen DC: *Haemophilus* vaccine associated with increased risk of diabetes: causality likely (Letter). *Diabetes Care* 23:872, 2000
3. Eskola J, Kayhty H, Takala AK, Peltola H, Ronnberg PR, Kela E, Pekkanen E, McVerry PH, Makela PH: A randomized prospective trial of a conjugate vaccine in the protection of infants and young children against invasive *Haemophilus influenzae* b disease. *N Engl J Med* 323:1381-1387, 1990
4. Karvonen M, Cepaitis Z, Tuomilehto J: Association between type 1 diabetes and *Haemophilus influenzae* type b vaccination: birth cohort study. *BMJ* 318:1169-1172, 1999
5. Verge CF, Gianini R, Kawasaki E, Yu L, Pietropaolo M, Jackson RA, Chase HP, Eisenbarth GS: Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA512/bc/IA-2 autoantibodies. *Diabetes* 45:926-933, 1996

### Consensus Development Conference on Diabetic Foot Wound Care

A randomized controlled trial does exist supporting use of adjunctive hyperbaric oxygen therapy

I read with interest the Consensus Development Conference Report published in the August 1999 issue of *Diabetes Care* (1). As an attendee and participant in that conference, I am puzzled and concerned about the statement in that report regarding the use of adjunctive hyperbaric oxygen (HBO) therapy: "There are no randomized controlled trials supporting the use of hyperbaric oxygen therapy to treat neuropathic foot wounds." I discussed the work of Faglia et al. (2) in which 70 diabetic patients were randomized to undergo either adjunctive HBO therapy or more traditional care. The groups were well matched and very carefully investigated and monitored. Sensory motor neuropathy was present in all 35 of the HBO-treated patients and in 31 of the control subjects ( $P = 0.23$ ); thus, neuropathy was a prominent feature in this study. Additionally, both groups were well matched for vasculopathy and corrective angioplasty or bypass surgery. This study clearly demonstrates an improvement in salvage in the HBO-treated group in whom Faglia et al. demonstrated a 9.1% (2 of 22 patients) amputation rate from Class IV Wagner lesions in the HBO-treated patients vs. a 55% (11 of 20 patients) amputation rate in the control group ( $P = 0.02$ ). These findings support, in a randomized and controlled trial, the benefit of HBO therapy as an adjunct in the treatment of the ischemic and/or infected neuropathic foot.

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References

1. American Diabetes Association: Consensus Development Conference on Diabetic Foot Wound Care (Consensus Statement).

*Diabetes Care* 22:1354-1360, 1999

2. Faglia E, Favales F, Aldeghi A, Calia P, Quarantiello A, Oriani G, Michael M, Campagnoli P, Morabito A: Adjunctive systemic hyperbaric oxygen therapy in treatment of severe prevalently ischemic diabetic foot ulcer. *Diabetes Care* 19:1338-1343, 1996

### Response to Cianci

Thank you for the opportunity to comment on the letter from Dr. Cianci (1) regarding the consensus statement "Consensus Development Conference on Diabetic Foot Wound Care" (2). As Dr. Cianci correctly points out, there is indeed a single randomized controlled trial in the literature describing the use of hyperbaric oxygen (HBO) therapy for diabetic patients with primarily ischemic lesions of the lower extremity (3). Although the mean values of vibration perception in the patients studied were within age-adjusted norms (4), the mean transcutaneous oxygen tensions were significantly below normal limits, >60% of the patients had gangrene, and the mean ankle brachial indexes were 0.65. Thus, the patients had primarily ischemic ulcers.

The consensus statement correctly stated that there were no trials of HBO in neuropathic patients, referring to patients whose lesions had a primarily neuropathic etiology and who were without significant lower-extremity vascular disease. Until evidence exists for the efficacy of HBO in healing primarily neuropathic foot ulcers, this treatment modality cannot be recommended for such patients, and relief of mechanical stress must be considered the primary approach to treatment.

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References

1. Cianci P: Consensus Development Conference on Diabetic Foot Wound Care: a randomized controlled trial does exist, supporting use of adjunctive hyperbaric oxygen therapy (Letter). *Diabetes Care* 23: 873, 2000



ing number of Hb variants can falsify HbA<sub>1c</sub> results with immunoassays. Only boronate affinity methods measure glycohemoglobin, regardless of the glycation site and may be more useful to reflect glycemic control in samples with Hb variants.

Shortened erythrocyte life span, as a reason for these low HbA<sub>1c</sub> results, makes sense in the patient reported (1), but we would suggest that Hb electrophoresis be performed to exclude a hemoglobin variant.

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References

1. Herranz L, Grande C, Janez M, Pallardo F: Red blood cell autoantibodies with a shortened erythrocyte life span as a cause of lack of relation between glycosylated hemoglobin and mean blood glucose levels in a woman with type 1 diabetes (Letter). *Diabetes Care* 22:2085–2086, 1999
2. Schnedl WJ, Krause R, Halwachs-Baumann G, Trinker M, Lipp RW, Krejs GJ: Evaluation of HbA<sub>1c</sub> determination methods in patients with hemoglobinopathies. *Diabetes Care* 23:339–344, 2000

## The 12-Item Well-Being Questionnaire

### Origins, current stage of development, and availability

**P**ouwer et al. (1) reported on the psychometric properties of a Dutch translation of the 12-Item Well-Being Questionnaire (W-BQ12) in a recent article in *Diabetes Care*. However, in their article,

no mention was made of the source of the original W-BQ12 and no information was given about other translations of the W-BQ12 or how to obtain permission to use the instrument. This information is provided below.

