OBSERVATIONS

Publication of Abstracts Presented at Diabetes Meetings

Presentation of abstracts at scientific meetings is a valid and a popular way to disseminate research findings. However, the ultimate way to have research methodology and results validated is publication in a peer-reviewed journal. No information is available in the diabetes literature regarding the ultimate fate of abstracts presented at national meetings.

The aim of this study was to determine the publication rates of abstracts presented at diabetes meetings.

Books of abstracts were obtained for three meetings: the 28th Annual Meeting of the European Association for the Study of Diabetes (EASD) in 1992, the 52nd Annual Meeting of the American Diabetes Association (ADA) in 1992, and the Annual Meeting of the Australian Diabetes Society (ADS) and the Australian Diabetes Educators Association in 1990. MEDLINE and Cumulative Index to Nursing and Allied Health Literature (CINAHL) searches were made of a random sample of the abstracts presented at each meeting to determine whether they were published in full.

At the ADA meeting, 710 abstracts were presented; of the 71 abstracts included in the search, 38 (53%) were published in full. At the EASD meeting, 840 abstracts were presented; of the 84 abstracts included in the search, 41 (49%) were published in full. At the ADS meeting, 126 abstracts were presented; of the 31 included in the search, 8 (26%) were published in full.

The publication rates of abstracts presented at diabetes meeting of 26% (Australia), 49% (Europe), and 53% (American) are consistent with reports in the literature for other disciplines (4–9).

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Influence of Glycemic Control on Zinc Urinary Excretion in Patients With Type 1 Diabetes

Several studies have found alterations in zinc status of patients with diabetes. Zinc and insulin interactions and the effects of diabetes on zinc metabolism have been particularly discussed (1). Zinc nutriture has been assessed by biochemical measurement in tissues and biological fluids. Abnormalities have been associated with duration of the disease, metabolic control, and presence of chronic complications in both types of diabetes (2,3). Hyperzincuria has been shown to be the most consistent finding (4,5). In type 2 diabetes, low serum zinc concentration was found in association with hyperzincuria and decreased gut absorption (6). Several evidences have suggested that zinc deficiency affects the progress of the disease and the development of microvascular complications (1). In a study on the kinetics of zinc in diabetic children, an inverse correlation was detected between zinc clearance, which could be secondary to glomerular hyperfiltration, and growth rate (7). The underlying mechanisms for hyperzincuria in diabetes are obscure, and the importance of glycemic control on zinc excretion is not completely elucidated. The aim of this study was to investigate the influence of the improvement in glycemic control on urinary zinc excretion of subjects with type 1 diabetes.

Twenty-three subjects aged 9–16 years, with a mean type 1 diabetes duration of 5.3 ± 2.0 years, were studied at an educational camp for diabetic children. Informed consent was obtained, and the experimental design was approved by the local ethics committee. No patient suffered from any disease other than type 1 diabetes, and none took medications other than insulin. Long-term complications were excluded (normal neurological examination, funduscopy, and serum creatinine; albumin excretion rate <20 μg/min). The subjects participated in a program of exercises and adequate diet. In addition to their usual NPH insulin injections, subjects were supplemented with regular insulin according to capillary glycemia measurements obtained with glucose oxidase strips. Anthropometric measurements were taken on the first and last mornings. Metabolic control was assessed by comparison of each subject's initial and final fasting glycemia, 24-h urinary glucose excretion, and insulin requirement. The same urine samples were collected in metal-free glassware. On the first day, a fasting blood sample was obtained for zinc and glucose determinations. Serum and urine aliquots were stored frozen at −20°C for further determinations. Urinary glucose was assayed by a colorimetric method. Zinc was measured by atomic absorption spectrophotometry. Quality control was tested by Community Bureau of Reference bovine liver, reference material 185. The reference value of the certificate of analysis was 142.0 ± 3.0 μg Zn/g and the mean value found in our lab was 145.0 ± 1.2 μg Zn/g. Food consumption from five randomly selected days was frozen at −20°C and was assessed by duplicate-portion technique. Nutrient intake was calculated by chemical analysis performed

according to the Association of Official Analytical Chemists (8). Statistical analysis included paired Student's *t* test, Wilcoxon's test, and Pearson's correlation coefficient.

Mean glycated protein at entry was 6.3 ± 2.3%. BMI (19.0 ± 2.0 kg/m^2) did not change during the period. Total energy intake was adequate (2,115 ± 230 kcal/day), and the distribution of calories from proteins (17.3%), lipids (36.1%), and carbohydrates (46.5%), and zinc intake (15.8 mg), were within the ranges recommended by the American Diabetes Association (9) and given in the Recommended Dietary Allowances (RDA) of the National Academy of Sciences (10). A mean serum zinc value of 105.5 ± 14.1 µg/dl was found. A negative correlation was detected between serum zinc and duration of type 1 diabetes ($r = -0.45$, $P = 0.024$) but not between serum zinc and the glycated protein levels. The 24-h urine volume was significantly correlated with glucose and zinc excretions. Also, urinary glucose and zinc were shown to be correlated ($r = 0.59$, $P < 0.005$). Initial urinary zinc loss was high and decreased (from 954.0 ± 370.3 to 667.2 ± 341.5 µg/24 h) at the end of the program, reaching values close to the normal range (300–600 µg/24 h). Such reduction was accompanied by improvement in glycemic control, which was expressed by a significant decrease in insulin requirement (from 31.6 ± 10.1 to 18.7 ± 11.2 U/day, $P < 0.001$), fasting glycemia (166 ± 84 to 128 ± 68 mg/dl, $P < 0.05$), and a tendency toward reduced urinary glucose (from 16.2 ± 18.0 to 13.2 ± 13.3 g/24 h).

