OBSERVATIONS

Plasma Interleukin-18 Concentrations Are Elevated in Type 2 Diabetes

We read with interest the article by Aso et al. (1) showing greater plasma concentrations of interleukin-18 (IL-18) in type 2 diabetic patients compared with matched control subjects. While the authors found no significant associations between fasting levels of IL-18 and homeostasis model assessment, a measure of insulin resistance, they found associations between IL-18 and C-reactive protein concentrations. Moreover, carotid intima-media thickness, a validated surrogate measure of atherosclerosis, was greater in diabetic patients with high IL-18 than in those with normal IL-18. As a whole, these data add to the mounting evidence that diabetes may be regarded as a chronic low-grade inflammatory state.

However, we disagree with the authors' conclusions that "the present study demonstrated for the first time that plasma IL-18 concentrations were significantly higher in type 2 diabetic patients than in age-matched control subjects" because we have reported similar findings in *Diabetes Care* (2). In that study, we demonstrated that 30 newly diagnosed, slightly overweight (BMI 26.9 ± 1.2 kg/m^2, means ± SD) type 2 diabetic patients without clinical or instrumental evidence of micro- and macrovascular complications presented higher circulating concentrations of IL-18 compared with nondiabetic subjects matched for sex, age, and body weight (205 ± 39 vs. 120 ± 25 pg/ml, $P < 0.01$). It is reassuring to see that the fasting values of IL-18 in the Japanese diabetic patients studied by Aso et al. (1) were quite similar (203 ± 153 pg/ml) to those of our Caucasian diabetic patients and that the relation between fasting plasma glucose and IL-18 concentrations was present in both studies ($r = 0.31$, $P < 0.05$; $r = 0.24$, $P < 0.02$, respectively). These results suggest that ethnicity does not to play a major role in these associations.

IL-18 is a potent proinflammatory cytokine reported to play a role in plaque destabilization (3) and predict cardiovascular death in patients with coronary artery disease (4). Although IL-18 is produced mainly by monocyte/macrophages, a contribution from adipose tissue has recently been suggested (5). In the light of the evidence that IL-18 circulating levels are higher in type 2 diabetic patients than matched nondiabetic subjects and correlate with fasting glucose levels, it does not seem hazardous to hypothesize that IL-18 may play a role in acute coronary syndromes through plaque destabilization (6). This working hypothesis, which deserves further investigation, also finds support in the observation that acute hyperglycemia may increase circulation levels of IL-18 in both normal subjects and patients with impaired glucose tolerance (7).

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Similar A1C Outcomes in Type 1 Diabetic Patients Undergoing Intensive Diabetes Management With Preprandial Rapid-Acting Insulin and Either CSII or Glargine

The importance of achieving and maintaining tight glycemic control in patients with type 1 diabetes is well known (1). Continuous insulin infusion therapy (CSII) has been available for many years, but only recently have reports of efficacy with rapid-acting insulin analogues been published. Similarly, multiple daily injection (MDI) therapy using glargine insulin in conjunction with premeal rapid-acting insulin is relatively new (2).

In our diabetes center, all patients with type 1 diabetes receive the same diabetes education, including instruction in carbohydrate counting. All patients are given the option of either CSII or MDI therapy and are encouraged to use whichever treatment maintains their blood glucose levels as close to normal as possible.

To assess our quality of care, we performed a random chart audit of 150 patients. To be included in the analysis, patients had to have type 1 diabetes and be treated for at least 6 months with either

CSII using rapid-acting insulin (lispro or aspart) or MDI with insulin glargine with premeal rapid-acting insulin. Patients who were pregnant, <15 years of age, or referred for treatment of severe recurrent hypoglycemia were excluded.

There were 103 patients who met our criteria; 58 were on CSII and 45 were on MDI therapy. Glargine was given in the morning in 11%, in the evening in 60%, and twice a day in 29% of patients. Age, duration of diabetes, and incidence of complications were similar in both groups. Duration of therapy was 16.0 ± 5.8 (means ± SD) (CSII) vs. 11.6 ± 3.8 months (MDI) ($P < 0.0001$). Most recent A1C levels were the same in both groups—6.79 ± 1.07 (CSII) vs. 6.84 ± 0.90% (MDI) ($P = 0.82$). One patient in the CSII group and two patients in the MDI group had an episode of severe hypoglycemia. One patient in the CSII group had two episodes of diabetic ketoacidosis.

Therefore, both CSII and MDI therapy can be used to treat patients with type 1 diabetes to target. Prospective data are needed to confirm the findings of this cross-sectional analysis.

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Matrix Metalloproteinase 2 May Be a Marker of Microangiopathy in Children and Adolescents With Type 1 Diabetes

Patients with type 1 diabetes develop microangiopathic complications such as retinopathy, peripheral neuropathy, and nephropathy (1), which are responsible for morbidity in adulthood. These complications usually have a prolonged asymptomatic phase, sometimes starting in adolescence, characterized by early subclinical functional and structural abnormalities (2).

Since matrix metalloproteinases (MMPs) represent a serum marker of vascular disease (4), the aim of our study was to detect MMP-2 and -9 levels and activity in type 1 diabetic children and adolescents.

Twenty-five children and adolescents (13 boys and 12 girls), median age 10.6 years (7.9−11.7), were longitudinally evaluated at clinical diagnosis and during a 5-year follow-up period.

Peripheral neuropathy, assessed by peroneal motor nerve conduction velocity, developed in 12 patients (6 boys and 6 girls) 5.7 years (3.7−6.5) from disease diagnosis. Background diabetic retinopathy (microaneurisms), assessed by fundus photography, developed in three patients (two boys and one girl) 6.6, 5.8, and 6.0 years from disease diagnosis, respectively.

For control subjects, we randomly chose 19 nondiabetic subjects (9 boys and 10 girls), median age 12.0 years (11.0−13.0), from among those reporting for their first hepatitis B virus vaccination and for the 5-year follow-up visit.

Informed consent was obtained from all parents. The study was approved by the local ethics committee. MMP-2 and -9 levels and activity were detected by ELISA (Amersham, Pharmacia Biotech); GAD antibody, IA-2 antigen, and insulin autoantibody levels were detected by radioimmunoassay (CIS Bio International). HbA_{1c} levels were evaluated by high-performance liquid chromatography (BioRad). All samples were stored at −80°C until analysis was performed.

No significant correlation was observed among MMP results (both levels and activity) and chronologic age, autoantibody, and HbA_{1c} levels.

At baseline, MMP-2 levels were significantly higher in type 1 diabetic patients and type 1 diabetic patients with complications than in nondiabetic subjects (1,100 [915–1,326], 1,742 [1,426–1,908], and 907 ng/ml [735–970], respectively), as was MMP-2 activity (31 [30–37], 152 [127–176], and 97% [88–101], respectively) ($P < 0.0001$). No significant differences were observed for MMP-9 level and activity.

Patients who developed microangiopathic complications during the follow-up period had significantly higher MMP-2 activity ($P < 0.001$) and levels ($P = 0.009$) than patients without complications.

At 5-year follow-up, MMP-2 levels were significantly higher in patients with microangiopathic complications compared with control subjects (1,782 [1,741–2,089] and 1,022 ng/ml [897–1,125], respectively; $P < 0.0001$), as was MMP-2 activity (116 [104–151] and 46% [37–68], respectively; $P < 0.0005$) and compared with patients without complications (1,371 ng/ml [1,197–1,479] and 31% [30–34] for levels and activity, respectively; $P < 0.0001$).

MMP-9 levels were significantly lower in patients with microangiopathic complications (44 ng/ml [30–63]) compared with control subjects (95 ng/ml [58–126]; $P = 0.024$) and patients without complications (82 ng/ml [46–99]; $P = 0.0013$), but no difference was found between control subjects and patients without complications. No difference was observed for MMP-9 activity among the three groups.

The three groups did not differ in terms of percentage change of MMP-2 levels and MMP-9 activity, but did differ in terms of percentage change of MMP-2 activity ($P = 0.0036$) and MMP-9 levels ($P = 0.009$).

Our results allow us to postulate that MMP-2 may be a good index of severity and stability of microangiopathy, and the literature reports MMP-9 as a marker of macroangiopathy (4).

The relationship between MMPs and the presence of diabetic complications needs to be elucidated; further studies are necessary to clarify their possible involvement in the onset or progression of diabetic complications.

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Plasma Adiponectin and Pregnancy-Induced Insulin Resistance

Lindsay et al. (1) recently published an article in *Diabetes Care* that claims adiponectin is present in the cord blood of the offspring of diabetic and nondiabetic pregnant mothers and that its level does not correlate with their birth weight and skinfold thickness. Our data, obtained from a case-control study of nondiabetic women and women with gestational diabetes mellitus (GDM), further support the role of adiponectin in insulin resistance.

