OBSERVATIONS

Ketosis-Onset Diabetes Without Islet-Associated Autoantibodies in a Patient With MELAS

It has been reported that excessive intake of sugar-containing soft drinks can result in diabetic ketoacidosis or ketosis in obese patients with type 2 diabetes (1). We describe herein soft drink ketosis–onset diabetes in a nonobese patient with mitochondrial myopathy, lactic acidosis, and stroke-like episodes (MELAS).

A 27-year-old Japanese man consulted a local otolaryngeal clinic with a history of tinnitus lasting for 3 weeks and a weight loss of 5 kg in 1 month. He was diagnosed as having sensorineural and apoplectiform deafness and was administered 15 mg prednisolone a day, after which he developed thirst and polydipsia and started drinking 1–3 l of sugar-containing soft drinks a day. After 1 week of prednisolone therapy, he was referred and admitted to our hospital with severe malaise on 6 December 1999. His mother also had diabetes, which was effectively controlled with nateglinide, and sensorineural deafness. The patient's height was 165 cm, and his weight was 44 kg (BMI 16.2 kg/m^2). He was lucid and of normal mental clarity. His urine was glucose positive, his blood glucose level was 28.2 mmol/l, and his HbA_{1c} was 8.6%. Blood gas analysis revealed metabolic acidosis (pH = 7.337, base excess = −3.0 mmol/l, and HCO_3 = 22.8 mmol/l). His urine was strongly positive for ketones, and the serum level of total ketone bodies was 2,232 μmol/l, of 3-hydroxybutylic acid 1,774 μmol/l, and of acetoacetate 458 μmol/l. Serum levels of lactate and pyruvate were 3.0 mmol/l and 167 μmol/l, respectively. The patient was negative for islet-associated autoantibodies, including anti-GAD antibody, anti–IA-2 antibody, islet cell antibody, and insulin autoantibody, and specific HLAs for type 1 diabetes in Japanese people. We diagnosed the patient as having diabetic ketoacidosis and treated him with continuous insulin infusion. After good glycemic control was achieved with intensive insulin therapy (38 U/day), urinary excretion of C-peptide was 8.28 μmol/day, and basal serum level of C-peptide was 0.46 nmol/l. His serum levels of C-peptide responded well to glucagon and arginine challenges, which achieved 1.08 and 2.81 nmol/l, respectively. The maternal transmission of his sensorineural deafness and diabetes prompted us to examine him for mitochondrial diabetes. A fragment of mitochondrial DNA containing nucleotide 3243 was amplified by means of a polymerase chain reaction using a rhodamine-labeled primer and the product was digested with *Apa*I. The digested product was then separated by acrylamide gel electrophoresis and each product was quantitated with the aid of a fluorescence analyzer. The ratios of heteroplasmic A-to-G mutation of the mitochondrial $tRNA^{Leu(UUR)}$ gene at position 3243 were 26% in the leukocytes and 66% in the biceps brachii muscle. Systemic skeletal muscle atrophy was evident and his gripping power was weak (right 24 kg, left 22 kg). Exercise at 15W for 15 min, as measured with an ergometer, increased serum lactate and pyruvate levels from 2.2 mmol/l and 158 μmol/l to 4.4 mmol/l and 183 μmol/l, respectively. Histological examination with Gomori-trichrome staining of a biopsy specimen from the biceps brachii muscle showed a moderate variation in fiber size and ragged red fibers (5–10%), indicating mitochondrial myopathy. On the 26th day of admission and just after lunch, the patient experienced a stroke-like episode, consisting of a sudden convulsion followed by syncope for 10 min. Blood gas analysis showed severe metabolic acidosis (pH = 7.108, base excess = −15.1 mmol/l, and HCO_3 = 14.2 mmol/l). Slow waves at ~5 Hz were scattered in an electroencephalogram obtained while the patient was conscious. Brain atrophy and calcification of the basal ganglia were not evident on computed tomography scan of the brain. Single-photon emission computed tomography showed a paradoxical increase in the cerebellar blood flow, even though the mean cerebral blood flow was normal. On the basis of these findings, we diagnosed the patient as having MELAS.

