

OBSERVATIONS

Hemoglobinopathies and HbA_{1c} Measurement

The measurement of glycosylated hemoglobin (HbA_{1c}) is one of the cornerstones of management of diabetes. Most physicians use HbA_{1c} values in the assessment of a patient's control of their blood glucose levels and as a reality check for home glucose-monitoring results. A target value of 7.0% is widely regarded as excellent glycemic control (1). Good glycemic control is associated with lower rates of microvascular complications from diabetes in both type 1 (1) and type 2 (2) diabetic patients.

Changes in therapy are often based on HbA_{1c} values. Some patients attending outpatient clinics for diabetes management were noted to have HbA_{1c} values that differed from what was expected based on home blood-glucose monitoring (hBGL) results. We have previously reported Hb Hamadan in one of our patients with an unexpectedly low HbA_{1c} value (3). The hypothesis was that a proportion of patients with an unexpected HbA_{1c} result would have a hemoglobinopathy. Hb electrophoresis (HbEPG) was performed on patients in whom the HbA_{1c} result differed from the expected value.

The study was a prospectively collected cross-sectional study of 30 consecutive patients whose HbA_{1c} values differed significantly from the expected results. The decision to perform HbEPG was made clinically by the investigators, on the basis of comparison of HbA_{1c} and hBGL results. Of the patients included in the study, 29 had HbA_{1c} values lower than expected, and 1 had a value higher than expected. Clinically, it is recognized that some patients underreport hBGL results. Thus, there was a bias toward testing unexpectedly low HbA_{1c} values.

The study started in May 1998 and concluded in November 1999. The computerized investigation report system used at our institution makes it possible to access every HbEPG performed by the investigators during the time of the study, thus eliminating recall bias. Subjects were classified as Caucasian or non-Caucasian according to their countries of birth.

Table 1—Patient characteristics and results

	Overall	Normal	Hemoglobinopathy
<i>n</i>	30	23	7
Type 1 diabetic patients	2	2	0
Type 2 diabetic patients	14	12	2
Patients with gestational diabetes	14	9	5
Age (years)	47 ± 17	48 ± 18	41 ± 11
HbA _{1c} (%)	6.1 ± 2.4	6.5 ± 2.4	4.6 ± 1.8*
Hb (g/l)	128 ± 23	128 ± 23	128 ± 26
MCV (fl)	85 ± 10	87 ± 10	81 ± 12
LDH (mmol/l)	184 ± 59	195 ± 63	147 ± 25†
Bilirubin (mmol/l)	9 ± 3	9 ± 4	9 ± 2
HbA ₂ (%)	2.9 ± 0.5	2.8 ± 0.2	3.0 ± 1.0
HbF (%)	1.1 ± 2.2	0.5 ± 0.3	2.8 ± 4.1‡

Data are *n* or means ± 2SD. **P* = 0.059; †*P* = 0.069; ‡*P* = 0.003.

Patient characteristics are shown in Table 1. The mean age of the subjects was 47 ± 17 years. Two of the patients had type 1 diabetes, 14 patients had type 2 diabetes, and 14 women had gestational diabetes. HbA_{1c} was measured by ion exchange high-performance liquid chromatography (HPLC) using the method of Jeppsson et al. (4). Of the patients, 27 had their full blood count, lactate dehydrogenase (LDH), and serum bilirubin measured. Results for HbA_{1c}, fetal Hb, and LDH measurements were not normally distributed.

Patient results are shown in Table 1. The proportion of pregnant subjects did not differ significantly between groups. Patients with abnormal HbEPG had higher fetal Hb than patients with normal Hb (2.8 ± 4.1 vs. 0.5 ± 0.3%, respectively; *P* = 0.003). There was a trend toward higher LDH in subjects with a hemoglobinopathy (*P* = 0.06). The mean corpuscular volume correlated with the total Hb (*P* < 0.01).

The patient with an HbA_{1c} higher than expected did not have a hemoglobinopathy. The 7 patients with abnormal HbEPG had lower than expected HbA_{1c} results. The abnormalities were HbE in 2 patients, hereditary persistence of fetal Hb (HPFH) in 2 patients, and Hb Hamadan, β-thalassemia, and HbS in one patient each. The first abnormality was detected in the subject with Hb Hamadan, as previously reported (3).

Of the 23 subjects with normal HbEPG, 10 (43.5%) were non-Caucasian, and of the patients with abnormal HbEPG, 3 of 7 had a hemoglobinopathy (42.9%) (NS). Considered by ethnicity, 4 of 17 Caucasian subjects (23.5%) and 3 of 13 non-Caucasian subjects (23.1%) had a hemoglobinopathy.

Pregnant women had lower HbA_{1c} values than nonpregnant subjects 4.5 ± 1.3 vs. 7.6 ± 2.4% (*P* < 0.001), respectively.

When only pregnant subjects were considered (15 subjects), the HbA_{1c} value in those with a hemoglobinopathy was 4.0 ± 1.7 vs. 4.9 ± 1.0% in those with abnormal Hb. When nonpregnant subjects were considered (15 subjects), the HbA_{1c} value in those with normal Hb was 6.3 ± 0.8 vs. 7.9 ± 2.5% in those with abnormal Hb.

Pregnancy is associated with lower blood glucose and higher LDH levels. Thus, the trend toward lower HbA_{1c} and higher LDH in the subjects with hemoglobinopathies may be related to the proportion of pregnant women. However, the difference in the proportion of pregnant women in each group was not significant. There is a trend toward lower HbA_{1c} values with hemoglobinopathy when pregnant women are considered separately and when nonpregnant subjects are considered separately, but the difference is not significant. A larger number of subjects may clarify this issue.

Hemoglobinopathies are known to affect HPLC measurement of HbA_{1c} (3,5,6). There are at least 2 methods by which abnormal Hb may affect HbA_{1c} values. One is the presence of an abnormal peak on chromatography, making the estimation of the fraction of HbA_{1c} unreliable. Second, some abnormal forms of Hb (e.g., β-thalassemia and sickle cell trait) make red blood cells more susceptible to hemolysis. Increased hemolysis corresponds with decreased red cell lifespan. This decreases the time available for glycosylation of Hb chains. The 2 effects may coexist.

Specific effects, which have been described, include decreased HbA_{1c} results

with homozygous or heterozygous HbS, HbC, HbG, and Hb Hamadan (3,5). Variably decreased or increased results are found with HbE and increased quantities of HbF including HbPFH.

Of the subjects, 43% were non-Caucasian. There was no difference in frequency of hemoglobinopathy between Caucasians and non-Caucasians. At this institution, only women with an abnormal glucose tolerance test during pregnancy or with pregestational diabetes have HbA_{1c} measurements performed during pregnancy. For women with abnormal glucose tolerance, figures for 1998 show that 41% were non-Caucasian.

Because of the importance placed on HbA_{1c} in the management of diabetes, it is important to consider hemoglobinopathy in patients when the HbA_{1c} value does not correlate with clinical expectations. If the value is artificially low, these patients may be at higher risk for complications of diabetes than the HbA_{1c} result would suggest, and they may require alterations in therapy.

It is well recognized that subjects with diabetes may underreport hBGL. However, it seems unlikely that they would report levels higher than they find during home monitoring. Thus, if the HbA_{1c} value is lower than expected, based on the results of hBGL, HbEPG should be performed. In subjects with a hemoglobinopathy, use of fructosamine to monitor diabetes may be more reliable.