We observed significantly decreased plasma adiponectin levels (7.55 ± 2.04 μg/ml [means ± SD]) using radioimmunoassay (Linco Research, St. Charles, MO) (intra-assay precision coefficient of variation [CV] 3.86%, interassay CV 8.47%) in 30 women with GDM, all of whom were treated with insulin (aged 28.12 ± 2.71 years, gestational age 27.35 ± 6.15 weeks), compared with 40 nondiabetic pregnant women tested with oral glucose tolerance test (OGTT) (total group 9.91 ± 3.32, $P < 0.01$, Mann-Whitney, aged 26.91 ± 2.65 years, gestational age 23.17 ± 10.91 weeks, 15 in the first, 12 in the second, and 13 in the third trimester) and 30 age-matched nonpregnant nondiabetic women (12.54 ± 3.76, $P < 0.01$, aged 28.42 ± 3.48 years). GDM was diagnosed with a 75-g OGTT according to the World Health Organization recommendations. In the nondiabetic pregnant group, plasma adiponectin levels were significantly lower in the second (9.30 ± 2.78) and third (8.06 ± 2.44) trimesters compared with women in the first trimester (12.30 ± 3.20, $P < 0.01$). After correction for gestational age and BMI, the differences still remained significant. No difference was found in plasma adiponectin levels before and after insulin treatment in the GDM patients.

In the GDM group, plasma adiponectin levels were in a significant negative linear correlation with serum tumor necrosis factor-α (TNF-α) ($r = -0.65$, $P < 0.0001$, Spearman's rank correlation test), leptin ($r = -0.75$, $P = 0.0004$), fasting C-peptide concentrations ($r = -0.83$, $P < 0.0001$), BMI ($r = -0.67$, $P < 0.0001$), and, as an indirect parameter of insulin resistance, fasting C-peptide/blood glucose ratio ($r = -046$, $P = 0.0109$). The same was found in the total group of nondiabetic pregnant women (TNF-α: $r = -0.56$, $P = 0.0002$; leptin: $r = -0.45$, $P = 0.003$; fasting C-peptide: $r = -0.70$, $P < 0.0001$; BMI: $r = -0.51$, $P = 0.0007$; C-peptide–to–blood glucose ratio: $r = -0.43$, $P = 0.0046$). In the nonpregnant nondiabetic control subjects, negative correlations with serum leptin ($r = -0.44$, $P = 0.0134$), fasting C-peptide concentrations ($r = -0.46$, $P = 0.01$), and BMIs ($r = -0.57$, $P = 0.0008$) were found.

Maternal plasma adiponectin levels correlated positively with the body weight of the neonates, in both the GDM (newborns, $n = 30$, 13 boys and 17 girls, maternal gestational age at delivery 38.22 ± 0.51 weeks, 14 Cesarean sections, body weight 3,151 ± 672 g, centile 55.87 ± 30.29%, $r = 0.4345$ Spearman's rank correlation test, $P = 0.0164$) and the nondiabetic pregnant group ($n = 20$, 9 boys and 11 girls, gestational age at delivery 38.92 ± 0.32 weeks, 6 Cesarean sections, body weight 3,562 ± 359 g, centile 63.46 ± 15.80%, $r = 0.6124$, $P = 0.0041$) after the correction for gestational age.

There is an association between adiponectin concentrations and insulin sensitivity. The protein is exclusively produced in adipocytes. Its inverse relationship with the increasing BMI, fasting C-peptide concentrations, and C-peptide–to–blood glucose ratio of nondiabetic pregnant women and patients with GDM underlines the importance of adipose tissue and its secreted products, such as adiponectin, in pregnancy-induced insulin resistance. The negative correlation between TNF-α, leptin, and adiponectin may suggest a negative regulatory role of these cytokines in the expression and secretion of adiponectin. The peroxisome proliferator–activated receptor system in adipocytes is a known regulator of fat cell differentiation and the production of TNF-α, leptin, and adiponectin (2). On the other hand, TNF-α may inhibit peroxisome proliferator–activated receptor function in adipocytes. Through this mechanism, the TNF system may have a suppressive effect on adiponectin production during the course of pregnancy (3).

Maternal insulin resistance and adipocytokines may influence different anthropometric parameters (body weight, body length, and head circumference) of the neonates. TNF-α and leptin were in a negative correlation with these parameters in neonates of mothers with GDM (4). However, analyzing the relationship between these parameters and maternal plasma adiponectin levels, the significant positive linear correlation between adiponectin, and the neonatal body weight, both in GDM and nondiabetic pregnant women, suggests a regulatory role of the protein in neonatal development.

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Vitiligo Associated With Subcutaneous Insulin Lispro Infusion in Type 1 Diabetes

Vitiligo vulgaris, the loss of skin pigmentation, is known to occur with increased frequency in patients with type 1 diabetes and, based on a preponderance of circumstantial evidence (1), presumed to be of autoimmune etiology. For example, 20% of 39 patients with vitiligo were found to have diabetes in a Romanian community study (2), and 9% of 457 consecutive Italian patients with diabetes had vitiligo in another study (including 54% of the type 1 patients) (3). However, the factors that can specifically precipitate vitiligo in type 1 diabetes are not known. Here, we present a case of focal vitiligo vulgaris precipitated and exacerbated by the subcutaneous infusion of the human insulin analog, insulin lispro.

A 32-year-old female with a 19-year history of type 1 diabetes began continuous subcutaneous insulin infusion (CSII) therapy 3.5 years before presentation. She had previously noted stable vitiligo vulgaris of the elbows and knees for ~10 years. After initiating CSII therapy with insulin lispro, she developed two symmetrical patches of depigmentation on her abdomen ~6 cm in diameter surrounding the insulin infusion sites bilaterally (Fig. 1). There was no known antecedent inflammatory skin disease.

The antibody response to rapid-acting human insulin analogs has been shown to be similar in magnitude to that triggered by human insulin (4,5). Most cutaneous allergies to insulin, however, manifest as IgE-mediated wheal and flare responses (6). In this case, the focal vitiligo was apparently induced by insulin infusion, raising questions about its pathogenesis. Possible mechanisms in-

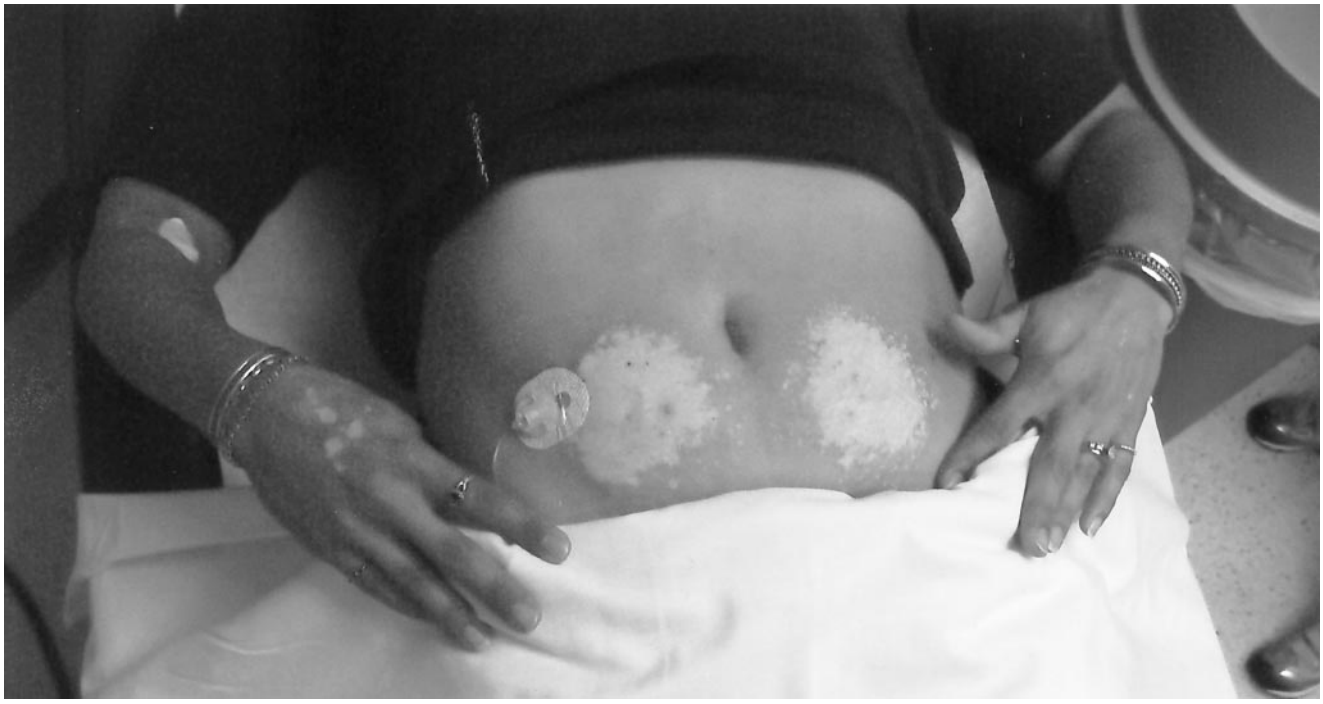

Figure 1—*Vitiligo vulgaris on the abdominal skin of a young woman associated with the subcutaneous infusion of insulin lispro.*

clude a postinflammatory, Koebner-type response in which depigmentation occurs in areas of mild injury or inflammation, but no evidence of skin damage or inflammation was present in the lesions. More likely, a local allergic reaction to the constituents of the insulin (or possibly the infusion catheter) may have precipitated an inflammatory response culminating in depigmentation. Other scenarios include molecular mimicry between the insulin lispro molecule and various melanocyte surface antigens, resulting in melanocyte destruction.