Soft drink–induced ketosis is often associated with obesity or a history of obesity (1,2). Such patients can recover endogenous insulin secretion after initial intensive insulin therapy, after which their blood glucose can be effectively controlled with diet therapy only. Our patient, however, had a slim build and no history of obesity. He needed intensive insulin therapy to maintain good glycemic control even after the initial treatment, whereas insulin responses to nonglucose secretagogues, such as glucagon and arginine, were comparatively well maintained, as reported previously (3). Islet-associated autoantibodies and specific HLAs for type 1 diabetes in Japanese people, which are reported to be occasionally associated with mitochondrial diabetes (4,5), were not observed in our patient. Glucose toxicity caused by steroid therapy and excessive intake of sugar-containing soft drinks and the impaired insulin secreting ability caused by mitochondrial 3243 mutation seem to have caused ketoacidosis-onset diabetes in this case. Nonobese patients with diabetic ketoacidosis caused by excessive intake of sugar-containing soft drinks should, therefore, be examined for the presence of mitochondrial gene mutation.

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Stability of Disease-Associated Antibody Titers in Pregnant Women With Type 1 Diabetes With or Without Residual β-Cell Function

Pregnancy has wide-ranging effects on maternal metabolism and the maternal immune system to facilitate the growth of the semiallogenic fetus. Changes in the immune system appear to affect autoimmune processes, and a number of studies have documented clinical changes in autoimmune diseases during pregnancy, including rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus (1). Earlier studies have shown an increased incidence of type 1 diabetes onset with pregnancy (2). We wished to determine whether changes in the immune system, secondary to pregnancy, could mediate β-cell destruction. Therefore, we selected pregnant women with type 1 diabetes and residual β-cell function and followed them throughout their pregnancies, hypothesizing that changes in C-peptide levels and humoral autoantibody levels could reflect changes in β-cell–destructive activity. β-Cell proliferation and growth are part of the normal adaptive changes associated with pregnancy (3), and the increased insulin secretion induced by pregnancy could increase the vulnerability of the remaining β-cells to autoimmune attack.

The study enrolled 97 pairs consisting of mothers and their newborns, which included 40 mothers with type 1 diabetes, 21 of whom had detectable C-peptide levels, and 57 healthy control subjects. Serum samples and clinical data were collected during gestation, at delivery, and postpartum. C-peptide levels and antibody titers against the 65kD isoform of GAD65, insulinoma antigen 2 (IA-2), and insulin were measured at weeks 7–10, 25–28, and 34–36 of gestation, at delivery, and 6 weeks and 6 months postpartum using techniques previously described (4–6). Antibody levels were expressed as an index related to a positive and negative internal standard, and cutoff values for positivity were based on the 99th percentile indexes of the pregnant nondiabetic control group. Antibody positivity was determined using the serum sample obtained at delivery; when not available, the next available time point was used. Differences in mean values between groups were compared using either a 2-sample Student's *t* test or the Wilcoxon rank-sum test, and correlations were determined using the Spearman rank-correlation coefficient.