It is reasonable to expect that otherwise clinically silent hemoglobinopathies may be present with greater frequency than currently realized. If discrepant results are found on an HbA_{1c} assay (either higher or lower than expected), hemoglobinopathy should be considered as a possible cause.

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References

1. 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

2. U.K. Prospective Diabetes Study (UKPDS) Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-853, 1998
3. Gunton JE, McElduff A: Heterozygous Hb Hamadan affects HbA_{1c} assay (Letter). *Diabetes Care* 22:177, 1999
4. Jeppsson JO, Jerntorp P, Sundkvist G, Englund H, Nyland V: Measurement of hemoglobin A1c by a new liquid-chromatographic assay: methodology, clinical utility, and relation to glucose tolerance evaluated. *Clin Chem* 32: 1867-1872, 1986
5. Wolfsdorf JJ, Anderson BJ, Pasquarello C: *Joslin's Diabetes Mellitus*. 13th ed. Philadelphia, Lea and Febiger, 1994

Improvement of Glycemic Control After Treatment With Mosapride for Diabetic Gastropathy

Upper gastrointestinal symptoms, such as postprandial nausea, vomiting, bloating, early satiety, fullness, and abdominal discomfort, are commonly found in patients with either type 1 or type 2 diabetes (1,2). Diabetic gastropathy has been found in ~50% of patients with type 1 diabetes and in ~30% of patients with type 2 diabetes (2). In addition to problems concerning quality-of-life issues (3), diabetic gastropathy may cause erratic and unpredictable blood glucose levels by reducing the effectiveness of dietary regimen and the absorption of oral medications, thereby causing difficulties in timing insulin peak with meals (3-6). However, no study has reported the effects of treatment for diabetic gastropathy on glycemic control, except for a preliminary observation of 8 Japanese subjects (6).

Various prokinetic agents, including the dopamine D2 antagonists metoclopramide and domperidone (3,4), the motilin agonist erythromycin (5), and a cholinergic mimetic cisapride (2) have been used to treat diabetic gastropathy. In the present study, we examined the effect of mosapride, a new prokinetic drug (a selective serotonin 5-HT₄ receptor agonist) (7), on glycemic control in patients with type 2 diabetes presenting with upper gastrointestinal symptoms typical of diabetic gastropathy. A total of 21 Japanese subjects (6 men and 15 women,

mean age 68.8 ± 5.6 years) with type 2 diabetes participated in the study. After a mean of 100.7 days of mosapride treatment (15 mg/day), gastrointestinal symptoms disappeared in all subjects. In 21 subjects, 14 showed a decrease in HbA_{1c}, although HbA_{1c} was increased or unchanged in 5 and 2 of them, respectively. HbA_{1c} changes showed a statistically significant decrease (7.6 ± 0.3 to 7.0 ± 0.3%, *P* < 0.05). Furthermore, there was a significant negative correlation between the HbA_{1c} change and the duration of mosapride treatment (*r* = 0.789, *P* < 0.0001).

This preliminary result suggests a possibility that treatment of diabetic gastropathy with mosapride results in better glycemic control and disappearance of gastrointestinal symptoms in patients with type 2 diabetes. Although the present study is limited because it was not a controlled study, it seems likely that better glycemic control may be attributable to an improvement of gastrointestinal activity, which is considered to result in better timing of the insulin peak with an increase in postprandial glucose, as proposed by other investigators (4,5). Prospective controlled trials may be justified in order to investigate whether mosapride treatment may improve glycemic control.

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References

1. Quigley EM: The pathophysiology of diabetic gastroenteropathy: more vague than vagal? *Gastroenterology* 113:1790-1794, 1997
2. Koch KL: Diabetic gastropathy: gastric neuromuscular dysfunction in diabetes mellitus: a review of symptoms, pathophysiology, and treatment. *Dig Dis Sci* 44: 1061-1075, 1999
3. Farup CE, Leidy NK, Murray M, Williams GR, Helbers L, Quigley EMM: Effect of domperidone on the health-related quality of life of patients with symptoms of diabetic gastro-

- Clarke WL: *Blood Glucose Awareness Training-III*. Charlottesville, VA, University of Virginia Health Science Center, 1994
4. American Diabetes Associates: Diabetes self-management education. In *Intensive Diabetes Management*. 1st ed. Alexandria, VA, American Diabetes Association, 1995
 5. Cox DJ, Irvine A, Gonder-Frederick L, Nowacek G, Butterfield J: Fear of hypoglycemia: quantification, validation, and utilization. *Diabetes Care* 10:617-621, 1987
 6. Snoek FJ, Pouwer F, Mollema ED, Heine RJ: De angst voor hypoglycemie vragenlijst (AHV): interne consistentie en validiteit. *Gedrag & Gezondheid* 24:287-292, 1996

Small-Bowel Bacterial Overgrowth in Diabetic Subjects Is Associated With Cardiovascular Autonomic Neuropathy

Gastrointestinal symptoms are present in 50–70% of patients with diabetes. Delayed gastric emptying, and disturbance of intestinal motility are frequent findings (1,2). Impaired intestinal motility is often followed by small-bowel bacterial overgrowth (SBBO), which can possibly lead to deconjugation of bile acids, diarrhea, steatorrhea, malabsorption of vitamin, and/or micronutrients and weight loss, as well as mucosal injury, bacterial translocation, and worsening of small-bowel motility (3). However, patients in whom bacterial overgrowth is found may also be asymptomatic (4).

Up until now, little attention has been devoted to the relationship among autonomic neuropathy, impaired intestinal motility, and SBBO in diabetic patients (3,5). The aim of our study was to evaluate the prevalence of bacterial overgrowth and its association with autonomic neuropathy in 50 diabetic outpatients with previously unknown diabetes-related gastrointestinal disorders (20 type 1 and 30 type 2 diabetic patients, mean age 47.3 ± 2.2 years, duration of diabetes 14.4 ± 1.3 years, HbA_{1c} $8.4 \pm 0.3\%$). Exclusion criteria consisted of a history of gastric or pancreatic surgery, celiac disease, inflammatory bowel disease, lactose intolerance, scleroderma, hypothyroidism, liver cirrhosis, and colonoscopy within the last 4 weeks. All of these conditions, as well as adminis-

tration of antibacterial medication within the last 4 weeks, are known to influence intestinal motility or small-bowel bacterial growth. Patients using β -blockers, H_2 blockers, proton pump inhibitors, corticosteroids, or other immunosuppressants, antidepressants, opioids, and metoclopramide were also excluded. All patients gave their informed consent for study participation. Testing for SBBO was performed at 8:00 A.M. with 80 g glucose dissolved in 200 ml of water. Samples of end-expiratory breath (20 ml) were taken at 0, 10, 20, 30, 40, 50, 60, 80, 100, and 120 min. H_2 breath concentration was measured by gas chromatography with thermal conductivity (GMI-Exhaled Hydrogen Monitor Medical; Stimotron, Wendelstein, Germany). An increase in breath H_2 concentration (H_2 exhalation) >20 parts per million was defined as indicative of SBBO. Cardiovascular autonomic nervous function was assessed with the following standardized tests: variation coefficient of 150 heart beat intervals in supine position, expiration-inspiration difference (heart rate variation during 6 deep breaths/min), the Valsalva ratio (maintaining a pressure of 40 mmHg for 15 s when blowing into the mouthpiece of a manometer), lying-to-standing ratio (heart rate response to standing up measured at the 15th and 30th heart beat), and orthostatic systolic blood pressure fall (systolic response to standing). The diagnosis of overall cardiovascular autonomic neuropathy was made if 2 or more of the 5 tests were abnormal. In a questionnaire addressing intestinal symptoms, patients were asked about diarrhea, flatulence, constipation, abdominal pain, and food intolerance. In addition, patients were asked to mark the leading symptom from which they suffered.