This case represents the first report of lispro insulin analog infusion as an etiologic factor in the development of focal vitiligo in diabetes. Aside from the standard treatment options for vitiligo, other options in this case include changing the type of insulin used, changing the type of infusion catheter used, and/or changing the site of insulin infusion. The patient was changed to insulin aspart and told to place her infusion catheter into an entirely new area of abdominal skin. Upon follow-up 6 months later, however, the original vitiligo lesions remained unchanged and new lesions were forming around the new infusion sites.

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Aseptic Peritonitis Revealed Through Recurrent Catheter Obstructions in Type 1 Diabetic Patients Treated with Continuous Peritoneal Insulin Infusion

Reiterated catheter obstructions thwart improved diabetes control with continuous peritoneal insulin infusion (CPII) from implantable pumps (1). Occlusions, from either fibrin clots or omental encapsulations, are promoted by CPII and diabetes duration and insulin instability (2,3). Pathological analysis of encapsulation tissues disclosed, among predominant collagen fibrosis, inflammatory reactions, including lymphocytes and amyloid-like deposits reacting to anti-insulin antibodies, surrounded by histiocytes or giant cells (2). However, catheter obstructions were not related to high plasma anti-insulin antibody levels (4,5). Enhanced migration toward insulin and the chemotactic peptide formyl-methionyl-leucyl-phenylalanine of monocyte-issued macrophages from three patients with previous catheter encapsulations suggested that higher macrophage chemotaxis might promote these events (5). We report two unique observations of aseptic peritonitis with predominant macrophagic reactions that occurred in patients using implantable pumps with recurrent catheter obstructions, supporting this hypothesis.

Case 1 is a 54-year-old woman, type 1 diabetes duration 42 years. After 2 years of CPII, she received an implantable pump (model MIP 2007; MiniMed, Sylmar, CA) in July 2000. In December 2000, a fibrin clot occluding catheter tip was removed using laparoscopy. A shorter replacement catheter was implanted when obstruction recurred in May 2001. Following surgery, CPII was ineffective and ketosis required intravenous insulin delivery. Computerized tomography scanning identified peritoneal fluid accumulation and diffuse thickening of mesenteric fat, suggesting possible neoplasic peritonitis. Laparoscopy revealed diffuse peritoneal inflammation but no cancer node. Neither bacterial infection nor cancer cells were found in peritoneal fluid, but a high content of fibrin, monocytes, lymphocytes, and macrophages were found. The catheter tip stuck to the peritoneum and was surrounded by predominant macrophages among an inflammatory cell reaction. CPII became effective again only after high doses of oral prednisone (1 mg $\cdot$ kg^{-1} $\cdot$ day^{-1}). Prednisone (15 mg/day) remained necessary to keep CPII effective, with each steroid interruption resulting in recurrent hyperglycemia. No catheter obstruction recurred thereafter.

Case 2 is a 62-year-old man, type 1 diabetes duration 30 years, using CPII since 1981 with previous implantable pump catheter encapsulations from 1990. He received a new implantable pump in December 2000. In June 2002, the catheter encapsulation needed peeling by laparoscopy. Removed tissue showed a predominantly macrophagic inflammatory reaction, including some lymphocytes, giant cells, and pseudoamyloid material among collagen fibrosis. Catheter obstruction recurred in January 2003. Laparoscopy revealed diffuse peritoneal inflammation with whitish urticaria-like plaques. Pathological analysis identified granulomatous peritoneal lesions with histiocytes, fibrosis, and pseudo-amyloid material unlabeled by anti-insulin antibodies. Similar histiocytic reaction was found in collagen fibrosis surrounding the catheter tip. Prednisone (20 mg/day) was prescribed to treat peritoneal reaction until pump replacement in July 2003 because an unexpected pump failure precluded assessment of steroid effect on CPII efficacy. CPII was effective with the new pump, and prednisone could be stopped 2 weeks after surgery.

In our experience with 87 patients using an implantable pump since 1990, such generalized peritoneal reactions have not been seen previously or reported elsewhere. In both cases, a predominant macrophagic reaction was disclosed, as previously described in encapsulation tissues (2,5). Long-term CPII or diabetes likely promoted these events in patients who appear to be specifically reactive to peritoneal infusion. Because pseudoamyloid material in Case 2 could not be labeled by anti-insulin antibodies, contribution of insulin in peritoneal macrophagic reaction cannot be argued. We recommend that recurrent implantable pump catheter obstructions should be explored by laparoscopy for peritoneal examination. Although steroid treatment appeared to be effective on peritonitis and restored CPII efficacy, continuation of CPII in such cases must be debated.

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Comparative Study of Prognostic Value for Coronary Disease Risk Between the U.K. Prospective Diabetes Study and Framingham Models

According to epidemiological and angiographic studies (1,2), diabetic patients present a two to four times greater risk for coronary artery disease (CAD) than nondiabetic individuals.

The Framingham model (3) estimates 10-year CAD risk based on the traditional risk factors, including age, sex, HDL and LDL cholesterol, hypertension, and smoking. In addition to these risk factors, the U.K. Prospective Diabetes Study (UKPDS) model, designed for people with type 2 diabetes (4), incorporates more specific variables, such as HbA_{1c}, age at diabetes diagnosis, and diabetes duration.

The aim of this analysis was to compare the accuracy of these models in the prediction of 10-year risk for CAD in diabetic patients.

Clinical information related to the above factors was retrieved from our diabetic outpatient database with 10-year clinical follow-up. Of the 339 participants (53% women and 47% men), 108 (32%) presented with CAD. We did not observe any statistically significant differences in their demographic characteristics. The diagnosis of CAD was established by coronary angiography. Diabetic patients without a history of CAD were not considered to have CAD; however, if they had presented symptoms of CAD, they would undergo a treadmill test and myocardium scanning with Th 201 (single photon emission computerized tomography).

Receiver operating characteristic curves were constructed for both models to evaluate their accuracy in the prediction of 10-year CAD risk (measured by the area under the receiver operating characteristic curve, range 0.5–1).

Areas under the curves were 0.61 ($P < 0.01$) and 0.65 ($P < 0.01$) for UKPDS and Framingham, respectively. The comparative analysis showed similar sensitivity (56 vs. 55%) between these models. A higher specificity in the Framingham model (65 vs. 56%) was noted. We also noted higher positive and negative predictive values of Framingham, 43 and 75%, respectively, compared with 37 and 73% in the UKPDS.

According to the results of this analysis, the Framingham model seems to be more appropriate for the prediction of CAD risk in diabetic patients. An explanation for this could be the small contribution of UKPDS variables (HbA_{1c}, age at diabetes diagnosis, and diabetes duration) to the 10-year risk for CAD.

Regarding the relation of HbA_{1c} to the development of CAD, data from the UKPDS 23 (5) indicated that for each 1% increment in HbA_{1c} there was a 1.11-fold increased risk of CAD, whereas for each 1-mmol/l increment in LDL concentration there was a 1.57-fold increased risk. It should also be noted that an HbA_{1c} increment from 6.5 to 11% (6) just doubles the risk of myocardial infarctions, whereas an HbA_{1c} increment of 1% multiplies the risk of microangiopathic incidents by 10. Additionally, recent studies (7,8) have confirmed the significant role of traditional risk factors in the prediction of CAD in contrast to the poor prognostic value of blood glucose concentration. In conclusion, comparing the above data, the association of HbA_{1c} with the risk for CAD is considered rather weak.

Regarding the contribution of age at diabetes diagnosis and diabetes duration to the 10-year risk, it is well known that in the early stages of the disease, when the symptoms are not apparent, diabetes is frequently underdiagnosed. Moreover, in some cases, although diabetes complications (micro- and/or macrovascular) have already presented, the existence of diabetes is still ignored by the patient. However, it is difficult to determine the exact age of diabetes onset and duration. Therefore, the evaluation of the exact duration of diabetes is potentially inaccurate, which may result in underestimation of CAD risk.

Future efforts should focus on the ultimate estimation and evaluation of the prognostic value of both models using randomized, prospective, comparative studies.

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The Biological Variation of Sex Hormone–Binding Globulin in Type 2 Diabetes

Implications for sex hormone–binding globulin as a surrogate marker of insulin resistance

Quantitative determination of insulin resistance is technically demanding and expensive. We have recently shown (1) that insulin resistance determined using the homeostasis model assessment of insulin resistance (HOMA-IR) has a significantly greater biological variability in individuals with type 2 diabetes than in healthy ones. A surrogate marker of insulin resistance that was reproducible, stable, and easily measured would be invaluable for both research and clinical practice, particularly for following insulin-sensitizing therapy, such as metformin and the thiazolidinediones. A low sex hormone–binding globulin (SHBG) concentration reflects hyperinsulinemic insulin resistance and has been proposed as such a surrogate measure (2–4). This study aimed to compare the biological variation of SHBG and insulin resistance in type 2 diabetes to determine the potential for SHBG as a surrogate marker of insulin resistance in type 2 diabetes.