Table 1—*Maternal and newborn characteristics*

	Type 1 diabetic subjects		Healthy control subjects
	C-peptide positive	C-peptide negative	
n	21	19	23*
Age at inclusion (years)	27.0 (18–33)	29.0 (19–37)	31.0 (21–41)
Diabetes duration (years)	11.0 (2–28)†	18.0 (6–33)	N/A
C-peptide (ng/ml)			
First	0.30 ± 0.38	0	0.71 ± 0.45
Second	0.32 ± 0.41	0	1.7 ± 0.59
Third	0.39 ± 0.55	0	1.3 ± 0.74
Delivery	0.22 ± 0.18	0.1 ± 0.03	1.2 ± 0.62
B-glucose (mmol/l)			
First	6.5 ± 2.3	7.1 ± 0.5	4.8 ± 0.6
Second	6.9 ± 2.6	8.0 ± 2.9	4.8 ± 1.0
Third	6.4 ± 2.9	7.6 ± 2.9	4.9 ± 0.7
Delivery	7.4 ± 2.3	6.2 ± 2.0	5.1 ± 0.9
HbA_{1c} (%)			
First	5.5 ± 0.7‡	6.1 ± 0.9	N/A
Second	5.0 ± 1.1‡	5.6 ± 0.9	N/A
Third	5.2 ± 0.7	5.5 ± 0.8	N/A
Delivery	5.1 ± 0.7	5.8 ± 1.4	N/A
Insulin dose IE/kg BW (24 h)			
First	0.61 ± 0.31‡	0.84 ± 0.22	N/A
Second	0.77 ± 0.24§	1.1 ± 0.28	N/A
Third	0.98 ± 0.33	1.3 ± 0.47	N/A
Delivery	0.97 ± 0.43	1.0 ± 0.5	N/A
GADAb			
Index	0.21 ± 0.06	0.21 ± 0.05	0.00 ± 0.002
% Positive	48	42	1.8
IA-2Ab			
Index	0.14 ± 0.05	0.05 ± 0.04	−0.004 ± 0.0004
% Positive	40	26	0.0
InsAb			
Index	11.9 ± 6.5	7.6 ± 2.9	1.0 ± 0.04
% Positive	52	68	1.8

Data are *n* or means ± SD, except for age at inclusion and diabetes duration, which are expressed as medians (ranges), and antibody indexes, which are expressed as means ± SEM. Test points refer to first (weeks 7–22), second (weeks 18–33), and third (weeks 28–39) trimesters, and delivery. *For antibody studies, the control group also included individuals studied only at delivery with a total $n = 57$. †$P < 0.01$, ‡$P < 0.05$, §$P < 0.001$ when compared with type 1 diabetic mothers who were negative for C-peptide.

As shown in Table 1, the median disease duration in the group of type 1 diabetic mothers with residual C-peptide was significantly lower than in mothers without C-peptide. At the first- and second-trimester time points, women with C-peptide had significantly lower average insulin requirements and HbA_{1c} values compared with women without C-peptide (Table 1). However, these differences were no longer significant at delivery. The findings are in agreement with previous reports and confirm that the residual insulin secretion in these individuals maintained biological activity (7,8).

Antibody indexes and the frequency of antibody positivity at delivery for the groups are shown in Table 1. There were no significant differences at delivery between the groups positive and negative for C-peptide. As expected, there was a significant negative correlation