In 17 of 50 diabetic patients, a pathological H_2 exhalation was found (indicating SBBO⁺), whereas H_2 exhalation was normal in 33 SBBO⁻ patients. Diabetic patients with and without SBBO were comparable according to age, sex, duration of diabetes, BMI, HbA_{1c} , and blood pressure. Only cardiovascular autonomic neuropathy was significantly found more often in SBBO⁺ as compared with SBBO⁻ (41.2 vs. 9.1%; $P < 0.01$), while frequencies of retinopathy, nephropathy, peripheral neuropathy, angiopathy, hyperlipidemia, and hypertension were comparable in both subgroups. There was no correlation between the presence of cardiovascular autonomic neuropathy and duration of

diabetes. Patients with SBBO reported suffering more frequently from intestinal symptoms as compared with patients with normal glucose H_2 breath tests (92.9 vs. 60.7%, $P < 0.05$). Flatulence and diarrhea were shown to occur more often in SBBO⁺ in comparison with SBBO⁻ patients, whereas constipation, food intolerance, and abdominal pain were equally distributed in both groups.

Scarpello et al. (6) described a pathological ^{14}C -glycocholate test, which is also considered indicative of SBBO in 4 of 7 diabetic patients suffering from diarrhea and other symptoms of autonomic neuropathy. SBBO was found in 43% of diabetic patients suffering from chronic diarrhea (3). To our knowledge, there are few other studies on animals or human that have focused on SBBO in diabetes (7).

Bacterial cultures of small intestinal aspirates are considered the "gold standard" in the diagnosis of SBBO (8). However, this procedure is invasive and requires specialized equipment. Therefore, noninvasive techniques are preferable in clinical practice. The glucose H_2 -breath test is reported to have a sensitivity of 62–91% and a specificity of 75–100%, both of which are comparable with those of other non-invasive tests (3,9). Dysfunction of intestinal motility has been shown to be the leading cause of small intestinal bacterial overgrowth in other conditions, such as progressive systemic sclerosis (10). Most studies have focused on the impairment of gastric emptying and rarely on the disturbance in intestinal motility in diabetic patients (11). Impairment in frequency and amplitude of motor-migrating complex in diabetic patients with symptoms of gastroparesis was shown by Björnsson et al. (12) and could also be demonstrated by intestinal manometry. In contrast to other studies, we found a significant association between SBBO and autonomic neuropathy diagnosed by pathological cardiovascular reflex tests (3). We believe that the association of SBBO and cardiovascular autonomic neuropathy is probably because patients with cardiovascular neuropathy are likely to suffer also from gastrointestinal neuropathy. Thus, SBBO may reflect intestinal dysmotility in patients with cardiovascular autonomic dysfunction. Although the pathogenesis of small-bowel motility dysfunction is not completely understood, it has been suggested that hyperglycemia, hyperinsulinemia, and autonomic neu-

ropathy may be involved. The presence of these conditions would lead to decreased concentrations of pancreatic polypeptide and motilin (11,12). Gastrointestinal symptoms were found to be only weakly associated with the grade of intestinal motility dysfunction in previous studies (2). In our study, patients with bacterial overgrowth suffered more often from gastrointestinal symptoms like flatulence as compared with patients without SBBO.

Taken together, we found SBBO in approximately one-third of patients with diabetes associated with cardiovascular autonomic neuropathy. Therefore, in diabetic patients suffering from unspecific gastrointestinal symptoms, bacterial overgrowth should be taken into diagnostic and therapeutic considerations.

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References

- Iber FL, Parveen S, Vandrunen M, Soo KB, Reza F, Serlovsky R, Reddy S: Relation of symptoms to impaired stomach, small bowel, and colon motility in long-standing diabetes. *Dig Dis Sci* 38:45–50, 1993
- Abrahamsson H: Gastrointestinal motility and diabetes. *J Intern Med* 237:403–409, 1995
- Virally-Monod M, Tielmans D, Kevorkian JP, Bouhnik Y, Flourie B, Porokhov B, Ajzenberg C, Warnet A, Guillausseau PJ: Chronic diarrhoea and diabetes mellitus: prevalence of small intestinal bacterial overgrowth. *Diabetes Metab* 24:530–536, 1998
- Camilleri M: Gastrointestinal problems in diabetes. *Endocrinol Metab Clin North Am* 2:361–378, 1996
- Roza AM, Edmiston CE, Frantzides C, Moore GH, Nowak TV, Johnson CP: Untreated diabetes mellitus promotes intestinal microbial overgrowth. *Am J Surg* 163:417–421, 1992
- Scarpello JH, Hague RV, Cullen DR, Sladen GE: The ¹⁴C-glycocholate test in diabetic diarrhoea. *Br Med J* 2:673–675, 1976
- Spengler U, Stellaard F, Ruckdeschel G, Scheuren C, Kruis W: Small intestinal transit, bacterial growth, and bowel habits in diabetes mellitus. *Pancreas* 4:65–70, 1989
- Riordan SM, McIver CJ, Wakefield D, Bolin TD, Duncombe VM, Thomas MC: Small intestinal bacterial overgrowth in the symptomatic elderly. *Am J Gastroenterol* 1:47–51, 1997
- King CE, Toskes PP: Comparison of the 1-gram [¹⁴C]xylose, 10-gram lactulose H₂, and 80-gram glucose H₂ breath test in patients with small intestine bacterial overgrowth. *Gastroenterology* 91:1447–1451, 1986
- Lock G, Holstege A, Lang B, Schölmerich J: Gastrointestinal manifestations of progressive systemic sclerosis. *Am J Gastroenterol* 92:763–771, 1997
- Camilleri M, Malagelada JR: Abnormal intestinal motility in diabetics with the gastroparesis syndrome. *Eur J Clin Invest* 14:420–427, 1984
- Björnsson ES, Urbanavicius V, Eliasson B, Attvall S, Smith U, Abrahamsson H: Effects of hyperglycemia on interdigestive gastrointestinal motility in humans. *Scand J Gastroenterol* 29:1096–1104, 1994

Thyroid Autoimmunity Starting During the Course of Type 1 Diabetes Denotes a Subgroup of Children With More Severe Diabetes

Thyroid autoimmunity (TAI) is the most prevalent immunological process affecting children and adolescents with type 1 diabetes (1–5). The susceptibility to develop multiple autoimmune diseases could be associated with disease-specific determinants. Several cross-sectional studies focused on clinical, genetic, and immunological differences that could distinguish patients with and without thyroid dysfunction, but no significant difference was found in either adult or pediatric studies (5–7).

Heterogeneity has been described in the natural history of TAI in type 1 diabetes: TAI may be diagnosed either at the onset of diabetes or during the follow-up, but no comparison has been made between these 2 populations.

In our longitudinal study, we analyzed 270 consecutive patients attending the Department of Pediatrics of the University

Federico II of Naples from 1992 to 1998. The mean age was 13.1 ± 4.3 years (range 1–18). Thyroid screening (TT3, TT4, thyroid-stimulating hormone, thyroglobulin, and thyroperoxidase antibodies) was performed at the end of the first admission and then yearly. Diagnosis of TAI was based on the presence of persistent elevated serum levels of thyroid autoantibodies and was confirmed by ultrasound images.