Subjects were initially recruited for a study to assess the biological variation of insulin resistance in individuals with type 2 diabetes (1). Postmenopausal Caucasian subjects ($n = 12$) with type 2 diabetes (median age 62 years, range 50–73, and median BMI 31.6 kg/m^2, range 25.1–35.7) and 11 age- and weight-matched, healthy, postmenopausal Caucasian control subjects (median age 56 years, range 48–70, and median BMI 32.0 kg/m^2, range 26.6–44.4) participated. Fasting blood samples were collected at 4-day intervals on 10 consecutive occasions. Plasma glucose was analyzed in singleton within 4 h of collection. Duplicate samples (i.e., two per visit) of stored serum were randomized and then analyzed (in a single continuous batch using a single batch of reagents) for SHBG and insulin (on a DPC Immulite 2000 analyzer; Euro/DPC, Llanberis, U.K.). The coefficient of variation for serum insulin and SHBG was 10.6% and 8.5%, respectively. The analytical sensitivity of the insulin assay was 2 μU/ml, and there was no stated cross-reactivity with proinsulin. All subjects were asked to have an unrestricted diet and instructed not to modify their eating patterns during the sampling period. The subjects were also advised to refrain from excessive physical exercise and alcohol before each fasting blood test.

The insulin resistance was calculated using the HOMA-IR method (HOMA-IR = [insulin × glucose]/22.5). Biovariability data were analyzed by calculating analytical, within-subject, and between-subject variances (5,6). The critical difference (i.e., the smallest percentage change unlikely to be due to biological variability) between two consecutive SHBG samples in an individual subject with type 2 diabetes was calculated using the formula $2.77(CV_I)$ (5), where CV_I is the within-subject biological coefficient of variation.

Figure 1 shows the mean and range of HOMA-IR and SHBG for the individuals in the two groups. In the type 2 diabetic group, SHBG concentrations were lower than those in the control subjects (mean ± SD; 38.8 ± 18.2 vs. 42.2 ± 17.1 nmol/l, $P = 0.001$), the insulin levels were higher (13.1 ± 5.4 vs. 9.42 ± 3.4 μIU/ml, $P = 0.0001$), and the HOMA-IR greater (4.33 ± 2.3 vs. 2.11 ± 0.79 units, $P = 0.0001$).

An inverse relationship was demonstrated between SHBG concentration and HOMA-IR in the group with type 2 diabetes ($r = -0.32$, $P = 0.001$) and in control subjects ($r = -0.28$, $P = 0.003$). The intraindividual variance of SHBG rose linearly with increasing SHBG concentrations ($r = 0.82$, $P = 0.0001$), and after accounting for analytical variation, the intraindividual variation of SHBG for the group with type 2 diabetes was similar to that seen in the control group (mean 2.35 vs. 2.44 nmol/l, $P = 0.93$). In contrast, the mean intraindividual variation of serum insulin (mean 2.38 vs. 1.45 μU/ml, $P = 0.016$) and HOMA-IR (mean 1.05 vs. 0.15 units, $P = 0.001$) was significantly greater in the group with type 2 diabetes than in the control subjects.

The critical difference between two consecutive SHBG samples in an individual patient with type 2 diabetes was 14.5% at any initial level of SHBG, indicating that a subsequent sample must rise

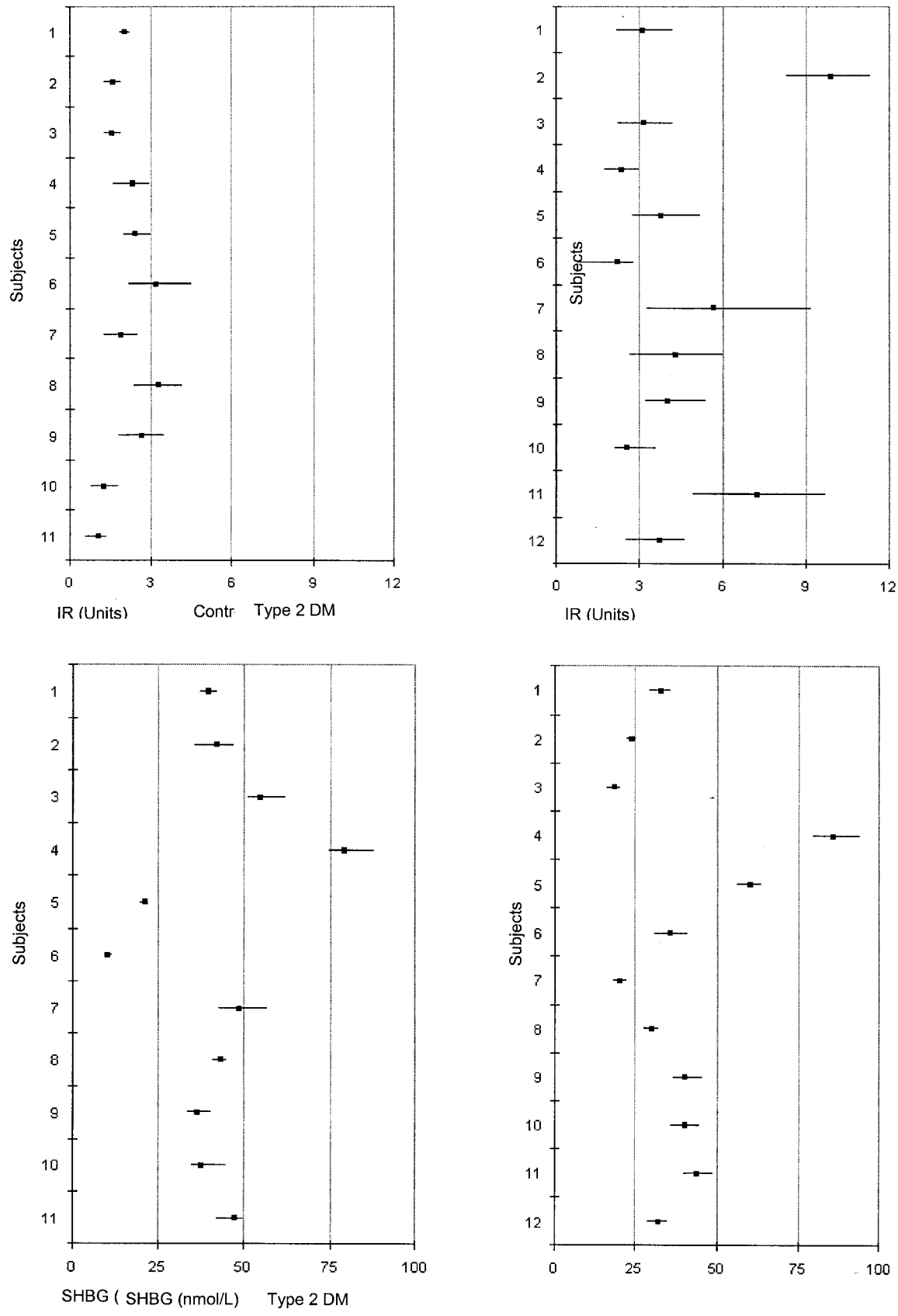

Figure 1— *Means (range) of insulin resistance and SHBG (unadjusted for analytical variation) in control subjects and type 2 diabetic subjects.*

or fall by >14.5% to be considered significantly different from the first.

The subjects with type 2 diabetes were hyperinsulinemic, insulin resistant, and demonstrated lower SHBG levels than control subjects. However, the more variable fasting insulin/insulin resistance in the subjects with type 2 diabetes was not reflected by similarly more variable SHBG readings compared with those of the control subjects. This suggests that a low SHBG concentration is a stable integrated marker of insulin resistance and therefore has the characteristics to be potentially used as a surrogate measure of insulin resistance, perhaps in monitoring the response of an individual to insulin sensitizers. However, although SHBG levels differed significantly between those with and without diabetes, the absolute mean difference was small, indicating that measurement of SHBG cannot be used as a simple test for insulin resistance in diabetes. A much larger study is required to investigate whether diagnostic cutoff values for low SHBG concentrations and insulin resistance in type 2 diabetes can be established. Without these parameters, the utility of a low SHBG concentration as a reflection of insulin resistance in type 2 diabetes will be for the serial monitoring of insulin resistance in individuals on treatment after the presence of insulin resistance has been established by conventional means. The low variation of SHBG compared with insulin resistance is likely due to the inherent temporal volatility of insulin and glucose levels as compared with SHBG. In conclusion, in the evaluation of serial measurements of SHBG concentration for an insulin-resistant individual with type 2 diabetes, such as before and after therapeutic intervention, the critical difference value of 14.5% reported here will identify whether any change is beyond that of natural biological variation and therefore a true response.

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Development of an Assessment Tool for Screening Children for Glucose Intolerance by Oral Glucose Tolerance Test

The American Diabetes Association (ADA) has recommended screening for type 2 diabetes by fasting plasma glucose (FPG) in children who are overweight (BMI >85th percentile) who have two of the following risk factors: at-risk ethnic minority origin, family history of diabetes in a first- or second-degree relative, or insulin resistance (acanthosis nigricans, polycystic ovarian syndrome, hypertension, or dyslipidemia) (1).

The case for refining the criteria for screening has been made previously (2). In that study, the sensitivity of the criteria was 24%, with a positive predictive value of 3%, i.e., 40 children needed to be tested to yield one abnormal result. In a response to this, Rosenbloom (3) highlighted the need to test the ADA criteria in high-risk populations to establish the strength and risk level of different factors that are influential in the development of type 2 diabetes.