The initial hyperzincuria found in our study is in agreement with the literature (1,4,5). The improvement in metabolic control was accompanied by a reduction in zinc excretion, and the positive correlation with urinary glucose suggested a role for glycemic control of zinc excretion. We speculate that hyperzincuria may be attributed at least in part to glomerular hyperfiltration, consequent to chronic hyperglycemia. It would be reasonable to expect that such a chronic exaggerated zinc excretion might lead to tissue depletion and lower circulating levels. In our study, subjects receiving a diet with a normal zinc content had serum zinc within the normal range. However, variable results have been shown in diabetic subjects, ranging from high to low levels (11). Although normal serum zinc concentrations were found, the inverse correlation detected with the duration of diabetes may suggest a deterioration of zinc nutriture as the disease progressed. However, in the course of the disease, persistent poor glycemic control has been associated with glycation of zinc-binding proteins, which could result in decreased affinity by this element (2). Zinc is shown to be important not only for immune responses in diabetic patients but also for reductions of the oxidative stress involved in the microvascular and neurological disease process (1,3). Therefore, in addition to the benefits of glycemic control per se in preventing chronic complications, the correction of the disturbed zinc metabolism could help in delaying their occurrence. In conclusion, our data demonstrated that near normalization of the exaggerated zinc excretion in unstable diabetes could be achieved by the improvement of glycemic control, both of which are desirable conditions for avoiding long-term diabetic complications.

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High Levels of Circulating Proinflammatory Cytokines and Leptin in Urban, but Not Rural, Indians

A potential explanation for increased risk of diabetes and coronary heart disease

The incidence of type 2 diabetes and coronary heart disease (CHD) is increasing in Indians, particularly in urban areas and abroad. Its cause is unclear, but this "epidemic" has been attributed to changes in diet and exercise leading to central obesity, and to stress. South Asian subjects show many features of the insulin resistance syndrome, and insulin resistance as well as its associated risk factors predicts the incidence of both type 2 diabetes and CHD.

Adipose tissue expresses the proinflammatory cytokine tumor necrosis factor-α (TNF-α), which has actions on insulin signaling and lipid metabolism and has been proposed as the link between obesity and insulin resistance. We have recently shown production of a similar cytokine, interleukin-6 (IL-6), by adipose tissue in vivo (1). We have found strong

Table 1—*Levels of IL-6, TNFα, leptin, and obesity in three Indian populations*

	Urban middle class	Urban slum	Rural village
n (M/W)	40 (30/10)	28 (15/13)	43 (39/4)
Age (years)	38 (34/41)	35 (31/38)	28 (25/40)
BMI (kg/m^2)	23.3 (20.1–27.0)*	22.3 (20.1–24.5)*	18.7 (17.5–21.0)
WHR	0.85 (0.79–0.91)†	0.86(0.75–0.93)*	0.83(0.78–0.87)
IL-6 (pg/ml)	7.15 (3.92–22.4)‡	23.5 (6.60–26.9)‡	2.50 (1.62–14.5)
TNF-α (pg/ml)	30.9 (7.44–41.9)‡	39.3 (10.3–41.6)‡	2.57 (1.27–5.52)
Leptin (pg/ml)	6.3 (3.7–13.5)‡	9.6 (6.6–12.3)‡	1.9 (1.4–2.6)

Data are medians (interquartile range). *$P < 0.001$, †$P < 0.05$ vs. rural subjects when data were controlled for age and sex; ‡$P < 0.001$ vs. rural subjects when data were controlled for age, sex, BMI, and WHR.

correlations of circulating concentrations of these cytokines with features of the insulin resistance syndrome in 107 Europid subjects, as well as with markers of endothelial damage (2), which plays a major role in atherothrombosis (3). We postulated that elevated levels of these cytokines may exist in urban Indians.

We recruited 111 subjects from three populations: villages near Pune (n = 43), urban slums in Pune (n = 28), and an urban middle-class residential area in Pune (n = 40). Subjects with intercurrent illness or clinically evident infections were excluded. Anthropometric and blood pressure measurements (Dinamapp; Criticon, Tampa, FL) were recorded, and fasting blood was collected to measure concentrations of glucose, lipids, TNF-α, IL-6 (R&D Systems, Minneapolis, MN), and leptin (Linco, St. Charles, MO).

In the rural subjects, levels of TNF-α and IL-6 were comparable to those found in an urban Europid population (2). In both urban groups, concentrations were significantly elevated (Table 1); TNF-α concentrations were similar between the groups, but IL-6 concentrations were higher in the subjects from the slums. Leptin concentrations were significantly related to BMI (r = 0.59, $P < 0.001$) and waist-to-hip ratio (WHR) (r = 0.18, P = 0.05), and were higher in women than in men ($P < 0.001$), but remained higher in urban Indians after controlling for confounders. Concentrations of TNF-α and IL-6 were also related to BMI (TNF-α: r = 0.36, $P < 0.001$; IL-6: r = 0.22, P = 0.025) but were not related to WHR. Levels of both were significantly higher in each urban group when data were controlled for age, sex, and adiposity, as evidenced by BMI, WHR, and leptin concentrations (TNF-α: $P < 0.001$ for subjects from the slums, $P < 0.001$ for middle-class subjects; IL-6: $P < 0.001$ for subjects from the slums, P = 0.003 for middle-class subjects). There was no relationship between the concentrations of any of the three molecules and the levels of blood pressure, glucose, or lipids.

The emergence of C-reactive protein as a cardiovascular risk factor has led to the recognition of the potential contribution to atherothrombosis from inflammatory cytokines. In addition, TNF-α has powerful metabolic effects, many of which are shared by IL-6, and may contribute to insulin resistance and dyslipidemia (4).

The origin of these cytokines remains speculative. Adipose tissue expresses all three molecules and is a major contributor to circulating IL-6 (1). Despite anthropometric adjustment for obesity, and for total fat mass using leptin concentrations (5), it is possible that regional differences in adipose tissue distribution or cytokine expression explain our findings. Urban Indians are more vulnerable to respiratory and gastrointestinal infections, including *Helicobacter pylori* infections, because of poor sanitation and crowding, especially in slum areas. Atmospheric pollution induces cytokine expression in alveolar macrophages and may contribute to circulating cytokine levels. Psychological stress can also elevate concentrations of IL-6. Determination of whether these mechanisms underlie the vulnerability of urban and migrant Indians to CHD and type 2 diabetes will require prospective studies.