Prevalence of TAI in our diabetic population was 18.1% (49 of 270 patients), and the female-to-male ratio was ~2:1 (32 females, 17 males). At the time of TAI diagnosis, 42 patients were euthyroid and 7 were hypothyroid (overt or subclinical). After a mean follow-up of 6.2 ± 3.8 years, a progression toward hypothyroidism was observed in 1 male subject and hyperthyroidism in 2 female subjects. Therefore, among TAI patients, the prevalence of hypothyroidism was 16% and that of hyperthyroidism was 4%. A family history of thyroid disorders was more prevalent among diabetic patients with TAI than among patients without TAI (33 vs. 9.4%, $P < 0.0001$).

Among diabetic patients with TAI, subjects with thyroid dysfunction presented a higher prevalence of a third autoimmune condition (celiac disease or chronic arthritis) than euthyroid patients and the diabetic control population (33 vs. 7.7 and 7.6%, respectively).

In 27 of 49 (55%) patients, TAI was diagnosed at the onset of diabetes (group A), whereas in the remaining 22 it was diagnosed after a mean duration of diabetes of 7 years (range 1.16–8.8 years) (group B). The remaining 221 diabetic patients, who were TAI negative, were the control group (group C). The age at diabetes onset was significantly higher in group A (9.9 ± 3.8 years) than in groups B and C (6.0 ± 3.6 and 7.6 ± 3.8 years, respectively; $P = 0.001$, analysis of variance [ANOVA]). Moreover, the mean age of group A at TAI diagnosis (9.9 ± 3.8 years) was lower than that of group B (13.1 ± 3.3 years, $P = 0.0001$). Pubertal stage was assessed at TAI diagnosis. In group A, 12 of 27 (44.4%) were prepubertal, 11 of 27 (40%) were pubescent, and 4 of 27 (14.6%) were postpubertal. In group B, 3 of 22 (13.6%) were prepubertal, 10 of 22 (45.5%) were pubescent, and 9 of 22 (40.9%) were postpubertal. Interestingly, group B patients presented a more severe form of diabetes, which was characterized by a higher prevalence of ketoacidosis at

diabetes onset (50 vs. 26 in group A and 33% in group C), a higher daily insulin dose (1.1 ± 0.1 vs. 0.7 ± 0.3 in group A and 0.8 ± 0.3 U · kg⁻¹ · day⁻¹ in group C; $P = 0.004$, ANOVA), and worse metabolic control assessed in the last year of the follow-up (HbA_{1c} [mean of the last 3 values], 8.3 ± 1.1 vs. 7.3 ± 1.2 in group A and $7.5 \pm 1.1\%$ in group C; $P = 0.003$, ANOVA).

Our study on children and adolescents with type 1 diabetes supports previous studies in terms of TAI prevalence, female predominance, and presence of family history of thyroid diseases. As previously reported (4), a higher prevalence of another autoimmune disease was found in patients with thyroid dysfunction.

By dividing patients according to the time of TAI diagnosis, it was possible to identify 2 subgroups with different clinical expressions of diabetes. In particular, patients with contemporary diagnoses of both diseases presented at an unusual age for diagnosis of both diabetes and TAI. In these patients, compared with our local diabetic population, presentation of diabetes was slightly delayed, but TAI was diagnosed earlier than that reported by Maenpaa et al. (8) in the general population (12.2 ± 0.58). On the contrary, TAI diagnosed during the course of diabetes seems to be the result of 2 serial hits: the first one is diabetes at prepubertal age and with more severe characteristics and the second one is TAI in the years of late puberty, when the physiological pubertal changes seem to play a triggering role.

Therefore, we recommend that if the initial thyroid screening is negative, future tests should be performed in type 1 diabetic patients, especially during pubertal ages, even in the absence of clinical signs.

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References

1. Riley WJ, MacLaren NK, Lezotte D, Rebecca P, Rosenbloom A: Thyroid autoimmunity in insulin-dependent dia-

betes mellitus: the case for routine screening. *J Pediatr* 98:350–354, 1981

2. McKenna M, Herskowitz R, Wolfsdorf J: Screening for thyroid disease in children with IDDM. *Diabetes Care* 13:801–803, 1990

3. Lorini R, D'Annunzio G, Vitali L, Scaramuzza A: IDDM and autoimmune thyroid disease in the pediatric age group. *J Clin Endocrinol Metab* 9:89–94, 1996

4. Roldan MB, Alonso M, Barrio R: Thyroid autoimmunity in children and adolescent with type 1 diabetes mellitus. *Diabetes Nutr Metab* 12:27–31, 1999

5. Hansen D, Bennedbaek FN, Hansen LK, Hoier-Madsen M, Jacobsen BB, Hegedus L: Thyroid function, morphology and autoimmunity in young patients with insulin-dependent diabetes mellitus. *Eur J Endocrinol* 150:512–518, 1999

6. McCanlies E, O'Leary L, Foley T, Kramer MK, Burke J, Libman A, Dorman J: Hashimoto's thyroiditis and insulin-dependent diabetes mellitus: difference among individuals with and without abnormal thyroid function. *J Clin Endocrinol Metab* 82:1548–1551, 1998

7. Fernández-Castañer M, Molina A, López-Jimenez L, Gomez JM, Soler J: Clinical presentation and early course of type 1 diabetes in patients with and without thyroid autoimmunity. *Diabetes Care* 22:377–381, 1999

8. Maenpaa J, Raatikka M, Rasanen J, Taskinen E, Wager O: Natural course of juvenile autoimmune thyroiditis. *J Pediatr* 107:898–904, 1985

Diabetic Ketoacidosis

A complication of type 2 diabetes in Canadian aboriginal youth

Diabetic ketoacidosis (DKA) is characterized by hyperketonemia, metabolic acidosis, and hyperglycemia (1). It is usually considered a complication of type 1 diabetes and can cause severe morbidity and mortality if not recognized and treated in a judicious manner. DKA is precipitated by an absolute or relative lack of insulin in combination with an increase in the catabolic hormones, which leads to an increased production of ketone bodies and glucose by the liver (1).

There have been several recent reports of DKA in adults with type 2 diabetes (2–6). Pinhas-Hamiel et al. (7) have reported its occurrence among obese African-American youth with typical insulin-resistant type 2

diabetes. There are several reports that described the increasing problem of type 2 diabetes in youth; these studies have also reported DKA at diagnosis in some of these youth. These reports do not, however, define DKA and/or include pH in their criteria for DKA (8–10). We present our experience with DKA in Canadian aboriginal children and youth with type 2 diabetes

We reviewed the charts of all individuals diagnosed with type 2 diabetes at the Winnipeg Children's Hospital (Winnipeg, Manitoba, Canada) for the 14-year period between January 1986 and December 1999 inclusive. All patients were 18 years of age or younger and resided in Manitoba or Northwestern Ontario. These regions are serviced by a single tertiary care pediatric center (Winnipeg Children's Hospital). It is possible that mild cases of DKA were treated in peripheral hospitals and were not referred to the Children's Hospital. Thus, this report generates a minimal prevalence for DKA in youth with type 2 diabetes in these regions.