Our institution covers a population where type 2 diabetes in childhood has emerged (4). We describe our experience of applying the ADA criteria and propose a clinical assessment tool to refine the selection of children for screening by the oral glucose tolerance test (OGTT).

In the last 4 years, 66 children have had OGTTs for suspected glucose intolerance. The characteristics of this population were mean age of 12.7 years (range 4.8–17.3), mean BMI standard deviation score 3.0 (0.0–4.6), 71% female, 83% ethnic minority origin (of whom 73% were South Asian and 9% African Caribbean), 88% had acanthosis nigricans, and 67% had a first- or second-degree family history of diabetes. Of these, 13 children had abnormal glucose tolerance (4 diabetes, 8 impaired glucose tolerance [IGT], and 1 impaired fasting glycemia).

Applying the ADA criteria, 11 of the 13 children with abnormal results would have qualified for screening, missing 1 child with diabetes and 1 with IGT. Screening these 11 with FPG as per the recommendations would have missed a further 7 children, 1 with diabetes and 6 with IGT, as only 1 of the children with IGT had impaired fasting glycemia. Overall, the sensitivity of the ADA criteria using FPG as a screening test in our population was 31%. Use of the ADA criteria to screen by OGTT would have identified 11 of the 13 abnormal results in this cohort, giving a sensitivity of 85% for the criteria and a specificity of 26%, with a positive predictive value of 22%, i.e., five children would need to be tested to yield an abnormal result.

Using the clinical characteristics of our cohort, we calculated the positive predictive value for each parameter singly and in combination. We used this data to weight each parameter and calculate a cumulative risk score, dividing children into low and high risk of abnormal glucose tolerance when tested by OGTT (Fig. 1). We then applied this risk score to our co-

						Score
Age (years) Points	<11 0	11+ 2				
Sex Points	Male 0	Female 3				
BMI SDS Points	<1 0	1 – 1.9 1	2 – 2.9 2	3 – 3.9 3	4+ 4	
Ethnicity Points	White UK 0	Asian or Mixed 2	African or Caribbean 3			
Any family history of type 2 diabetes Points (total 4)	Negative 0	Positive 2	Parent +1	Both sides of family +1		
Signs of insulin resistance Points (total 8)	Acanthosis Nigricans +2	Polycystic ovaries* +4	Hypertension† +2			
					Total	

Figure 1—*Assessment tool for the selection of children at risk of abnormal glucose tolerance. The assessment tool does not apply to children referred with raised fasting blood glucose values or with suspected glucose intolerance posttransplantation or secondary to other diagnoses. Children are scored on the clinical characteristics as shown. The scores for family history and insulin resistance are cumulative, giving a maximum score of 24. *Definite diagnosis on scan or irregular menses and hirsutism; †systolic or diastolic blood pressure >95th percentile for age (5). Scores: 0–11, low risk; 12–24, high risk (these children should be screened by OGTT).*

hort: 36 children scored as high risk, which included all 13 children with abnormal glucose tolerance. The sensitivity of this scoring system in our cohort is 100%, specificity 57%, and positive predictive value 36%, i.e., three children need to be tested to identify one abnormal result. Prospective validation of this assessment tool is now underway in our clinic. We suggest that other units develop risk assessment tools for their populations so that screening at-risk children for glucose intolerance becomes more feasible and is not "a lost battle."

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Vinegar Improves Insulin Sensitivity to a High-Carbohydrate Meal in Subjects With Insulin Resistance or Type 2 Diabetes

The number of Americans with type 2 diabetes is expected to increase by 50% in the next 25 years; hence, the prevention of type 2 diabetes is an important objective. Recent large-scale trials (the Diabetes Prevention Program and STOP-NIDDM) have demonstrated that therapeutic agents used to improve insulin sensitivity in diabetes, metformin and acarbose, may also delay or prevent the onset of type 2 diabetes in high-risk pop-

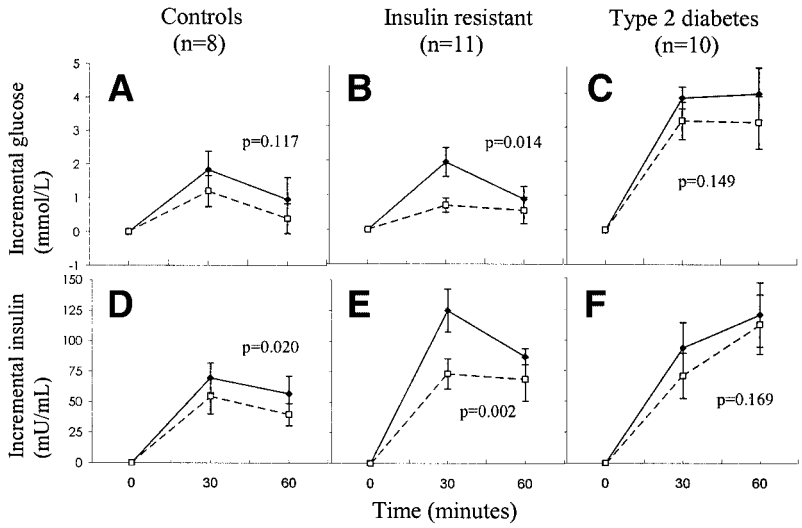

Figure 1—*Effects of vinegar (□) and placebo (◆) on plasma glucose (A–C) and insulin (D–F) responses after a standard meal in control subjects, insulin-resistant subjects, and subjects with type 2 diabetes. Values are means ± SE. The P values represent a significant effect of treatment (multivariate ANOVA repeated-measures test).*

ulations. Interestingly, an early report showed that vinegar attenuated the glucose and insulin responses to a sucrose or starch load (1). In the present report, we assessed the effectiveness of vinegar in reducing postprandial glycemia and insulinemia in subjects with varying degrees of insulin sensitivity.

Our study included nondiabetic subjects who were either insulin sensitive (control subjects, $n = 8$) or insulin resistant ($n = 11$) and 10 subjects with type 2 diabetes. Subjects provided written informed consent and were not taking diabetes medications. Fasting subjects were randomly assigned to consume the vinegar (20 g apple cider vinegar, 40 g water, and 1 tsp saccharine) or placebo drink and, after a 2-min delay, the test meal, which was composed of a white bagel, butter, and orange juice (87 g total carbohydrates). The cross-over trial was conducted 1 week later. Blood samples were collected at fasting and 30 and 60 min postmeal for glucose and insulin analyses. Whole-body insulin sensitivity during the 60-min postmeal interval was estimated using a composite score (2).

Fasting glucose concentrations were elevated ~55% in subjects with diabetes compared with the other subject groups ($P < 0.01$, Tukey's post hoc test), and fasting insulin concentrations were elevated 95–115% in subjects with insulin resistance or type 2 diabetes compared with control subjects ($P < 0.01$). Compared with placebo, vinegar ingestion raised whole-body insulin sensitivity during the 60-min postmeal interval in insulin-resistant subjects (34%, $P = 0.01$, paired t test) and slightly improved this parameter in subjects with type 2 diabetes (19%, $P = 0.07$). Postprandial fluxes in insulin were significantly reduced by vinegar in control subjects, and postprandial fluxes in both glucose and insulin were significantly reduced in insulin-resistant subjects (Fig. 1).

These data indicate that vinegar can significantly improve postprandial insulin sensitivity in insulin-resistant subjects. Acetic acid has been shown to suppress disaccharidase activity (3) and to raise glucose-6-phosphate concentrations in skeletal muscle (4); thus, vinegar may possess physiological effects similar to acarbose or metformin. Further investigations to examine the efficacy of vinegar as an antidiabetic therapy are warranted.

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Sleep Disturbance and Onset of Type 2 Diabetes

Sleep disturbance, which is often observed among patients with diabetes (1), is possibly caused by impaired glucose metabolism or physical and psychological discomfort due to the disorder. In addition, a recent prospective study of women has indicated an interesting association between sleep patterns and later-onset type 2 diabetes, with a greater incidence among both short-term (<6 h) and long-term (>8 h) sleepers (2). Disturbance in sleep quality may also affect the later onset of overt diagnosis of type 2 diabetes. We investigated the association between sleep disturbance and the subsequent onset of type 2 diabetes in a group of Japanese male employees.

We analyzed the database of an 8-year prospective study of male employees of an electrical company in Japan (3). We followed 2,649 male employees with no medical history of diabetes or other chronic illnesses at baseline for 8 years from 1984 to 1992. Data from 2,265 (86%) male respondents, who were thoroughly followed, were analyzed. All subjects received a medical checkup once a year during the follow-up to identify those with type 2 diabetes according to World Health Organization criteria (4). A mailed questionnaire was used to assess sleep disturbance in the previous month at baseline. Two single-item questions

were asked concerning difficulty initiating sleep ("Did you have trouble falling asleep?") and difficulty maintaining sleep ("Did you often wake up in the middle of the night?"). The subjects were classified into one of two categories: low for those who indicated "seldom" or "sometimes" and high for those who indicated "often" or "almost everyday" in response to the questions.