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Enteroviral RNA and IgM Antibodies in Early Pregnancy and Risk for Childhood-Onset IDDM in Offspring

The prevalence of diabetes is very high in individuals with the rubella embryopathy syndrome, and recent studies have shown increased levels of enteroviral, and specifically coxsackie IgM, antibodies during pregnancy among mothers whose children later developed diabetes compared

Table 1—*Mothers with enterovirus RNA or IgM antibodies: case/control status, GAD antibodies index, and age at onset of proband*

Case/control status	PCR status	Entero IgM antibody titer	GAD index	Age at onset of IDDM (years)
Case	Negative	Positive	0.022	10
Case	Negative	Positive	0.072	5
Case	Positive	Negative	0.001	9
Case	Positive	Negative	0.038	8
Case	Negative	Positive	0	5
Case	Positive	Negative	0	2
Control	Positive	Negative	0	—

Cutoff for the entero IgM antibody titer is ≥0.05 absorbance units. For the GAD index, the 99th centile of control subjects = 0.144.

with that found in control subjects (1–3). We have now analyzed the prevalence of enteroviral RNA and IgM antibodies in the first trimester of control mothers and of mothers whose children became diabetic. We also analyzed antibodies to glutamic acid decarboxylase (GAD), a β-cell antigen that has been shown to share an amino acid sequence with one of a coxsackie virus protein (4). Serum samples collected routinely during the first trimester of pregnancies in 85 mothers whose children developed diabetes before the age of 15 as recorded in the Swedish Childhood diabetes register (5) were compared with sera from 172 mothers whose children had not developed diabetes. Control subjects were selected at random in strata by county and year of birth. The distribution of month of birth was similar for both groups of subjects. Enterovirus RNA was analyzed using a nested polymerase chain reaction (PCR) (6). Enterovirus IgA, IgM, and IgG titers were analyzed using conventional enzyme-linked immunosorbent assay (7) and GAD antibodies by radioimmunoassay (8).

Three mothers of diabetic patients and one mother of control subjects were RNA positive. In addition, mothers of three patients had IgM antibodies to coxsackie B virus, but no control mother was positive. Thus 6 of 85 mothers of diabetic children had signs of an ongoing or recent enterovirus infection in early pregnancy compared with 1 of 172 control mothers, equal to an odds ratio of 12.9 (95% CI 2.43–69.52). IgG and IgA antibody concentrations did not differ between the groups. The distribution of birth months was similar among the two groups. The mean age at onset of diabetes was 7.01 ± 3.5 years among all patients, and the age at onset of patients with possible intrauterine infection was not significantly different (6.5 ± 3.0 years). The mean GAD index was higher among mothers of diabetic patients compared with mothers of control subjects ($P = 0.03$), but when setting the cutoff for GAD positivity at 99% of control subjects, there was no difference in positivity rate between mothers of diabetic patients and control subjects. None of the PCR^+ mothers were GAD^+ (Table 1).

This latter finding does not rule out the possibility of antigen mimicry, but there are other possible mechanisms to explain an association between fetal virus infection and later development of type 1 diabetes. One could be that the perinatal infection leads to a persistent low-grade infection that might slowly hit the β-cell directly and/or lead to peri-insular infection. In the latter case, the resulting inflammation could then act on the β-cell according to the so-called "innocent bystander" theory.

To our knowledge, for the first time, enterovirus RNA has been detected early in pregnancy in mothers of children who later become diabetic in a higher frequency than that found in mothers of control subjects. The numbers are low and therefore need confirmation, preferably by large prospective cohort studies, where sera could be analyzed immediately to ensure that RNA is not destroyed during long-term storage. Taken together with the higher frequency of coxsackie IgM positivity reported in this study and others (2,3), and with the previous demonstration of fetal rubella infection as a strong risk factor for type 1 diabetes, evidence is accumulating that fetal viral infections might be etiologically associated with childhood type 1 diabetes, introducing new possibilities for primary prevention.

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American Diabetes Association Criteria for Diabetes Diagnosis

Another perspective

We read with interest article by Harris et al. (1) on diagnosis of diabetes by 1997 American Diabetes Association (ADA) criteria (2) compared with 1980–1985 World Health Organization (WHO) diagnostic criteria (3,4).

We agree with the finding, given in the article (1), that the diagnosis of diabetes and impaired fasting glucose (IFG) should be determined by the ADA criterion of fasting plasma glucose (FPG) measurement in the U.S. population (2). The loss of sensitivity is only ~2%, and more cases of diabetes could be diagnosed through better compliance to evaluation by FPG.

We studied FPG and 2-h post–glucose load plasma glucose (2-h PG) values in 400 apparently healthy subjects and 75 patients with angiographically proven coronary artery disease (CAD) from the armed forces (serving and retired) who were 35–75 years of age.

We observed that diabetes was diagnosed in 0.5 (n = 2) and 8% (n = 6) of apparently healthy and CAD patients, respectively, by ADA criteria. Diabetes was diagnosed in 2.25 (n = 9) and 21.3% (n = 16) of apparently healthy individuals and CAD patients, respectively, by WHO criteria (FPG and 2-h PG). However, based on WHO FPG criteria alone (FPG ≥140 mg/dl), only 1 of 9 and 6 of 16 diabetic patients were identified in the healthy and CAD groups, respectively.

There is a significant difference in the sensitivity of diagnosing IGT/IFG by WHO (2-h PG) and ADA (FPG) criteria. The incidence of IFG by the ADA criterion is 1.7 (n = 6) and 8% (n = 6) among apparently healthy subjects and CAD patients, respectively, while by WHO criteria, IGT was detected in 7.5 (n = 30) and 17.3% (n = 13) of apparently healthy subjects and CAD patients, respectively.