Diabetes was diagnosed according to the guidelines of the Canadian Diabetes Association (11). Type 2 diabetes was diagnosed in individuals who were able to be maintained without exogenous insulin for >6 months and who had clinical features typical of type 2 diabetes. These included a positive family history, obesity, acanthosis nigricans, and absence of any medication or underlying illness that might predispose to secondary diabetes. We have recently reported that the First Nation youth with diabetes seen at our institute lack evidence of autoimmunity (12). Two of the subjects reported here (subjects 11 and 12) were included in that report and were negative for islet cell antibodies, GAD antibodies, and insulin autoantibodies.

DKA was defined as pH ≤7.35 and HCO₃ ≤15 mEq/l in the presence of hyperglycemia. Total glycosylated hemoglobin was measured by an affinity chromatography method (Isolab) from 1986 to 1996 and by the Abbott Imx Analyzer from 1996 and thereafter. Results are reported as calculated HbA_{1c} values (normal range 4.4–6.4%).

Between 1986 and 1999, 120 type 2 diabetic children and adolescents 6–18 years of age were seen at our center. Of these children, 118 (98%) were of self-declared aboriginal origin, 90 (75%) were girls, and 13 (10.8%) experienced episodes of DKA. All 13 episodes were experienced by children of aboriginal descent; 5 (38.5%) of

Table 1—Clinical presentation of DKA

Case	Sex	Age at diagnosis (years)	Age at DKA (years)	BMI (kg/m ²) at DKA* (percentile)	pH	HCO ₃ (mmol/l)	BHOB (normal 0.0–0.3 mmol/l)	Blood glucose (mmol/l)	HbA _{1c} (%)
1	F	9	12	24 (>95th)	7.35	13.0	1.9	27.0	12.6–15.3†
2	F	12	14	28 (>95th)	6.90	NA	NA	32	10.9–12.2†
3	F	11	14	23 (>75th)	7.10	NA	NA	22.2	16.2
4	F	16	16	36 (>95th)	7.02	NA	NA	NA	NA
5	F	14	17	35 (>95th)	7.25	7.7	6.1	22.5	14.6
6	F	15	15	35 (>95th)	7.10	15.0	6.2	32.6	9.5
7	F	11	13	22 (>75th)	7.00	2.7	15.0	31.0	14.3
8	F	13	15	28 (>95th)	7.00	1.0	8.0	16.8	18.6
9	F	15	15	27 (>95th)	7.31	14.7	6.9	28.8	NA
10	F	12	12	29 (>95th)	7.17	3.0	8.3	37.7	NA
11	M	12	12	26 (>95th)	7.28	9.9	8.9	58.2	13.8
12	M	13	13	34 (>95th)	7.09	5.8	5.4	54.9	NA
13	F	10	17	27 (>90th)	7.22	7.8	8.8	24.1	12.7

Cases 1–3 were observed between 1986 and 1990. Cases 4–9 were observed between 1991 and 1995. Cases 10–13 were observed between 1996 and 1999. *BMI percentiles are listed in the article by Hammer (17); †because HbA_{1c} values were unavailable at the time of DKA, they were obtained 6 months before the episode of DKA. BDHB, betahydroxybutyrate; NA, not available.

these episodes occurred at the time of type 2 diabetes diagnosis. Thus, DKA occurred in 4.2% (5 of 120) of all presentations of type 2 diabetes seen at our institute. A female predominance was seen (11 of 13 [84.6%]) as a slight overrepresentation compared with the sex distribution within our clinic. Mean age at DKA was 14.2 ± 1.8 years. This is similar to the mean age of our current caseload with type 2 diabetes (14.9 ± 2.1 years). As a group, the subjects were obese, having a mean BMI of 28.8 (± 5.0 kg/m²).

Obvious precipitants for the episode of DKA were found in 3 individuals (pneumonia, gonococcal septicemia, and a severe culture-negative systemic illness resembling sepsis). One young woman was pregnant at the time of DKA and subsequently had a spontaneous miscarriage. Glycemic control at the time of DKA was uniformly poor in all patients in whom an HbA_{1c} was available (mean HbA_{1c} 13.9 ± 2.6%). Currently, ~50% of the youths with type 2 diabetes seen in our center maintain an HbA_{1c} value <7.0%. There were 5 of 13 (38.5%) individuals (all girls) who had a second documented episode of DKA.

A positive family history of type 2 diabetes was found in all of the subjects. Of 13 patients, 11 had an affected first-degree relative; all 13 subjects had many affected second-degree relatives. This is typical of the population seen at our institute with type 2 diabetes. Details of their clinical presentation are shown in Table 1.

The prevalence of type 2 diabetes is increasing in the children and adolescents

of Canada's aboriginal people (13,14). In another Native North American population, there is evidence that the prevalence, and not just the detection of this problem, is increasing (15). In our institute, the majority of cases with type 2 diabetes are in youth of aboriginal origin. The diagnosis of type 1 diabetes is very rare in this group and occurs in young children of mixed ancestry.

The distinction between type 1 and type 2 diabetes can be difficult in the pediatric population, particularly when DKA is the presenting feature. However, this distinction is important because of differing education and long-term treatment strategies. The implications for family members for the risk of diabetes also differ. Those young people with type 2 diabetes may well be able to discontinue insulin once their condition has stabilized; therefore, they may not have to contend with injections and the side effects of insulin e.g., weight gain, and hypoglycemia.

DKA has been previously reported in type 2 diabetes, predominantly in adults (2–6). Many of the previous reports demonstrate a predominance of males (2,4,5,16). A lower prevalence of obesity has been noted in some reports (3–5). In our population, females predominate and the majority of BMIs were in the obese range (>85th percentile for age and sex).

Pinhas-Hamiel et al. (7) reported the occurrence of DKA among obese African-American youth with typical insulin-resistant type 2 diabetes. Our population is also obese and had their episode of DKA at

a mean age of 14.0 years, similar to the African-American adolescents. The sex distribution in our population, compared with that reported by Pinhas-Hamiel, is more skewed, having a greater female predominance. Four older adolescents (aged 15–17 years) with type 2 diabetes presenting in DKA have been reported from Japan, all of whom were obese males with a history of exceptionally large intakes of sugared drinks (16).

A precipitating illness was found in the minority (3 of 13) of our population, contrary to other reports (5,6,16). This is similar to the population reported by Pinhas-Hamiel et al. (7), who found an acute illness in only 4 of 12 episodes of DKA in their series. Glycemic control in our population was uniformly poor (mean HbA_{1c} 13.79 ± 2.6%) and is likely a contributing factor to DKA.

Five of the subjects in this report had at least 1 documented repeat episode of DKA. Despite this, we remain confident that they have type 2 diabetes on the basis of clinical criteria and the significant periods of time without insulin therapy, weight loss, symptoms of hyperglycemia, or acute metabolic decompensation. Continued poor long-term glycemic control was the factor common to all these cases.

The occurrence of DKA in type 2 diabetes in aboriginal youth emphasizes the importance of screening youth at risk for diabetes (e.g., aboriginal origin, positive family history, or obesity). In this article, 38.5% of patients who had an episode of

DKA had DKA at presentation of diabetes. Screening at-risk populations may prevent presentation of individuals in DKA and thus prevent a potentially fatal complication of diabetes. Screening will also provide for earlier diagnosis, thereby allowing introduction of education and treatment at an earlier stage and potentially decreasing the chronic complications of diabetes.

In summary, DKA occurs in aboriginal children and youth with type 2 diabetes and represents a potentially life-threatening complication of this disorder. DKA may occur at the presentation of the disease or during the disease course. Thus, the presence of an episode of DKA cannot be used to support the diagnosis of type 1 diabetes in this population or, alternatively, as evidence against the diagnosis of type 2 diabetes.