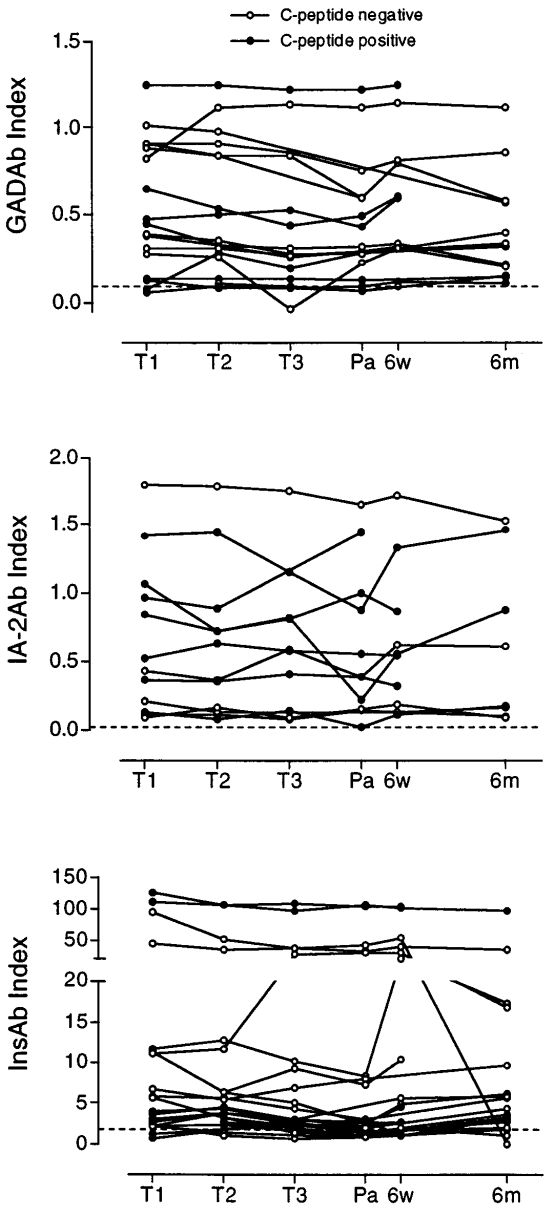

Figure 1—*Longitudinal measurements of diabetes antibody markers during pregnancy in women with type 1 diabetes.*

between the GAD antibody index at delivery and diabetes duration ($r = -0.35$, $P = 0.03$).

Longitudinal measurements of antibody levels for individual mothers classified as antibody positive are shown for each of the 3 antibodies in Fig. 1. There was no difference in mean antibody levels at any of the time points between the C-peptide–positive group and the C-peptide–negative group for any of the antibodies. Individual antibody levels for GAD65, IA-2, and insulin generally remained constant during pregnancy, and there were no significant differences in mean values between time points for any of the antibodies for either of the C-peptide groups. There appeared to be a small decrease in antibody titers at the third trimester, perhaps as a result of hemodilution during pregnancy.

This general stability in antibody levels throughout pregnancy and postpartum suggests that the mechanisms by which autoantibodies are produced continue unabated, despite the significant immunological changes occurring as pregnancy develops. Interestingly, there does not appear to be any effect of residual β-cell function on autoantibody levels in our study. The generation of autoantibodies may be independent of antigen levels in individuals with a long history of diabetes. Alternatively, these low levels of β-cell secretion, though biologically active, may not be sufficient to significantly alter the chronic autoimmune processes generating autoantibodies.

In the C-peptide–positive mothers, we examined the changes in C-peptide levels during gestation. Of the 21 mothers, 11 showed increases in C-peptide levels from the first to third trimester, whereas the remaining 10 showed decreases. The mean change in C-peptide levels for the 2 groups was $+0.32 \pm 0.15$ ng/ml and -0.19 ± 0.08 ng/ml, respectively. Comparing antibody indexes in mothers from the 2 groups found a higher mean insulin antibody index in women with decreasing C-peptide levels during pregnancy. This difference was statistically significant at partus ($P = 0.04$).

The absence of a generalized increase in C-peptide levels during pregnancy suggests that residual β-cell function in a number of these women does not follow traditional regulation. Moreover, in many patients the changes were small, putting into question their clinical significance. The significance of increased insulin antibodies in a subgroup of these women is unclear but may suggest that insulin antibodies at sufficiently high levels can be associated with impaired β-cell function.

In conclusion, this study demonstrates a general stability of autoantibody titers during pregnancy in women with type 1 diabetes. In women with residual β-cell function, pregnancy had varying effects on β-cell output, with approximately equal numbers of women showing increases and decreases in C-peptide levels from the first to third trimesters. We found no relationship between residual β-cell function during pregnancy and changes in autoantibody titers. This suggests that the increased risk of developing type 1 diabetes during pregnancy is not likely to be explained by enhanced autoimmune β-cell destruction but rather by an increased insulin demand caused by insulin resistance.

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Coxsackie B Virus–Induced Autoimmunity to GAD Does Not Lead to Type 1 Diabetes

Long-suspected environmental causes of insulin-dependent diabetes include a number of viruses. Among these viruses, Coxsackie B viruses are thought to play a prominent role (1) because of the striking sequence similarity between their P2-C protein and the β-cell autoantigen GAD65 (2), which is essential for the development of autoimmune diabetes in NOD mice (3,4) and antibodies and cellular immune reactions, which are among the earliest and most reliable predictive markers of type 1 diabetes in humans (5,6).