We also found that all of the individuals in the healthy and CAD groups could be correctly classified according to WHO criteria into normal GTT, IGT, and diabetic, based on 2-h PG value alone.

In the Indian group studied, abnormal glucose tolerance (diabetes and IGT/IFG) by WHO criteria is 9.75% for apparently healthy individuals and ~38.6% for those in the high-risk CAD group, while by ADA criteria it is only ~2.2 and ~16% in the healthy and CAD groups, respectively.

Therefore, 7.5% of the apparently healthy individuals and 22.6% of those in the CAD group have been diagnosed with normal glucose tolerance test in the presence of either IGT or diabetes. This may be due to the fact that South Asians and Indians are reported to have a higher incidence of insulin resistance and hyperinsulinemia. The impaired release of insulin in response to glucose load and insulin resistance develops early in the course of type 2 diabetes, and even a small decrease in insulin secretory reserve or action impairs ability to handle a glucose load. Therefore, 2-h PG (WHO) is probably a more sensitive index than the FPG (ADA) criterion for the presence of disease (diabetes and IGT).

We recommend that for the diagnosis of diabetes or IGT, either WHO recommendations (FPG and 2-h PG) be followed, or if a single value is considered for diagnosis, it should be the 2-h PG value rather than the FPG value, as recommended by the ADA, in ethnic groups known to have high prevalence of insulin resistance and hyperinsulinemia. Following 2-h PG, the number of individuals known to have abnormal carbohydrate metabolism would not remain undiagnosed and would not progress to hyperglycemic complications, such as increased incidence of CAD. Of CAD patients, 24% have normal FPG, according to the ADA criterion (FPG <110 mg/dl), but an abnormal 2-h PG value of >140 and <199 mg/dl in 16% (n = 12) and >200 mg/dl in 8% (n = 6). We are also studying the incidence of abnormal 2-h PG values and normal FPG in other patients in whom hyperglycemia is an associated risk factor, such as those who develop cataracts at an early age.

Therefore, we emphasize that the ADA recommendation for using FPG to diagnosis IFG and diabetes, based on U.S. population studies, should be further studied in comparison to 2-h PG in ethnic groups such as Indians, who are known to have a high prevalence of hyperinsulinemia and insulin resistance, so that no individual who is presently classified to have an abnormal glucose tolerance according to WHO criteria is classified as normal. Detection of individuals with such an abnormal carbohydrate metabolism may delay or prevent complications in these individuals, and at the same time would not impart a false sense of security associated with normal FPG in the presence of an abnormal 2-h PG value.

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Pneumatosis Cystoides Intestinalis After Treatment With an α-Glucosidase Inhibitor

Side effects of α-glucosidase inhibitor (α-GI) include flatulence and abdominal distention resulting from fermentation of unabsorbed carbohydrates by intestinal bacteria; it has rarely caused ileus (1,2). We report here for the first time pneumatosis cystoides intestinalis (PCI) as a side effect of α-GI.

A 64-year-old woman was admitted to our hospital in late June 1998 because of abdominal distention. She denied constipation, nausea, and vomiting. For 20 years, she had been receiving insulin ther-

apy for type 2 diabetes: 22 U Penfil 30R s.c. daily at the time of admission. Except for an antilipemic agent, she received no other drugs until April 1998, when the HbA_{1c} level was found to be 8.0%, and an α-GI, voglibose (0.6 mg/day), was prescribed in addition to the insulin at the beginning of May. Excessive flatulence was noted 1 month later. By the middle of June, abdominal distention had appeared and gradually progressed. The patient was considered to have moderate diabetic neuropathy, without such symptoms as numbness or orthostatic hypotention, and mild diabetic retinopathy; however, these complications were stable. She had no diabetic nephropathy. The patient's past surgical history included a Cesarean section. She was 149 cm in height and 53 kg in weight. Bowel sounds were normal. Fasting plasma glucose concentration was 8.3 mmol/l, and the HbA_{1c} level was 7.0%. An abdominal radiograph showed distention of the ascending and proximal transverse colon with cystic radiolucencies, indicating intramural gas. Abdominal computed tomography indicated subserosal cystic areas of gas and distention of the involved segments. A barium enema study showed translucent areas of gas clustered along the distorted contours of the ascending and transverse colon. A diagnosis of PCI was made, voglibose was discontinued, and fasting was imposed. Flatulence and abdominal distention then improved, and cystic gas-filled loculations disappeared from radiographs on the 4th day.

PCI is a condition characterized by the presence of gas-filled cysts in the submucosa or subserosa of the gastrointestinal tract (3). Since diseases previously associated with PCI were ruled out and PCI improved when the α-GI was discontinued, we had little doubt that in this instance PCI was related to the α-GI. Although diabetes is rarely associated with PCI, we have previously written that worsening of diabetic neuropathy could be connected with PCI (4). Neuropathy, however, was not worsening in this case. Because lactulose administration or jejunoileal bypass surgery may result in PCI from bacterial fermentation (5,6), α-GI treatment may also be a cause of PCI. In fact, treatment with α-GI reportedly has caused ileus in diabetic patients, particularly in those with previous abdominal surgery and relatively advanced age (1,2), at least in part from excessive gas formation. Since the symptoms of PCI and the side effects of α-GI both involve nonspecific gastrointestinal complaints, we should consider the possibility of PCI when an α-GI is given to diabetic patients, especially in the contexts of advancing age, past abdominal surgery, and possible autonomic neuropathy.

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COMMENTS AND RESPONSES

Do the New Screening and Diagnostic Criteria of Diabetes Proposed by the American Diabetes Association Really Match?