The W-BQ12 was first developed by members of the Diabetes Research Group at Royal Holloway, University of London, in collaboration with Dr. Ishii and his colleagues at the Tenri Hospital, Nara, Japan. A Japanese version of the Well-Being Questionnaire was used with a sample of people with diabetes attending the Tenri Hospital diabetes clinic. This work, sponsored by Eli Lilly Japan, was documented in a 1996 internal report to Eli Lilly (2) and in a published abstract by Riazi et al. (3). The W-BQ12 consists of selected items from the longer parent instrument, the 22-Item Well-Being Questionnaire (W-BQ22) (4,5). The W-BQ12 achieves a balance of positively-worded versus negatively-worded items with subscales of equal length, thereby improving on the structure of the W-BQ22, as well as providing a welcome short form.

The W-BQ22 is available in >20 translations, in addition to Japanese (2,3) and Dutch (1). In work sponsored by Hoechst Marion Roussel, Germany (now Aventis Pharma Deutschland), the psychometric properties of 8 translations of the W-BQ12 (including English for use in the U.S.) have now been examined, and the factor structure and reliability were shown to be excellent for all but 1 language, in which further investigation with a larger sample size was needed (6). Thus, it would appear that the selection of items made to produce the W-BQ12 in the Japanese translation is also producing a psychometrically sound instrument in other translations, at least in terms of internal consistency, reliability, and factor structure. Reanalysis of previous data sets using the W-BQ22 and further use of the W-BQ22/12 in clinical trials and other intervention studies are now needed to establish the new W-BQ12's sensitivity to change in comparison with that of the parent instrument.

As the copyright holder of the W-BQ22 and W-BQ12 and their translations, potential users of these instruments may contact me for permission to use them in any of the translations currently available. Readers wishing to reanalyze existing data sets from the W-BQ22 to further investigate the properties of the new W-BQ12 are welcome to contact me for further details.

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C.B. has received grants from Eli Lilly Japan and Hoechst Marion Roussel and consulting fees from Hoechst Marion Roussel (now Aventis Pharma).

References

1. Pouwer F, van der Ploeg HM, Ader J, Heine RJ, Snoek FJ: The 12-Item Well-Being Questionnaire: an evaluation of its validity and reliability in Dutch people with diabetes. *Diabetes Care* 22:2004–2010, 1999
2. Bradley C: Well-being Questionnaire (W-BQ): translation and development of a Japanese version, the W-BQ12 (Japanese). Report to M. Wada. Kobe, Japan, Eli Lilly Japan KK, 1996
3. Riazi A, Ishii H, Barendse S, Bradley C: Well-Being Questionnaire (W-BQ): translation and psychometric development of a short form (W-BQ12) in Japanese (Abstract). *Proc Brit Psychol Soc* 7 (Suppl. 1):34A, 1999
4. Bradley C, Lewis KS: Measures of psychological well-being and treatment satisfaction developed from the responses of people with tablet-treated diabetes. *Diabet Med* 7:445–451, 1990
5. Bradley C: The Well-Being Questionnaire. In *Handbook of Psychology and Diabetes: A Guide to Psychological Measurement in Diabetes Research and Practice*. Bradley C, Ed. Chur, Switzerland, Harwood Academic Publishers, 1994, p. 89–109
6. Plowright R, Witthaus E, Bradley C: Evaluating the 12-item Well-Being Questionnaire for use in multinational trials. *Qual Life Res* 8:650, 1999



# Errata

**Rohlfing CL, Little RR, Wiedmeyer H-M, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE: Use of GHb (HbA<sub>1c</sub>) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:187–191, 2000**

The authors have asked that 2 sentences in column 3 on page 188 be corrected. The sentence starting on line 10, which reads “Sensitivity at each possible HbA<sub>1c</sub> cutoff level was calculated as  $[TP/(TP + FN)] \times 100$ , where TP = true positive (diabetic fasting plasma glucose and HbA<sub>1c</sub> cutoff level) and FN = false negative (diabetic fasting plasma glucose,  $\leq$ cutoff level HbA<sub>1c</sub>),” should instead read, “Sensitivity at each possible HbA<sub>1c</sub> cutoff level was calculated as  $[TP/(TP + FN)] \times 100$ , where TP = true positive (diabetic fasting plasma glucose and  $>$ cutoff level HbA<sub>1c</sub>) and FN = false negative (diabetic fasting plasma glucose,  $\leq$ cutoff level HbA<sub>1c</sub>).”

The sentence that begins on line 16, which reads, “The sensitivity represents the percentage of those with fasting plasma glucose  $<7.0$  mmol/l who are classified as positive according to HbA<sub>1c</sub>,” should read, “The sensitivity represents the percentage of those with fasting plasma glucose  $>7.0$  mmol/l who are classified as positive according to HbA<sub>1c</sub>.”

**Fujimoto WY: Background and recruitment data for the U.S. Diabetes Prevention Program. *Diabetes Care* 23 (Suppl. 2):B11–B13, 2000**

The author of the above paper should have been listed as the Diabetes Prevention Program Research Group. Dr. Wilfred Y. Fujimoto prepared the paper on behalf of the DPP Research Group.