Cellular and humoral immunity to determinants common to GAD and Coxsackie B virus have been demonstrated in subjects with, or at increased risk for, type 1 diabetes (7,8). However, prospective investigation in people with Coxsackie B virus infections, although crucial to prove or disprove the hypothesis that molecular mimicry between viral antigens and autoantigens induces type 1 diabetes, are lacking.

We have prospectively followed 18 patients with acute Coxsackie B virus infections and evaluated humoral and cellular immunity to GAD peptides in relation to the eventual development of glucose intolerance or overt diabetes.

Thirteen subjects (5 girls, aged 5–14 years, mean age 9 years) had Coxsackie B4 virus–induced pharingitis (IgM anti-Coxsackie B4-positive) and 5 (1 girl, aged 9–15 years, mean age 13 years) had Coxsackie B1 virus–related meningitis (IgM anti-Coxsackie B1-positive).

Anti–GAD65 antibodies were determined by enzyme-linked immunoassay and radioimmunoassay (Nuclear Laser Medicine, Settala, Italy); T-cell proliferation (lymphocyte transformation) assays were performed as previously described (9) using as antigens the synthetic GAD peptides 247–266 and 260–279, which at positions 257, 260–265, 270, and 272–273 have identity with aminoacids 35, 38–43, 47, and 49–50 of Coxsackie virus P2-C protein. Anti-GAD antibodies were detected in 8 subjects with Coxsackie B4 and in 3 subjects with Coxsackie B1 infections. Cellular immunity to GAD peptides 247–266 was found in 6 individuals with Coxsackie B4 and in 2 with Coxsackie B1 infections, whereas T-cell reactivity to GAD peptides 260–279 was demonstrated in 3 subjects with Coxsackie B4 and in 3 with Coxsackie B1 infections. No individuals showed cellular immunity to both of the antigens, and all of the subjects with T-cells reactive to GAD peptides had anti-GAD antibodies.

Within 1 year after Coxsackie B virus infection, all of the subjects lost antibodies to GAD, and no one showed cellular immunity to GAD peptides. After a mean follow-up of 28 months (range 20–47 months), no individual has developed glucose intolerance or overt type 1 diabetes.

Our findings demonstrate that Coxsackie B virus infections are frequently associated with immune reactions to GAD, but such reactions are transient, and type 1 diabetes does not follow. Therefore, molecular mimicry between viral antigens and GAD is not involved per se in the immunopathogenesis of type 1 diabetes in humans, which is similar to recent suggestions in an animal model (10). For type 1 diabetes to develop, additional mechanisms (e.g., defects in the control of immune responses to GAD leading to perpetuation of such autoimmune reactions, autoimmune reactivity to other crucial pancreatic β-cell antigens) must, therefore, operate.

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Does the Choice of Treatment for Type 2 Diabetes Affect the Physiological Response to Hypoglycemia?

The risk of severe hypoglycemia is less in patients with type 2 diabetes compared with those with type 1 diabetes (1), but those treated with insulin suffer more severe hypoglycemic episodes than those treated with sulfonylureas (2).

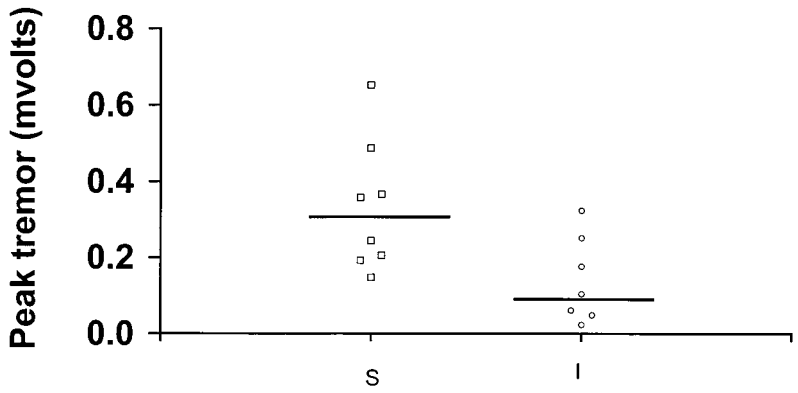

Figure 1—*Individual peak tremor responses (*$P < 0.02$*) and peak sweating responses (*$P < 0.02$*). Bar = median. S, sulfonylurea group; I, insulin group.*