The new criteria proposed by the American Diabetes Association (ADA) for the diagnosis of diabetes (1) have recently been applied to a large sample of obese subjects by Mannucci et al. (2) and have resulted in an increased prevalence of both diabetes and impaired fasting glucose (IFG). When these authors used fasting plasma glucose (FPG) on its own to estimate the prevalence of diabetes, their diagnosis, when compared with the oral glucose tolerance test (OGTT) criteria advanced by the ADA, failed for 57% of the population under study. Therefore, we planned to verify the effective correlation between FPG and 120-min glycemia after administration of a glucose oral load (75 g) for both the screening and the diagnosis of diabetes in a consecutive series of 414 outpatients (327 female and 87 male subjects), ranging from normal in weight to obese and attending the program for weight control at the Catholic University of Rome's section of metabolic disease. They had no previous history of diabetes, and they had the following characteristics: BMI, 34.65 ± 8 kg/m^2; age, 38.1 ± 14 years; cholesterol, 185.4 ± 43.3 mg/dl; and triglycerides, 138 ± 67.9 mg/dl. They were tested for FPG and 120-min glycemia after an oral glucose load (75 g). According to the new ADA diagnostic criteria, using the OGTT as the diagnostic procedure, 261 patients (63.7%) were found to be normal. The prevalence of diabetes was 8.5% (35 patients), that of impaired glucose tolerance (IGT) was 27.8% (115 patients), and the diagnosis of IFG was established for only 3 obese patients (0.7%). Conversely, when FPG was used alone for diabetes screening, diabetes was diagnosed in only four obese patients (1%). Because both BMI and plasma glucose levels (basal values and those measured 120 min after the oral glucose load) had normal distributions, Pearson's correlation was calculated. A positive significant correlation was found between BMI and basal glycemia ($R = 0.34$; $P < 0.0001$) and between BMI and 120-min glycemia ($R = 0.21$; $P < 0.0001$). Therefore, because Mannucci et al. (2) studied only obese subjects, they overestimated the prevalence of diabetes, IGT, and IFG.

In conclusion, using the 120-min OGTT screening procedure, the prevalence of type 2 diabetes was similar to that currently referred by literature (10–15%), while FPG clearly underestimated the prevalence of diabetes mellitus. The Expert Committee on the Diagnosis and Classifi-

cation of Diabetes Mellitus of the ADA recommends FPG as a screening procedure, and the test of 120-min glycemia after glucose load as a diagnostic procedure (1), but these criteria have resulted in different estimates of diabetes prevalence in both obese and nonobese subjects.

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Response to Gniuli et al.

The principal aim of our study (1) was to compare the estimates of diabetes prevalence among obese patients based on either the World Health Organization's (2) or the American Diabetes Association's (ADA) (3) criteria. The sample studied was composed of obese outpatients seeking treatment for weight loss, and it was not intended to represent the general population. In fact, the prevalence of diabetes is known to be significantly higher in obese patients when compared with normal-weight subjects; this difference is confirmed by the positive correlation between BMI and plasma glucose described by Gniuli et al. in their sample. Therefore, we think that this sample was adequate for the purpose of the study. In this regard, it should be observed that the sample selected by Gniuli et al., which was composed of subjects seeking weight loss and having a mean BMI of 34.6 kg/m^2, is equally unrepresentative of the general population.

We reported that the adoption of fasting plasma glucose (FPG) as the only screening method for diabetes while maintaining 2-h postload plasma glucose >200 mg/dl among the diagnostic criteria, as suggested by the ADA's Expert Committee on the Diagnosis and the Classification of Diabetes Mellitus (3), could lead to a remarkable underestimate of diabetes prevalence in obese patients (1). This result was confirmed by Gniuli et al. in a different, more heterogeneous sample. They enrolled in their study all patients seeking treatment for weight loss, irrespective of their actual weight; therefore, their sample also included normal-weight and underweight individuals, many of whom were probably affected by eating disorders and could be scarcely representative of normal-weight subjects in the general population. Considering that the mean BMI was well above 30 kg/m^2, even if the number of normal-weight and underweight patients is not specified, the vast majority of the sample was probably composed of obese individuals. Therefore, the conclusions of Gniuli et al. about the ineffectiveness of FPG in the screening of diabetes can be applied to overweight subjects, but not to normal-weight subjects. Further studies are needed to assess the sensitivity and specificity of FPG in the screening of diabetes in the general population.

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HbA_{1c} Determination in Patients With Hemoglobinopathies

There is an increasing number of hemoglobin (Hb) variants, causing false HbA_{1c} results in determination of HbA_{1c} in diabetic patients of all ethnic groups. We read with interest the study of Roberts et al. (1) on the effects of sickle cell trait and HbC trait in a black study population on determination of HbA_{1c} by an immunoassay method (DCA 2000, Bayer Diagnostics, Elkhart, IN). In patients with HbC trait, the immunoassay method showed significant positive bias compared with that found with high performance liquid chromatography (HPLC) (Diamat, Bio-Rad Clinical Labs, Hercules, CA) (1). Using a cation-exchange chromatography, hemoglobinopathies C and S are already reported to falsely lower glycohemoglobin values in diabetic patients (2). Although rare in Caucasians, HbC and HbS are, with a prevalence of up to 3 and 9%, a common occurrence in African-Americans (1).

We report an estimated 0.6‰ prevalence of silent hemoglobinopathies detected in 15,000 HbA_{1c} determinations during a time period of 6.5 years. This means that there were nine diabetic patients of Caucasian origin with silent Hb variants (3,4). There are several reports on hemoglobinopathies and wrong HbA_{1c} results in diabetic patients of Asian origin (5). Despite advances in the standardization of methods for glycohemoglobins, several hemoglobinopathies cause false results in HbA_{1c} determinations (3). HbA_{1c} is highly sensitive to blood glucose elevations, and a deviation of 1% in HbA_{1c} results reflects a change of 1.4–1.9 mmol/l in average blood glucose. If the clinical impression and HbA_{1c} test results do not match, in case of inappropriate HbA_{1c} results or additional peaks in HPLC chromatograms, it is suggested to determine the HbA_{1c} values with a second method based on a different principle (4). Since blood glucose measurements and fructosamine results are not always well corre-

lated (6), correct HbA_{1c} values and mean blood glucose are the most appropriate methods, but only if the contribution of Hb variants is properly accounted for. With respect to local appearances of Hb variants, and concerning the ethnic origin of a population, every individual laboratory still has to establish and secure its own assay method. Calculation of mean blood glucose and/or measurement of fructosamine (7) may be alternative methods for long-term control in diabetic patients with hemoglobinopathies.