During the 18,006 person-year observation, 38 incidents of type 2 diabetes were identified (an incidence rate of 1.68 per 1,000 person-years). Those who experienced a high frequency of difficulty initiating sleep had a significantly higher age-adjusted hazard ratio (2.98, 95% CI 1.36–6.53) for type 2 diabetes compared with those who experienced low-frequency difficulty initiating sleep. A similar hazard ratio was observed for difficulty maintaining sleep (2.23, 1.08–4.61). These hazard ratios were almost identical and were statistically significant after controlling for other factors relevant to type 2 diabetes (i.e., age, education, occupation, shift work, BMI, leisure-time physical activity, smoking, alcohol consumption, and family history of diabetes).

Our analysis demonstrated that those who had sleep disturbances showed a two- to threefold higher risk of later onset type 2 diabetes. The association was independent of known risk factors for type 2 diabetes and was not attributable to treatment for sleep disturbance. Sleep disturbance at baseline was unlikely due to complications or disability from the treatment of diabetes because we excluded subjects with known diabetes at baseline. A possible explanation is that an increased sympathetic nervous activity associated with sleep disturbance (5) causes glucose intolerance (6) and increases the risk of type 2 diabetes. Physicians may need to pay more attention to patients with sleep disturbance because it may indicate a higher risk for type 2 diabetes.

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Non-HDL Cholesterol Contributes to the "Hypertriglyceridemic Waist" as a Cardiovascular Risk Factor

The Hoorn Study

Lemieux et al. (1) described the "hypertriglyceridemic waist" as a marker of the atherogenic metabolic triad (hyperinsulinemia, hyperapolipoprotein B, and small, dense LDL) in men. In 287 men, those with a waist circumference ≥90 cm and with triglyceride levels ≥2 mmol/l had an odds ratio of 3.6 (95% CI 1.17–10.93) for having angiographically diagnosed coronary artery disease compared with those with smaller waists and lower triglyceride concentrations. We recently reported (2) the predictive value of non-HDL cholesterol and triglyceride concentrations for 10-year cardiovascular disease incidence in the Hoorn Study, a population-based cohort study of glucose tolerance. In people with abnormal glucose metabolism, high triglyceride concentration was associated with the risk of cardiovascular disease, particularly in people with high non-HDL cholesterol, but not in those with normal glucose metabolism (3).

We used the Hoorn Study data to prospectively investigate whether the risk associated with the hypertriglyceridemic waist differs between subjects with normal and abnormal glucose metabolism. Additionally, we studied whether non-HDL cholesterol adds to the predictive power of the hypertriglyceridemic waist in predicting cardiovascular disease. The Hoorn Study is a cohort study among 2,484 subjects in the Netherlands that started in 1989. Cardiovascular disease was defined as first new cardiovascular fatal or nonfatal event (3). Because the cutoff points for waist girth in men used by Lemieux et al. (1) are not applicable for women, we used waist ≥94 cm in men and ≥80 cm in women, according to the European Group of Insulin Resistance definition (4). The results show that in particular the combination of a large waist and a high triglyceride level (≥2 mmol/l) was associated with cardiovascular disease in subjects with both normal and abnormal glucose metabolism (hazard ratio 1.82 [95% CI 1.27–2.62] and 2.68 [1.89–3.81], respectively). These findings concur with those of Lemieux et al. (1). In addition, we observed that after stratification for non-HDL cholesterol, defined as the difference between total cholesterol and HDL cholesterol concentration, the risk associated with the hypertriglyceridemic waist was further increased by 50% in the presence of high non-HDL cholesterol concentrations (above the median, i.e., 5.2 mmol/l for men and 5.3 mmol/l for women) (Fig. 1). The hazard ratio for subjects with a combination of large waist, high triglycerides, and high non-HDL cholesterol concentrations was 2.94 (2.06–4.19).

Non-HDL cholesterol is closely linked to visceral obesity (5). Non-HDL cholesterol includes all cholesterol in po-

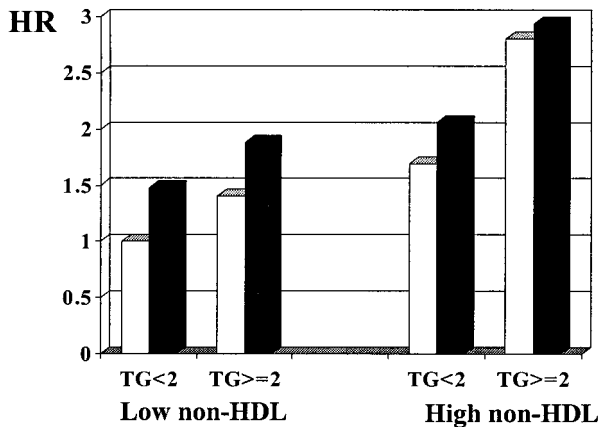

Figure 1—*Hazard ratios (HRs) of cardiovascular disease for combined categories of triglycerides and waist circumference, stratified for non-HDL cholesterol.* □, *waist <94/80 cm;* ■, *waist ≥94/80 cm.*

tentially atherogenic triglyceride-rich lipoproteins and may be a better predictor for the "bad" triglycerides, which are associated with increased risk of cardiovascular disease (6).

In conclusion, in the Hoorn Study, non-HDL cholesterol contributes considerably to the risk associated with the hypertriglyceridemic waist. Further studies are clearly required to evaluate the clinical relevance of monitoring these particular variables for the assessment of cardiovascular risk.

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Improvement of Temperature and Flow in Feet of Subjects with Diabetes With Use of a Transdermal Preparation of L-Arginine

A pilot study

Circulatory impairment and its sequlae have long been known to be major complications of diabetes. It has been shown that in diabetes, the functionality of the endothelial nitric oxide (NO)/nitric oxide synthase (eNOS) system is impaired (1–3). NO is generated in the endothelium through the oxidation of the amino acid L-arginine by the enzyme eNOS. NO causes vascular smooth muscle to relax, resulting in increased blood flow. In addition to being a substrate of eNOS, L-arginine facilitates the dimerization of two identical subunits, forming a homodimer. The enzyme is only active in the dimeric form. Under proper conditions, dimerization occurs rapidly, on a timescale of minutes. Once formed, the dimer is stable (4).

Subjects with diabetes have abnormally low levels of L-arginine (5) and elevated levels of the eNOS inhibitor asymmetric dimethylarginine (ADME) (6) in their plasma. Though the value of increasing L-arginine levels in cases of impaired circulation is now recognized, practical schemes for therapeutic use of L-arginine have been elusive. In this pilot study, we sought to determine whether supplying L-arginine transdermally would improve vascular function of the feet in patients with diabetes as indicated by flow and temperature.

The study was designed as a double-blind, vehicle-controlled, two-period, crossover protocol with washout periods of 1 week. Sixteen subjects were enrolled, and 13 completed the study (aged 56 ± 8 years). After analyzing the data, it was clear that the effect of L-arginine persisted throughout the washout periods (Tables 1 and 2). Because of this, except for the initial exposure of L-arginine virgin feet, the analysis was altered to determine the effect from cumulative exposure to L-arginine throughout the protocol. Flow was measured at the metatarsal and Achilles area using a Doppler flow meter (7), and temperature was measured at the metatarsal and big toe areas using an infrared thermometer. The active cream was a water-based moisturizing vehicle containing 12.5% L-arginine hydrochloride in a hostile biophysical environment comprised of high concentrations of choline chloride, sodium chloride, and magnesium chloride. The vehicle control was identical except that the L-arginine was omitted.

At the first visit, after baseline measurements were made each subject rubbed active cream (4 mg L-arginine/cm^2) into one foot and vehicle into the other. After 30 min, measurements were made again. A 1-week washout period followed. Patients returned after the

Table 1—*Effect of transdermal L-arginine cream on temperature*

	Metatarsal (°F)	*P* vs. visit 1	Big toe (°F)	*P* vs. visit 1
Visit 1	82.0 ± 2.3		74.4 ± 4.2	
Visit 2	84.1 ± 3.4	0.004	77.7 ± 5.3	0.01
Visit 3	87.0 ± 2.4	<0.0001	83.6 ± 4.9	<0.0001
Visit 4	86.1 ± 2.4	<0.0001	80.6 ± 5.4	<0.0001
Visit 5	86.9 ± 2.4	<0.0001	82.4 ± 4.8	<0.0001

Data are means ± SD.

washout period and flow and temperature measurements were made. They were then randomly given either active or placebo cream and told to rub it into their feet in the morning and evening every day for 2 weeks. At the end of 2 weeks, subjects returned and again measurements were made. A second 1-week washout period followed that third visit. At the end of that period subjects returned and measurements were made. They were given the crossover product and told again to rub it into their feet morning and evening for 2 weeks. The subjects returned for final flow and temperature measurements at the end of that period.