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References

1. Krentz AJ, Natrass M: Acute metabolic complications of diabetes mellitus: diabetic ketoacidosis, hyperosmolar non-ketotic syndrome and lactic acidosis. In *Textbook of Diabetes*. Vol. 1. 2nd ed. Pickup J, Williams G, Eds. London, Blackwell Science, 1997, p. 39.1–39.23
2. Umpierrez GE, Casals MMC, Gebhart S, Mixon PS, Clark WS, Phillips LS: Diabetic ketoacidosis in obese African-Americans. *Diabetes* 44:790–795, 1995
3. Banerji MA, Chaiken RL, Huey H, Tuomi T, Norin AJ, Mackay IR, Rowley MJ, Zimet PZ, Lebovitz HE: GAD antibody negative NIDDM in adult black subjects with diabetic ketoacidosis and increased frequency of human leukocyte antigen DR3 and DR4. *Diabetes* 43:741–745, 1994
4. Wilson C, Krakoff J, Gohdes D: Ketoacidosis in Apache Indians with non-insulin-dependent diabetes mellitus. *Arch Intern Med* 157:2098–2100, 1997
5. Westphal SA: The occurrence of diabetic ketoacidosis in non-insulin-dependent

- diabetes and newly diagnosed diabetic adults. *Am J Med* 101:19–24, 1996
6. Sharma SC, Bhattacharyya A: Diabetic ketoacidosis in non-insulin-dependent diabetes mellitus. *JR Soc Med* 91:34–35, 1998
7. Pinhas-Hamiel O, Dolan LM, Zeitler PS: Diabetic ketoacidosis among obese African-American adolescents with NIDDM. *Diabetes Care* 29:484–486, 1997
8. Glaser N, Jones KL: Non-insulin-dependent diabetes mellitus in children and adolescents. *Adv Pediatr* 43:359–396, 1996
9. Scott CR, Smith JM, Craddock MM, Pihoker C: Characteristics of youth-onset non-insulin-dependent diabetes mellitus and insulin-dependent diabetes mellitus at diagnosis. *Pediatrics* 100:84–91, 1997
10. Neufeld ND, Raffel LJ, Landon C, Chen YDI, Vadheim CM: Early presentation of type 2 diabetes in Mexican-American youth. *Diabetes Care* 21:80–86, 1998
11. Melzer S, Leiter L, Daneman D, Gerstein HC, Lau F, Ludwig S, Yale JF, Zinman B, Lillie D: 1998 Clinical practice guidelines for the management of diabetes in Canada. *Can Med Assoc J* 159 (Suppl. 8):S1–S29, 1998
12. Sellers E, Eisenbarth G, Young TK, Dean HJ: Diabetes-associated autoantibodies in aboriginal children. *Lancet* 355:1156, 2000
13. Dean HJ: NIDDM-Y in First Nation Children in Canada. *Clin Pediatr* 37:89–96, 1998
14. Harris SB, Perkins BA, Whalen-Brough E: Non-insulin-dependent diabetes mellitus among First Nations children: new entity among First Nations people of northwestern Ontario. *Can Fam Physician* 42:869–876, 1996
15. Dabelea D, Hanson RL, Bennett PH, Roumain J, Knowler WC, Pettitt DJ: Increasing prevalence of type 2 diabetes in American Indian children. *Diabetologia* 41:904–910, 1998
16. Yamada K, Nonaka K: Diabetic ketoacidosis in young obese Japanese men (Letter). *Diabetes Care* 19:671, 1996
17. Hammer LD, Kraemer HC, Wilson DM, Ritter PL, Dornbusch SM: Standardized percentile curves of body-mass index for children and adolescents. *Am J Dis Child* 145:259–263, 1991

Interaction of Gliclazide and Rifampicin

We describe a patient with type 2 diabetes who required an increase in daily dosage of gliclazide after rifampicin administration. To our knowledge, this is the first report of the interaction between gliclazide and rifampicin.

A 65-year-old man with type 2 diabetes had been treated with diet (30 kcal/kg) and gliclazide (80 mg/day) without problems for 2 years. In mid-February 1998, he was diagnosed with atypical mycobacteriosis caused by *Mycobacterium gordonae* and was treated with rifampicin, isoniazid, ethambutol, and clarithromycin (450, 400, 750, and 400 mg/day, respectively). The fasting plasma glucose (FPG) concentration was 6.4 mmol/l; HbA_{1c} was 5.4%; and 1,5-anhydroglucitol was 17.9 µg/ml before the commencement of treatment for atypical mycobacteriosis. FPG was found to be increased 11 days later, and after treatment on day 17 it was further elevated up to 11.3 mmol/l. Although the dose of gliclazide was increased to 120 mg/day on day 20, FPG was still >9 mmol/l. Finally, the dose was increased up to 160 mg/day on day 32. The plasma concentration of gliclazide 2 h after an oral dose of 80 mg gliclazide was 1.4 µg/ml on day 75, but it increased up to 4.7 µg/ml after discontinuation of 7 months of rifampicin treatment. Therefore, the dose of gliclazide was reduced to 80 mg/day, and HbA_{1c} diminished to 5.4–5.6%.

This case strongly suggests an interaction between rifampicin and gliclazide. Rifampicin has been reported to interact with several drugs, such as oral anticoagulants, glucocorticoids, digitoxin, quinidine, ketoconazole, and verapamil (1). Some oral hypoglycemic agents have also been reported to interact with rifampicin. For example, the half-life and serum concentration of tolbutamide were decreased after rifampicin treatment in both healthy volunteers and patients with tuberculosis (2,3). In patients receiving treatment other than rifampicin for tuberculosis, no significant changes in serum levels of tolbutamide were observed (2). Self and Morris (3) reported a diabetic patient who required higher doses of chlorpropamide when treatment with rifampicin was initiated. The serum chlorpropamide concentration diminished during rifampicin therapy, but rose dramatically on discontinuation of the antibiotic with a decrease in blood glucose level (3). In patients with diabetes treated with glibenclamide, plasma glucose levels increased after administration of rifampicin (4,5), and dose modification of glibenclamide was required because of poor control of diabetes (4,5). Plasma glucose concentration in these patients returned to the normal range by day 6 after discontinuation of rifampicin therapy (4).

pose a limit corresponding to a high sensitivity, estimated at $HbA_{1c} \geq 6.0\%$ in our survey. In opposition to the findings of Rohling et al., use of this value resulted in a high sensitivity (93%) and a moderate specificity (74%). These results, as compared with those using FCPG alone, contributed to the overall effectiveness of using HbA_{1c} to screen for diabetes.

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References

- Rohlfing CL, Little RR, Wiedmeyer H-M, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE: Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:187-191, 2000
- The Expert Committee on the Diagnosis and Classification of Diabetes Mellitus: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 20:1183-1197, 1997
- DECODE Study Group, on behalf of the European Diabetes Epidemiology Study Group: Will new diagnostic criteria for diabetes mellitus change phenotype of patients with diabetes? Reanalysis of European epidemiological data. *BMJ* 317:1343-1353, 1998
- World Health Organization: *Diabetes Mellitus: Report of a WHO Study Group*. Geneva, World Health Org., 1985 (Tech. Rep. Ser., no. 727)

Use of GHb (HbA_{1c}) to Screen for Undiagnosed Diabetes in the U.S. Population

Rohlfing et al. (1) used data from the Third National Health and Nutrition Examination Survey (NHANES III) to evaluate the use of glycohemoglobin (GHb) as a screening test for undiagnosed

diabetes. There are 3 issues that need to be considered when interpreting their results.