We conclude that in treatment of diabetic patients the knowledge of hemoglobinopathies influencing HbA_{1c} determination methods is essential (8) and needs to be investigated thoroughly because Hb variants contribute to mismanagement of diabetic patients due to false HbA_{1c} results.

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Response to Schnedl et al.

We appreciate the authors' interest in our findings (1,2). We agree that hemoglobin variants may pose a potential problem in ascertaining glycemic control, particularly when working with populations that contain a high prevalence of them. The effect of a particular hemoglobin variant on glycohemoglobin measurements is highly method-dependent (3). Correlation of glycohemoglobin values with other measures of glycemic control, such as fructosamine or mean blood glucoses obtained from patient self-monitoring, may help identify those patients with spurious results due to hemoglobin variants. Further investigation into the impact of their presence on decision-making in the management of diabetes is needed. The data from our paper and this letter underscore the need for additional studies on the effects of variant hemoglobins on the numerous glycohemoglobin methods used in clinical laboratories and physician offices.

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Fasting Glucose and Progression of Impaired Glucose Tolerance

I write in response to the article by Chou et al. (1) published in the July issue of *Diabetes Care*. The authors of this article examine progression to diabetes in a cohort of subjects according to their baseline glucose tolerance, which is subdivided according to normal glucose tolerance (NGT), impaired glucose tolerance (IGT), and persistent fasting hyperglycemia (PFH) (defined as fasting plasma glucose [FPG] 5.6–7.8 mmol/l). They conclude that PFH may be a "transitional condition that precedes" IGT and is intermediate between NGT and IGT

I am concerned, however, that the selection bias inherent in the design of the study does not allow this conclusion to be drawn.

Selection bias was introduced by the use of an initial FPG cutoff value of 5.6 mmol/l to decide who required an oral glucose tolerance test (OGTT). In our experience among Hong Kong Chinese, the mean FPG value of subjects with IGT is only 5.3 mmol/l—well below the cutoff value used in this study. Thus many, quite possible the majority, of subjects with IGT based on World Health Organization criteria may have been excluded from the outset, and the IGT group would have been preselected for a higher FPG. These patients would therefore constitute a high-risk subgroup of IGT, not necessarily reflecting the behavior of IGT as a whole.

Also, it is not clear how those subjects with both PFH (FPG 5.6–7.8) and diabetes (2-h plasma glucose >11.1) were handled. I estimate that these patients account for ~20% of the PFH group.

Putting it another way, all of the subjects by definition have PFH, since subjects with FPG <5.6 mmol/l were excluded as normal and subjects with FPG >7.8 mmol/l were diagnosed as having diabetes. Thus, what the authors have

found is that subjects with PFH are more likely to progress to type 2 diabetes if they also have a 2-h glucose in the IGT range. While interesting, this tells us nothing about the progression of IGT as a whole as compared with PFH. To answer this point, direct comparison would need to be made between an IGT group unselected by FPG and a PFH group selected by OGTT for 2-h glucose <7.8 mmol/l.

Thus I would disagree with the wording of the conclusions by the authors, since the IGT group is biased toward "more severity," while the PFH group may be biased in the other direction.

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Response to Cockram

We would like to thank Dr. Cockram (1) for his response to our study (2). The major concern of his letter is that the method of our study design may have led to bias in the selection of study subjects. However, in the title of our study, we clarified that our study subjects were from a high-risk group rather than from the general population. The main objective of our study was to explore the conversion rate of people among a high-risk group to type 2 diabetes. Thus, there should be no selection bias introduced by the use of an initial fasting plasma glucose (FPG) value of 5.6–7.8 mmol/l to decide who required an oral glucose tolerance test (OGTT). What we were more concerned with was possible selection bias introduced by inevitable losses between the two-stage screening steps. As mentioned by Hamman (3), this was one of the disadvantages of the two-stage screening approach to identifying people with diabetes.

In our study, glucose tolerance classification was based on currently used World Health Organization (WHO) criteria with a 75-g OGTT (4). Those with a history of diabetes were excluded first. Impaired glucose tolerance (IGT) was defined as FPG <7.8 mmol/l and a 2-h plasma glucose (PG) ≥7.8 and <11.1 mmol/l. Type 2 diabetes was defined as either FPG ≥7.8 mmol/l or a 2-h PG ≥11.1 mmol/l. Persistent fasting hyperglycemia (PFH), which patients used to be classified as having normal glucose tolerance (NGT) according to WHO criteria, was defined as FPG of 5.6–7.8 and a 2-h PG <7.8 mmol/l. NGT, PFH, IGT, and diabetes were all mutually exclusive. Therefore, there is no overlapping among these groups. In fact, we did make direct comparison between an IGT group and a PFH group based on OGTT.

What we showed in this study was that, among the high-risk groups in Kin-Chen, Kinmen, those with PFH are more likely to develop diabetes than those with NGT but less likely than those with IGT, suggesting that PFH may precede IGT in the progression toward type 2 diabetes. We agree with Hamman (3), who mentions that it is difficult to determine from a single follow-up OGTT the temporal sequence of fasting versus postchallenge glucose elevations. Additional prospective studies of sequential tests over longer time periods are certainly needed.

Our study has provided useful data for exploring the natural history of type 2 diabetes from Chinese patients with fasting hyperglycemia. Researchers should continue to seek the best methods and cutoff levels to permit early detection and appropriate treatment for high-risk populations.