At the first visit, flow was increased at the Achilles area in the foot with active cream from 8.1 ± 3.3 to 11.5 ± 5.5 AU ($P = 0.05$) 30 min after application. In the foot that received placebo cream, flow failed to increase (8.1 ± 1.4 vs. 8.3 ± 2.2 AU). Furthermore, at the last visit the temperature at the metatarsal area had risen from the initial value of 82.0 ± 2.3 to 86.9 ± 2.4°F ($P < 0.0001$), and the temperature of the big toe had risen from the initial visit value of 74.4 ± 4.2 to 82.4 ± 4.8°F ($P < 0.0001$). And at the last visit the flow at the metatarsal area had risen from 8.7 ± 4.3 to 11.6 ± 5.5 AU ($P < 0.0001$), and flow at the Achilles area had risen from 8.4 ± 2.5 to 11.4 ± 5.5 AU ($P = 0.02$). While the failure of the L-arginine effect to wash out removed the opportunity for placebo control, the improvement in temperature and flow were substantial and highly statistically significant. Though puzzling, one explanation of the persistence of the L-arginine effect is that the local tissue concentration of L-arginine becomes high enough to cause inactive monomers of eNOS to form active dimers.

We conclude that in the patients we studied with diabetes, treatment of their feet with a transdermal preparation of L-arginine improved both flow and temperature, and this effect was surprisingly long lasting. Such improvement of compromised local blood flow should be beneficial and could reduce the complications of the disease.

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Table 2—*Effect of transdermal L-arginine cream on flow*

	Metatarsal (AU)	*P* vs. visit 1	Achilles (AU)	*P* vs. visit 1
Visit 1	8.7 ± 4.3		8.4 ± 2.5	
Visit 2	10.8 ± 5.9	NS	8.5 ± 3.9	NS
Visit 3	10.8 ± 4.8	0.05	9.2 ± 3.9	NS
Visit 4	11.6 ± 8.3	NS	10.0 ± 4.2	0.06
Visit 5	11.6 ± 5.5	<0.0001	11.4 ± 5.5	0.02

Data are means ± SD.

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Increased Prevalence of Enteroviral RNA in Blood Spots From Newborn Children Who Later Developed Type 1 Diabetes

A population-based case-control study

Virus infections during fetal life may lead to persistent infections due to unresponsiveness of the immature immune system and by different mechanisms inducing autoimmunity in the β-cell (1). In a previous study, we found group-reacting antibodies to enterovirus more frequently increased in serum at delivery in a cohort of mothers whose children later developed diabetes than in control subjects (2). It has been argued that mothers of later diabetic children might have increased immune responses to certain viruses (3). Therefore, detection of the virus nucleic acid would be important to confirm.

Enteroviral infections typically occur as epidemics with a short viremic phase; therefore, they are detectable for only a short time in peripheral blood (4). Consequently, large series of blood or serum samples would be necessary. Since 1973, blood spots routinely taken on days 2–4

of life for analysis of inherited metabolic diseases in all newborns in Sweden are stored in a biobank. From this biobank, we collected blood spots from 600 children in the Swedish childhood diabetes register who were born during the years 1986–1995 and who had diabetes onset before 1996. For each case, a control sample was included from a child born on the same date and not found in the Swedish childhood diabetes register, and the control sample was stored adjacent to the case filter.

Nested enterovirus PCR was performed essentially according to Puig et al. (5). To exclude the possibility of false-positivity due to contamination, we excluded all case-control pairs with double positivity and each step of RNA extraction and analysis included positive and negative controls. All case-control pairs were analyzed in the same run. As references, representatives of the most common congenital viral infections were chosen for analysis: CMV (6) and parvo B19 virus (7). A total of 542 pairs of samples were valid and analyzed for enterovirus RNA.

Twenty-seven diabetic cases were RNA positive, as compared with 14 control subjects (odds ratio 1.98 [95% CI 1.04−3.77], two-tailed $P = 0.04$). Due to limited material, typing of enteroviral RNA by sequencing was not possible . For CMV and parvo B19 viruses, no differences were shown between cases and references in samples of 208 and 180 pairs, respectively.

The findings support the hypothesis that early enteroviral infections may play a role in the pathogenesis of type 1 diabetes. In our previous study of maternal sera, the etiological fraction of enteroviral infection in pregnancy (4) was calculated at 27%. Thus, early fetal or neonatal infections may explain a fraction of childhood diabetes cases.

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Physician Attitudes Toward Foot Care Education and Foot Examination and Their Correlation With Patient Practice

Foot complications are one of the most serious causes of morbidity, disability, poor quality of life, and resource use among diabetic people (1). The adoption of preventive strategies to reduce the rate of foot problems thus represents an important priority. In fact, a strategy that includes prevention, patient and staff education, multidisciplinary treatment of foot complications, and close monitoring has been demonstrated to be very effective in reducing amputation rate (2).

In the context of a nationwide outcomes research program (the QuED project), we investigated several aspects related to foot care in 3,564 patients with type 2 diabetes enrolled by 125 diabetes outpatient clinics and 103 general practitioners.

Details on study design have been reported elsewhere (3). Briefly, all patients with type 2 diabetes were considered eligible, irrespective of age, duration of diabetes, and treatment. Foot complications included ulcers, gangrene, nontraumatic amputations, and claudicatio intermittens.

Patients filled out a questionnaire investigating whether they had received information about foot care, how often they had had their feet examined in the last year, and how often they usually checked their feet. Analyses were adjusted for patient case mix and physician-level clustering using multivariate multilevel logistic regression models (4).

The prevalence of lower limb complications was 6.8%. Seventy-two percent of the patients declared that they had received foot education, but only 49% reported that they had had their feet examined in the last year. Patients with ≤5 years of school education (odds ratio [OR] 1.3, 95% CI 1.1–1.6) and those with low income (≤$12,000) (1.2, 1.0–1.4) were more likely not to receive foot education. The presence of foot complications, peripheral vascular disease, cardiac-cerebrovascular disease, and diabetic neuropathy were not independently associated with a greater chance of receiving foot education. Foot examination was more likely to be performed in low-income patients (1.3, 1.1–1.6) and in those with foot complications (1.5, 1.1–2.1) but not in those with diabetic neuropathy, peripheral vascular disease, or cardiac-cerebrovascular disease. Foot examination tended to be performed less frequently by general practitioners and other specialists in diabetes outpatient clinics as opposed to diabetologists, even though the statistical significance was reached only for the comparison between

general practitioners and diabetologists (0.6, 0.4–1.0).

Overall, 33% of the patients declared that they never checked their feet. Patients who had received foot education (OR = 2.5, 95% CI 2.0–3.0) and those who had had their feet examined by their physician (1.7, 1.4–2.0) were more likely to check their feet regularly. Similarly, patients with foot complications (2.2, 1.5–3.2), but not those with peripheral vascular disease, cardiac-cerebrovascular disease, or diabetic neuropathy, were more likely to check their feet.

In conclusion, the attention to foot complications is generally poor, and a substantial proportion of type 2 diabetic patients is not offered foot education and examination, even in those subgroups showing a significant increase in the risk of foot complications. Even in the presence of foot complications or major risk factors, one-quarter of the patients did not pay any attention to foot care. Those patients who had received foot education and had had their feet examined were significantly more likely to regularly check their feet. This finding underlines the crucial role of physicians in orienting patient practices.

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Hepatocyte Growth Factor in the Vitreous Fluid of Patients With Proliferative Diabetic Retinopathy

Its relationship with vascular endothelial growth factor and retinopathy activity

The role of hepatocyte growth factor (HGF) in the etiopathogenesis of proliferative diabetic retinopathy (PDR) remains to be elucidated. Others and we (1–5) have found high intravitreous concentrations of HGF in patients with PDR. However, in these previous reports, the main confounding factors that could lead to misinterpretation of the results (vitreous hemorraghe, intravitreal protein concentration, and plasma HGF levels) have not been fully considered. In the present study, we consider all these confounding factors in order to evaluate the vitreous levels of HGF in patients with PDR and to investigate its relationship with vascular endothelial growth factor (VEGF) and retinopathy activity.

A total of 28 diabetic patients with PDR, in whom a vitrectomy was performed, were included in the study. Thirty nondiabetic patients with other conditions requiring vitrectomy but in whom the retina was not directly affected by neovascularization served as a control group. Patients in whom intravitreous hemoglobin was detectable by spectophotometry were excluded. HGF and VEGF were determined by enzyme-linked immunosorbent assay (R&D Systems, Abingdon, U.K.). The results are expressed as the median and range.

Vitreal levels of both VEGF and HGF were higher in diabetic patients with PDR than in the control group (1.34 [0.16–6.2] vs. 0.009 ng/ml [0.009–0.003] and 19.38 [0.4–80] vs. 6.04 mg/ml [1.8–17.34], respectively, $P < 0.0001$). These differences remained highly significant after adjusting for serum levels ($P < 0.0001$). To explore the influence of the breakdown of the blood-retinal barrier and, in consequence, the increased serum diffusion that occurs in PDR patients, the levels of both HGF and VEGF were normalized for total vitreal protein concentration. After correcting for total vitreous protein concentration, the ratio of VEGF-to-vitreal proteins remained significantly higher in diabetic patients with PDR than in the control group (0.34 [0.01–2.3] vs. 0.01 ng/mg [0.003–0.03], respectively, $P < 0.0001$). However, the ratio of HGF-to-vitreal proteins was lower in diabetic patients than in nondiabetic control subjects (5.03 [2–20] vs. 7.52 ng/mg [1.9–26], $P = 0.02$). The lower intravitreous levels of HGF obtained after correcting for intravitreal proteins in patients with PDR in comparison with nondiabetic control subjects suggest that serum diffusion largely explains the differences detected in the intravitreous HGF levels between these groups.