First, the authors chose to evaluate GHb as a screening test for undiagnosed diabetes based on fasting plasma glucose (FPG) ≥ 7.0 mmol/l alone. Reliance on FPG for the diagnosis of diabetes differentially misses substantial numbers of subjects with isolated post-challenge hyperglycemia who have rates of microvascular complications (particularly diabetic retinopathy) and mortality similar to those of other diabetic subjects (2-4). Of the 6,615 subjects in the NHANES III data set with all 3 glucose measures, 1,272 had diabetes based on either an FPG ≥ 7.0 mmol/l or a 2-h (75-g oral glucose load) plasma glucose (2-h PG) ≥ 11.1 mmol/l. Of the individuals with diabetes, 485 were diagnosed on the basis of both FPG and 2-h PG, 82 on the basis of FPG alone, and 705 on the basis of 2-h PG alone. Thus, 705 (55%) patients would not have been identified if only the FPG criterion had been used. If GHb is compared with FPG as the gold standard, a cutoff value of GHb $\geq 6.0\%$ provides sensitivity of 0.862 and specificity of 0.850. However, if GHb is compared with 2-h PG ≥ 11.1 mmol/l as the gold standard, GHb $\geq 6.0\%$ is associated with sensitivity of 0.627 and specificity of 0.880. If GHb is compared with FPG ≥ 7.0 mmol/l or 2-h PG ≥ 11.1 mmol/l, GHb $\geq 6.0\%$ is associated with sensitivity of 0.613 and specificity of 0.885. Thus, GHb does not perform as well in predicting diabetes based on FPG and 2-h PG criteria.

Secondly, studies have demonstrated that GHb does not perform as well as 2-h PG or FPG in diagnosing diabetes. We have previously demonstrated that in the Egyptian population, 2-h PG and FPG both perform better than GHb in minimizing the overlap of the components of the bimodal distributions (5). Using receiver-operating characteristic analyses, we also demonstrated that both 2-h PG and FPG perform significantly better than GHb in predicting the prevalence of diabetic retinopathy (5).

Lastly, GHb may be unsuitable as a screening test for other reasons. GHb tends to be more expensive than other glucose measures. The lack of widely used laboratory standard reference materials and variation in the reference method remain a limitation; however, as the authors note (and in a large part, due to their efforts), many advances have been made in this area. Finally, a study in a small number of nor-

moglycemic individuals failed to find a relationship between fasting venous glucose and HbA_{1c} values (6). Others have found that only 2-30% of the variance in GHb in nondiabetic individuals can be explained by fasting or postload glucose; the remainder is presumably related to factors independent of glycemia, such as differences in the rate of glycation and in red cell survival (7,8).

In summary, the performance of GHb for the diagnosis of diabetes based on a gold standard that includes FPG and 2-h PG criteria is substantially less sensitive than reported. A number of factors further limit the suitability of GHb as a screening test.

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References

- Rohlfing CL, Little RR, Wiedmeyer H-M, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE: Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:187-191, 2000
- Borch-Johnsen K for DECODE Study group on behalf of the European Diabetes Epidemiology Study Group: Will new diagnostic criteria for diabetes mellitus change phenotype of patients with diabetes? Reanalysis of European epidemiological data. *BMJ* 317:371-375, 1998
- The DECODE Study Group, on behalf of the European Diabetes Epidemiology Study Group: Is fasting glucose sufficient to define diabetes? Epidemiological data from 20 European centers. *Diabetologia* 42:647-654, 1999
- The DECODE Study Group, on behalf of the European Diabetes Epidemiology Study Group: Consequences of the new diagnostic criteria for diabetes in older men and women. *Diabetes Care* 22:1667-1671, 1999
- Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, Badran A, Sous ES, Ali MA: Comparison of fasting and 2-hour glucose and HbA_{1c} levels for diagnosing diabetes. *Diabetes Care* 20:785-791, 1997

6. Kilpatrick ES, Maylor PW, Keevil BG: Biological variation of glycated hemoglobin: implications for diabetes screening and monitoring. *Diabetes Care* 21:261-264, 1998
7. Yudkin JS, Forrest RD, Jackson CA, Ryle AJ, Davie S, Gould BJ: Unexplained variability of glycated hemoglobin in nondiabetic subjects not related to glycemia. *Diabetologia* 33:208-215, 1990
8. Modan M, Meytes D, Roseman P, Yosef SB, Sehayek E, Yosef NB: Significance of high HbA_{1c} levels in normal glucose tolerance. *Diabetes Care* 11:422-428, 1988

Response to Herman et al. and Papoz et al.

We appreciate the interest of Herman et al. (1) and Papoz et al. (2) in our study of the use of glycohemoglobin (GHb) as a screening test for diabetes (3). Our purpose was not to debate the validity of the current American Diabetes Association criteria for diabetes diagnosis, which will undoubtedly be a topic of discussion for some time. Our data simply show that GHb is both sensitive and specific in detecting diabetes when compared with fasting plasma glucose (FPG).

As noted in our report, some cross-sectional studies comparing GHb with FPG and/or the oral glucose tolerance test have concluded that GHb is a useful screening test, while others, including the study cited by Herman et al. (4), have suggested the opposite. However, prospective studies have shown a high correlation between GHb and the presence of microvascular complications (5-7).

Regarding the issue of cost, it has been noted that because GHb does not require special patient preparation, it may actually be more cost effective than FPG in some screening situations (8). With respect to the issue of GHb standardization, we agree that considerable progress has been made in this area. Since 1996, the National Glycohemoglobin Standardization Program (NGSP) has certified many GHb assay methods that have passed a rigorous precision- and bias-testing protocol comparable with the Diabetes Control and Complications Trial. Proficiency-testing data from the College of American Pathologists have documented excellent comparability of results between laboratories when NGSP-certified methods were used (9). With

respect to the issue of interindividual variation of GHb, we are unaware of any data suggesting that normoglycemic individuals are at significant risk for development of diabetic complications as long as GHb levels remain within the nondiabetic range. Moreover, numerous studies have shown a strong correlation between GHb and plasma glucose levels in individuals with diabetes (10-12).

Interestingly, Papoz et al. found higher sensitivity and lower specificity than we did for detecting diabetes at a GHb cutoff of 6.0% (our cutoff was 6.1%). Different GHb cutoff levels can be selected for screening based on the sensitivity/specificity desired, but this in turn depends on the assay method used and the characteristics of the population being screened; as we showed, there are differences in sensitivity and specificity between ethnic groups. Based on our assay method and study population, we chose a cutoff of 2 standard deviations above the normal mean, which resulted in moderate sensitivity but very high specificity. Given that GHb levels <7.0% confer low risk for complications, we believe this cutoff would identify almost all individuals at significant risk for complications, while resulting in very few false positives.