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Meningeal Syndrome in Diabetic Ketoacidosis

Consider cerebral edema

We read with great interest the case reported by Albareda et al. (1). They described a case of diabetic ketoacidosis (DKA) occurring in a 32-year-old woman who presented with headache and nuchal rigidity, symptoms of meningeal irritation. Cerebrospinal fluid (CSF) analysis revealed a raised protein with no evidence of infective cause. Albareda et al. subsequently concluded that DKA and meningeal syndrome are causally related, but they cannot specify any metabolic abnormality to account for this (1).

In this case, we believe that the diagnosis of cerebral edema should have been considered. Cerebral edema is an uncommon complication of DKA that occurs mainly in children (2), although it has also been reported in the adult population (3). In fact, a significant number of patients with DKA had subclinical cerebral edema confirmed by computed tomography (CT) (4). A CT brain scan in this patient before the lumbar puncture (LP) would have been very valuable to detect brain swelling, as well as to exclude other causes of raised intracranial pressure, which would make LP hazardous. CSF pressure, which would have given some indication of whether cerebral edema was present, was not documented. In DKA-associated cerebral edema, the presence of white cells in CSF (absent in this case) can occur in the absence of infection, which may create a diagnostic problem. It would also be valuable for the authors to include values of plasma electrolytes, particularly plasma sodium in this patient, given that

she had been on diuretic therapy (spironolactone). It has been shown that hyponatremia is a predicting factor in the development of this potentially serious complication of DKA (6). Symptoms of meningeal irritation often settle after the restoration of fluid and electrolyte balance; however, it may also deteriorate during early treatment (7). Several mechanisms have been proposed for the development of cerebral edema in DKA, although the precise cause remains speculative (8).

Cerebral edema should always be considered in patients with DKA who have meningeal symptoms not only at presentation but also during early rehydration. CT of the brain is mandatory to establish early diagnosis because this complication has a very high mortality (2).

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Response to Chan et al.

Is diabetic ketoacidosis a cause of meningeal syndrome?

We have carefully read the comments of Chan and colleagues (1) on our report of diabetic ketoacidosis (DKA) as a potential cause of meningeal syndrome (2). We agree that cerebral edema (a well-known complication of DKA [3]) can be responsible for symptoms of meningeal irritation (4). However, this was not the case in the patient we reported. Before lumbar puncture was attempted, an ophthalmoscopy documented the absence of papilledema, and a cranial computed tomography scan did not disclose any signs of cerebral edema. The cerebral fluid pressure was not measured. So, we can affirm that in the reported patient, meningeal syndrome was not due to cerebral edema (not even subclinical) and, thus, must have been due to something else.

We would also like to add that the clinical course of the reported patient did not suggest at all the presence of cerebral edema. This complication is infrequent, mainly occurs in children (3), and is exceptional before the initiation of therapy (5). Even more important, the patient's status improved steadily after the initiation of treatment, which is the opposite of what one would expect in the case of cerebral edema.

In conclusion, we agree that cerebral edema has to be considered as a potential cause of meningeal syndrome in DKA. However, DKA per se seems to be sufficient to produce it.

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Analytic Accuracy of Calculated Low-Density Lipoprotein Cholesterol Concentrations

We would like to discuss the conclusions of Branchi et al. (1) regarding the reason for inaccurate LDL cholesterol values in diabetic subjects calculated with Friedewald's formula. Although there was a good correlation between the calculated and determined LDL cholesterol concentrations (r = 0.95 and 0.97 in samples with triglyceride [TG] concentrations $<$4.52 mmol/l), the bias was frequently large, especially in the diabetic subjects and in subjects whose plasma TG concentrations were $\geq$2.26 mmol/l. Consequently, ~25% of the subjects were misclassified for coronary heart disease risk using cutoff points of the National Cholesterol Education Program.

The report of Branchi et al. (1) adds to numerous other studies published on this subject. All of these reports suggest that the deviating chemical composition of the VLDL and LDL fractions must be the major reason for the observed deviations. However, it can be reasoned that this argument is of limited value for clinical decision making. Previously, we found that the cholesterol content in atherogenic remnants, as present in familial dysbetalipoproteinemia (FD), is included in the calculated LDL cholesterol value, thereby more accurately reflecting the clinical risk than the determined LDL cholesterol value (2). This finding can be explained by the fact that in

these patients, the plasma TG concentration is relatively low. Consequently, the calculated VLDL cholesterol concentration is only a fraction of the actual cholesterol content in the density <1.006 g/ml containing mainly remnants. In subjects with remnant accumulation not typical for FD, i.e., in those with the apoprotein (apo) E phenotypes E2/E3 or E2/E4, a similar clinical risk correction can be expected. In addition, we found in two independent studies that the ratio of VLDL cholesterol to plasma TG was relatively independent of the plasma TG up to a TG concentration of 8 mmol/l (usually, 400 mg/dl [4.52 mmol/l] is considered the limit).

In our recent study (3), we suggest that the use of imprecise and inaccurate methods for measurement of plasma lipids and HDL cholesterol may be the main reason for the reported limited value of Friedewald's approach in other laboratories. This possibility is supported by the higher correlation coefficients (r = 0.97 to >0.98) we obtained comparing calculated and determined LDL cholesterol concentrations in subjects with plasma TG concentrations up to 8 mmol/l (2,3). Besides, there are other methodological concerns in the study of Branchi et al. (1): quality control data of the methods used are lacking, and the reference HDL cholesterol method is based on ultracentrifugation, which is inaccurate because the density fraction >1.063 g/ml contains most of the lipoprotein(a) present in plasma (4). Other lessons of our two independent studies on the reliability of Friedewald's equation are as follows: *1*) In each evaluating study, the relatively small VLDL cholesterol value needed to calculate the reference LDL cholesterol concentration must be determined directly instead of indirectly as the difference of two large analytes (total cholesterol minus cholesterol in the density fraction >1.006 g/ml). The use of a tube-slicing technique instead of direct aspiration is thus problematic. Direct aspiration and analysis improve the precision (coefficient of variation [CV]) of the reference VLDL cholesterol value. *2*) An HDL cholesterol method with an uncomplicated precipitation of the apoB-containing lipoproteins in sera with a TG concentration >4.5 mmol/l should be used, e.g., the PEG-6000, the phosphotungstate/Mg^{2+}, or a direct HDL cholesterol method (5). Surprisingly, the reference for the HDL cholesterol method used by Branchi et al. (1) is not indicated. The use of an inadequate HDL cholesterol method contributes to a relatively high imprecision, not only of the reference but also of the calculated LDL cholesterol value. *3*) Methods to measure TG and cholesterol (the latter also at low levels, as present in the HDL fraction) should be maximally precise and accurate. In this respect, the linearity of the TG determination should be taken into account: most methods commonly used require a dilution at concentrations >4.0 mmol/l. When a dilution is needed, the CV of the analyte increases severalfold.