The vitreous concentrations of VEGF were higher in patients with active PDR than in patients with quiescent PDR (1.89 [0.2–6.2] vs. 0.78 ng/ml [0.1–1.7], respectively, $P = 0.004$). By contrast, vitreous HGF was not related to PDR activity (active vs. quiescent, 17.1 [7.3–46.6] vs. 23 ng/ml [0.4–80], respectively, $P =$ NS). Previous studies have found higher HGF concentrations in patients with active PDR than in those with quiescent PDR (1,2). However, after carefully considering the main confounding factors that could lead to a misinterpretation of the results, we did not observe any relationship between PDR activity and intravitreous HGF concentrations.

Finally, we did not find a relationship between intravitreous levels of HGF and VEGF. This was true in absolute terms and after normalizing for vitreal proteins. The different response of HGF and VEGF to hyperglycemia (6) and hypoxia (7) could explain the lack of relationship detected between these growth factors in the vitreous fluid of patients with PDR.

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Autoimmune Hypoglycemia in a Type 2 Diabetic Patient With Anti-Insulin and Insulin Receptor Antibodies

There are two types of autoimmune hypoglycemia, one due to autoantibodies acting against the insulin receptor and the other due to autoantibodies acting against insulin itself in individuals who have or have never received exogenous insulin, respectively (1). Both types are rare and can produce fasting and postprandial reactive hypoglycemia.

A 72-year-old woman with frequent severe hypoglycemia was admitted to the emergency room, presenting with loss of consciousness. Three weeks before her admission, she was diagnosed with diabetes and received insulin at a local hospital. In the emergency room, her blood glucose level was 40 mg/dl. She had been in good general health, except for hypertension for 30 years and postmenopausal osteoporosis 10 years before admission. She has no evidence of other diseases associated with altered immunity.

Three weeks ago, her biceps tendon ruptured when she slipped and fell, and during treatment, her blood glucose levels were >400 mg/dl. She was treated with insulin. However, she stopped insulin treatment because of frequent hypoglycemic events. Although she had had intravenous glucose injections, she had frequent hypoglycemic attacks, such as disorientation, loss of consciousness, palpitation, and diaphoresis. Her blood glucose levels had been <40 mg/dl on every hypoglycemic event, especially during fasting hypoglycemia.

Physical examination revealed normal vital signs except for a chronically ill appearance. Her HbA_{1c} was 6.3% (range 3–6%), plasma glucose 40 mg/dl, insulin 103.7 μU/ml, C-peptide 4.1 ng/ml, GAD autoantibody levels 0.01 units/ml (normal range 0–1.45 units/ml; RSR, Cardiff, U.K.), and insulinoma-associated protein 2 autoantibody 0.01 units/ml (normal range 0–1.1 units/ml; RSR). Her thyroid, liver, and adrenal function studies were normal. She was anemic, with a hemoglobin level of 9.2 g/dl. Her creatinine level was 0.8 mg/dl. Tests for anti-nuclear antibody, anti-DNA antibody, anti-smooth antibody, and anti-microsomal antibody were all negative. Insulin antibody levels were 58.5% (nonspecific binding, normal range <7%, measured by radioimmunoassay [Cobra 5010; Biosource Europe, Nivelles, Belgium]), and she was positive for insulin receptor antibodies (measured by radioreceptor assay [LKB 1261; BML, Tokyo, Japan]).

She was prescribed glucocorticoids and glucose tablets. Treatment with prednisone and glucose tablets was accompanied by the resolution of hypoglycemic episodes within 48 h. Six months later, her insulin antibody level was 67%, and she was negative for insulin receptor antibodies.

Most cases of insulin autoimmune hypoglycemia described in Asian races (2,3) have shown a strong correlation with certain HLA systems, suggesting the existence of a predisposing genetic component. This subject's HLA typing result was HLA-DRB1*. Autoimmune hypoglycemia is associated with certain HLA systems, such as DR4 and DQw3, and especially DRB1*0406/DQA1*0302/DQB1*0302. There have been 190 cases of insulin autoimmune syndrome reported over the past 20 years. It is noteworthy that HLA-DRB1*0406 is quite prevalent in Japanese patients (2,3). Our patient's HLA typing is predisposed to autoimmune hypoglycemia.

Several types of insulin antibodies have been reported and are most frequently seen in patients who receive insulin injections, but there have also been reports of them in nondiabetic patients with such autoimmune disease. Postprandial hypoglycemia is more common with

this syndrome than fasting hypoglycemia (1), and the course of this condition is benign and self-limited, with remission usually occurring within 1 year.

The insulin receptor antibody is associated with the inhibition of insulin binding to insulin receptors, accelerated receptor degradation, receptor downregulation, and extreme insulin resistance and hyperglycemia (4). Insulin receptor antibodies act as agonists or antagonists to the insulin receptor. Insulin receptor antibodies may also inhibit insulin binding, thereby inhibiting insulin clearance and elevating levels of plasma insulin. The most important laboratory test in autoimmune hypoglycemia is a direct assay for the presence of antibodies directed against the insulin receptor or insulin.

Patients with this condition have low circulating insulin, C-peptide levels, and refractory hypoglycemia. Antibody titers generally decrease over time and remission eventually occurs in most patients. However, because of the severity of the hypoglycemia, aggressive treatment is indicated. High-dose glucocorticoids, plasmapheresis, and alkylating agents have been tried with varying success (5).

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COMMENTS AND RESPONSES

Phenotypic Heterogeneity and Associations of Two Aldose Reductase Gene Polymorphisms With Nephropathy and Retinopathy in Type 2 Diabetes

Response to Wang et al.

Wang et al. (1) recently examined aldose reductase as a susceptibility gene for diabetic nephropathy among type 2 diabetic Chinese in Hong Kong. Although there was a small increase in the frequencies of the risk alleles of the $(CA)_n$ dinucleotide repeat and C-106T polymorphisms, analysis of the genotype distribution failed to detect any significant association between these polymorphisms and diabetic nephropathy (1). This negative result persisted despite confining the statistical analyses to control subjects who were normoalbuminuric with at least 5 years of known diabetes duration and case subjects with both diabetic nephropathy and concomitant diabetic retinopathy.

By and large, this study does not confirm earlier findings, which had implicated aldose reductase as a genetic risk factor for diabetic nephropathy among Caucasians with type 1 diabetes (2). Although the two studies were done on patients with different types of diabetes, drawn from separate human populations, which may arguably provide a basis for the discordant findings, a distinct possibility relates to the differential definitions of diabetic nephropathy. In this Boston study, diabetic nephropathy was defined on the basis of persistent proteinuria, i.e., ≥1+ on Multistix or albumin-to-creatinine ratio (ACR) ≥300 mg/g, or end-stage renal disease due to diabetic nephropathy (2). In contrast, the definition used in the current Hong Kong study was less stringent and included patients with microalbuminuria (albumin excretion rate ≥20 μg/min or ACR ≥3.5 g/mmol) (1). This latter criterion poses some concern in terms of misclassification because regression of micro- to normoalbuminuria is likely to be a common phenomenon, as recently demonstrated in type 1 diabetic patients (3). In genetic epidemiological studies, such misclassification can diminish the power of a study to detect an association. Therefore, the study by Wang et al. does not negate the hypothesis that aldose reductase could be a susceptibility gene for advanced diabetic nephropathy in type 2 diabetes. This possibility might be addressed by reanalyzing their data using a stricter definition based on advanced diabetic nephropathy.

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Phenotypic Heterogeneity and Associations of Two Aldose Reductase Gene Polymorphisms With Nephropathy and Retinopathy in Type 2 Diabetes

Response to Ng et al.

We thank Ng et al. (1) for their response to our recent article on the associations of two aldose reductase gene polymorphisms, a (CA)n microsatellite at the 5′ region and a promoter C/T polymorphism with nephropathy and retinopathy in type 2 diabetes (2).

However, we hold the view that Ng et al. have misinterpreted our data and inadvertenly commented that our "negative" results were related to our less stringent definitions of nephropathy, when in fact, we have provided clear evidence to show that both the z-2 and T allele of the aldose reductase gene polymorphisms were risk factors for diabetic nephropathy in Chinese type 2 diabetic patients.

In the consecutive cohort analysis involving all 738 type 2 diabetic patients, those with the T allele had higher albuminuria than noncarriers (30.2 vs. 21.9 μg/min) (2). This difference remained significant after adjustment for age, duration of disease, blood pressure, and HbA_{1c}.

We then excluded patients with a short duration of disease (<5 years) and used a case-control study design to further test the hypothesis. We defined case subjects as diabetic patients with both diabetic retinopathy and nephropathy, whereas patients who had no complications were selected as control subjects. Using this design, we found that both z-2 (odds ratio 2.64) and T alleles (odds ratio 2.48) were independent risk factors for the coexistence of diabetic nephropathy and retinopathy. The other predictors were age, blood pressure, HbA_{1c}, triglyceride, and male sex (2).

Hence, contrary to the comments made by Ng et al. that we failed to confirm results from previous studies on aldose reductase as risk genotypes for diabetic nephropathy in type 1 diabetes, our study has indeed provided corroborative evidence to support these findings.

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