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References

1. Herman WH, Engelgau MM, Zhang Y, Brown MB: Use of GHb (HbA_{1c}) to screen for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:1207-1208, 2000
2. Papoz L, Favier F, Clabé A, Sanchez A, Le Moullec N: GHb (HbA_{1c}) is more sensitive than fasting plasma glucose as a screening test for diabetes mellitus. *Diabetes Care* 23:1206-1207, 2000
3. Rohlfing CL, Little RR, Wiedmeyer HM, England JD, Madsen R, Harris MI, Flegal KM, Eberhardt MS, Goldstein DE: Use of GHb (HbA_{1c}) in screening for undiagnosed diabetes in the U.S. population. *Diabetes Care* 23:187-191, 2000

4. Engelgau MM, Thompson TJ, Herman WH, Boyle JP, Aubert RE, Kenny SJ, Badran A, Sous ES, Ali MA: Comparison of fasting and 2-hour glucose and HbA_{1c} levels for diagnosing diabetes: diagnostic criteria and performance revisited. *Diabetes Care* 20:785-791, 1997
5. The Diabetes Control and Complications 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
6. U.K. Prospective Diabetes Study Group: Intensive blood-glucose control with sulphonylureas or insulin compared with conventional treatment and risk of complications in patients with type 2 diabetes (UKPDS 33). *Lancet* 352:837-853, 1998
7. McCance DR, Hanson RL, Charles MA, Jacobsson LTH, Pettitt DJ, Bennett PH, Knowler WC: Comparison of tests for glycohemoglobin and fasting and two hour plasma glucose concentrations as diagnostic methods for diabetes. *BMJ* 308:1323-1328, 1994
8. Knowler WC: Screening for NIDDM: opportunities for detection, treatment, and prevention. *Diabetes Care* 17:445-450, 1994
9. Little RR: Recent progress in glycohemoglobin testing (Editorial). *Diabetes Care* 23:265-266, 2000
10. The Diabetes Control and Complications Trial (DCCT) Research Group: DCCT: results of feasibility study. *Diabetes Care* 10:1-19, 1987
11. Little RR, England JD, Wiedmeyer HM, McKenzie EM, Pettitt DJ, Knowler WC, Goldstein DE: Relationship of glycosylated hemoglobin to oral glucose tolerance: implications for diabetes screening. *Diabetes* 37:60-64, 1988
12. Nathan DM, Singer DE, Hurxthal K, Goodson JD: The clinical information value of the glycosylated hemoglobin assay. *N Engl J Med* 310:341-346, 1984

Transcutaneous Glucose Measurements Using Near-Infrared Spectroscopy

Validation of statistical calibration models

A recent article on a noninvasive transcutaneous assay for blood glucose using near-infrared spectroscopy was published by Gabriely et al. (1). The authors reported on mean absolute pre-

diction errors for blood glucose concentrations as low as 2.6 mg/dl (0.14 mmol/l). This approach would provide a splendid analytical performance for the hypoglycemic range, which has never been achieved, even with far less complex in vitro samples using near-infrared spectroscopy. Unfortunately, their results based on statistical partial least-squares (PLS) calibrations are extremely questionable.

An important and sensitive issue in statistical calibrations is the validation of calibration models. Problems related to such measurements were recently discussed by Arnold et al. (2), elucidating the pitfalls of statistical calibrations using tissue phantoms without glucose being present. Their conclusion, which we support, was that more rigorous testing strategies have to be applied to prove the applicability of such calibration models.

Details on the calibration and validation design are essential for judging the scientific value of the calibration models used. Such rules were not followed in the article by Gabriely et al. (1). The authors omitted any information concerning spectral quality, range, resolution, and the number of spectral variables used for their calibrations. Most results were from the calibration fit itself, which is known to give better results for sensitive linear equation systems compared with independent predictions. For such a validation, the authors picked 6–10 masked data pairs from each of their individual calibration populations. However, it is not clear whether these data were left out one at a time (similar to leave-one-out crossvalidation) or as a complete package. Furthermore, the calibration experiments were made up from 2 glucose concentration profiles of nearly constant slope, prone to run parallel to other drift effects. Without more sophisticated validation strategies, their results cannot be accepted. We calculated from their results (Fig. 3C) a standard error of prediction (SEP) of 5.4 mg/dl (0.3 mmol/l) (mean absolute error 3.7 mg/dl) for the pooled masked values ($n = 75$, SD 17 mg/dl, mean reference concentration 76 mg/dl).

For comparison, the illustrative results presented below were obtained within our previous feasibility studies from in vivo data as measured during oral glucose tolerance testing (3). Different PLS calibration models, based on a selected spectral interval, were considered. For validation, different methods, such as crossvalidation with leaving 1 or 10 samples out, as well as using day-to-day

testing were applied in our case (H.M.H., P.L., unpublished observations). The results demonstrate the dangers of overfitting when too many variables are taken into account for modeling. For calculating robust calibration models, the parsimony principle with respect to the number of spectral variables is of great importance. We have seen an improvement in prediction performance with a reduction of spectral variables (3). Our best result with a mean absolute error of 30 mg/dl (1.7 mmol/l) (SEP 37 mg/dl, SD [reference data] 168 mg/dl) was obtained with leave-one-out crossvalidation for our complete 2-day series. However, we also tested the robustness with respect to transferability of the calibration models to data of other separate days (H.M.H., P.L., unpublished observations).

Another test demonstrated that any simple artificial glucose concentration profile can be fitted with good precision, using the same validation tools as applied by Gabriely et al. Instead of using the actual blood glucose profiles, we calculated a PLS regression against a running spectrum number (equivalent to a straight line through the origin) during our 2-day test (SD [reference data] 38.2, mean value 66.5 [dimensionless]). For example, using 115 spectral variables, a splendid fit was obtained, leading to an SEP value of 7.1 and 8.2 by crossvalidation with leave-one-out and leave-ten-out strategies, respectively. With calibration models calculated from data of 1 day only, the predictions using the spectral measurements of the other day failed completely and were therefore in opposition with the results obtained from measuring blood glucose concentration profiles.

In regard to the work of Gabriely et al. (1), it is essential to know if the calibrations calculated from measurements with decreasing blood glucose, successfully predict the concentrations observed on the increasing blood glucose range and vice versa. To acquire such knowledge, more day-to-day tests must be conducted. To avoid chance correlations and overfitting with many spectral variables, more sophisticated calibration design and validation experiments than previously applied in many investigations are essential.

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References

1. Gabriely I, Kaplan J, Wozniak R, Aharon Y, Mevorach M, Shamon H: Transcutaneous glucose measurement using near-infrared spectroscopy during hypoglycemia. *Diabetes Care* 22:2026–2032, 1999
2. Arnold MA, Burmeister JJ, Small GW: Phantom glucose calibration models from simulated noninvasive human near-infrared spectra. *Anal Chem* 70:1773–1781, 1998
3. Heise HM, Bittner A, Marbach R: Clinical chemistry and near-infrared spectroscopy: technology for non-invasive glucose monitoring. *J Near Infrared Spectrosc* 6:349–359, 1998

Transcutaneous Glucose Measurement Using Near-Infrared Spectroscopy During Hypoglycemia

We appreciate the opportunity to respond to the issues raised in this issue by Heise and Lampen (1) concerning our article “Transcutaneous Glucose Measurements Using Near-Infrared Spectroscopy: Validation of Statistical Calibration Models” (2).

First, we should emphasize that differences in the accuracy of glucose prediction using near-infrared spectroscopy depend on both the hardware used and spectral data processing to construct calibration models. The goal of achieving improved prediction and accuracy by using this technology will ultimately need optimization of both components to develop the biological models that reflect relevant physiology in humans.

More specifically, Heise and Lampen misinterpreted our data and thus reached some erroneous conclusions. Their assumption that “most results were from the calibration fit itself” is incorrect; all of the results given, other than those for the “masked” values, were obtained by leave-one-out crossvalidation, the same technique used by Heise et al. (3). In addition