From the above discussion, it is obvious that at TG concentrations >4.0 mmol/l, several factors increase the imprecision of both the measured and the calculated LDL cholesterol concentrations. In our opinion, this problem is a major cause of the presumed imprecision of Friedewald's formula (3). This view is supported by the similar under- and overestimation percentages of 16–19% in the study of Branchi et al. (1), in both the patient and the control subject groups. Note that under- or overestimation was most frequent at low concentrations of LDL cholesterol (≤3.9 mmol/l), which were characteristic for sera with increased plasma TG concentrations.

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Response to Demacker and Stalenhoef

In their letter, Demacker and Stalenhoef suggest that the imprecision of LDL cholesterol calculation using Friedewald's formula is due mainly to the inaccuracy of methods for measurement of plasma lipids and HDL cholesterol rather than to variations in VLDL composition. Their opinion is based on two observations: *1*) the high correlation coefficients they found between measured and calculated LDL cholesterol and *2*) the relative constancy of the ratio of VLDL cholesterol to plasma triglycerides (TGs) up to a plasma TG level of 1,240 mg/dl (14 mmol/l) (1). In our series, too, and in other studies (2), measured and calculated LDL cholesterol values were highly correlated (r = 0.95 in 151 diabetic patients and r = 0.97 in 405 nondiabetic patients); however, a good correlation may mask the prevalence of clinically significant errors. Despite the high correlation between the two methods, we found a difference ≥10% between measured and calculated LDL cholesterol values in 34% of samples from diabetic subjects and in 26% of samples from nondiabetic subjects, in accord with previous reports (2).

VLDL composition has a major impact on LDL cholesterol calculation using Friedewald's formula, which is based on the assumption of a fixed relationship between VLDL cholesterol and serum TGs. Demacker and Stalenhoef claim that the ratio of VLDL cholesterol to plasma TGs is relatively constant and independent of the plasma TG level, although in the figure accompanying their article (1), the ratio of VLDL cholesterol to plasma TG is scattered from <0.1 to >0.7, for a TG level up to 5

mmol/l. In our series, too, the VLDL cholesterol–to–plasma TG ratio showed a wide variability, ranging from 0.05 to 0.9. The great variability of the ratio is expected because the serum TG level results not only from VLDL concentration but from the sum of the TG content of all the lipoproteins, and the TG content of all the lipoprotein families, including VLDL, changes when TG metabolism is altered (3). On the other hand, it is widely accepted that variations in serum TG concentration are the main source of error in Friedewald's empirical equation (2).

We agree that the method used in determining HDL cholesterol is critically important in the estimation of LDL cholesterol. As stated in our article, we determined the cholesterol content of the density fraction >1.063, which contains some of the lipoprotein(a) present in the plasma, as correctly pointed out by Demacker and Stalenhoef. HDL cholesterol determined in such a way should be overestimated; however, this is of minor importance in the final results of our study, because the same value of HDL cholesterol was used when reference ultracentrifugation method was used as when Friedewald's equation was used. Therefore, the difference between measured and calculated LDL cholesterol was entirely due to a difference in the estimation of VLDL cholesterol.

Demacker and Stalenhoef observe that our article lacks data on the reproducibility of determinations. We apologize for our omission; the coefficient of variation was 1.76% for cholesterol, 3.54% for TGs, 7.01% for VLDL cholesterol, 4.57% for LDL cholesterol, and 4.19% for HDL cholesterol. VLDL cholesterol was calculated as the difference between total cholesterol and cholesterol of density >1.006, according to the method of Bronzert and Brewer (4). Demacker and Stalenhoef observe that this method of determining VLDL cholesterol is less accurate than the direct measurement of cholesterol in the lipoprotein fraction. In fact, in their study (1), the resulting coefficient of variation of VLDL cholesterol was low. In our experience, the Bronzert and Brewer method is not less suitable than the direct determination of VLDL cholesterol in the aspirated fraction, which often has a very low cholesterol concentration (therefore increasing imprecision and inaccuracy) and needs to be corrected for recovery.

Obviously, we agree that methodology of lipid determination plays a key role in both the reference method and Friedewald's calculation, but we believe that imprecision of Friedewald's equation in some conditions, such as hypertriglyceridemia, is unlikely to be due to only imprecision and inaccuracy of methods used in lipid determination. A number of studies using different methodologies are in accord with our conclusions.

The main purpose of our study was to evaluate the reliability of Friedewald's equation in type 2 diabetic patients with respect to nondiabetic subjects. As expected, we found a greater error in LDL cholesterol estimation in diabetic patients than in nondiabetic subjects, because of the more frequent abnormalities in TG metabolism in the former group. The limitations of the estimation of LDL cholesterol with Friedewald's formula are well known. However, in our experience, the method proved useful in the correct assignment of risk classes of coronary disease in >75% of nondiabetic and diabetic patients.

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Erratum

Sánchez-Margalet V, Lobón JA, González A, Fernández-Soto ML, Escobar-Jiménez F, Goberna R: Increased plasma pancreastatin-like levels in gestational diabetes. *Diabetes Care* 21:1951–1954, 1998

Maria Luisa Fernández-Soto, MD, was accidently omitted as an author of the above article. The corrected list of authors is printed above.