This may be related to the more variable insulin levels of those on insulin, in contrast to the lower and more stable profiles of those on sulfonylureas (3). Alternatively, the risks may be related to the integrity of the physiological defenses to hypoglycemia. Because high insulin concentrations are associated with reduced endocrine responses to hypoglycemia (4), patients on insulin may acquire impaired counterregulatory defenses. We have tested the hypothesis that patients with type 2 diabetes treated with insulin have a diminished physiological response to hypoglycemia compared with those on sulfonylureas.

We approached patients participating in the U.K. Prospective Diabetes Study at 4 centers. None had ketonuria at diagnosis and all had been randomized within 3 months of diagnosis to receive either sulfonylurea or insulin therapy (2). Fifteen agreed to participate: 8 (2 women) sulfonylurea-treated (group S) and 7 (1 woman) insulin-treated patients (group I), age (mean ± SEM, S vs. I) 54 ± 2 vs. 48 ± 2 years, $P = 0.10$; duration of diabetes 7 ± 1 vs. 7 ± 1 years, $P = 0.85$; BMI, 28.1 ± 0.9 vs. 25.4 ± 1.4 kg/m^2, $P = 0.09$; and HbA_1, 8.3 ± 0.6 vs. 9.2 ± 0.5%, $P = 0.24$ (HbA_1 nondiabetic range −4.5 to 8.5%). We also recruited matched healthy control subjects (group C; $n = 9$), all men, age (mean ± SEM) 50 ± 4 years ($P = 0.41$), BMI 25.6 ± 1.3 kg/m^2 ($P = 0.13$).

All subjects underwent a stepwise hyperinsulinemic-hypoglycemic glucose clamp after overnight glucose control (5). Soluble insulin was infused at 120 mU · m^{-2} · min^{-1} with blood glucose maintained at 5.0, 4.0, 3.5, and 3.0 mmol/l for 40 min and 2.5 mmol/l for 20 min.

The mean arterialized venous whole-blood glucose concentrations, glucose infusion rates, and insulin concentrations were not significantly different between the 2 groups. Mean peak hormone responses above baseline (S vs. I) were as follows: glucagon 41 ± 7 vs. 54 ± 23 ng/l, $P = 0.56$; and epinephrine 4.9 ± 1.1 vs. 4.1 ± 1.2 nmol/l, $P = 0.65$. Glycemic thresholds for rises in glucagon were 2.6 ± 0.1 vs. 2.7 ± 0.1 mmol/l, $P = 0.53$; and epinephrine 3.9 ± 0.2 vs. 3.4 ± 0.1 mmol/l, $P = 0.07$. Mean peak tremor response (6) (S vs. I) was 0.33 ± 0.06 vs. 0.14 ± 0.04 V, $P < 0.02$ (Fig. 1); and sweating (7) (median [range]) was 171 (20 to 376) vs. 16 (−2 to 157) g · m^{-2} · h^{-1}, $P = 0.02$. Glycemic thresholds for increases in tremor or sweating were not different. There was a significant negative correlation between frequency of hypoglycemic episodes and peak tremor ($r = 0.6$, 95% CI, −0.11 to −0.86; $P < 0.02$) and sweating ($r = -0.51$, 95% CI, −0.82 to 0.02; $P = 0.05$). There was no difference in glucose threshold for deterioration in reaction time or symptom scores. No differences were found between control and diabetic subjects except for the threshold for epinephrine release (C vs. S) 3.2 ± 0.1 vs. 3.9 ± 0.2 mmol/l, $P < 0.05$; and area under the curve sweating response (C vs. I) 157 ± 55 vs. 10 ± 16 g/m^2; $P < 0.05$.

Our data show that counterregulatory responses were similar in type 2 diabetes patients randomized to either insulin or sulfonylureas, with comparable glycemic control. We found lower tremor and sweating response in those treated with insulin compared with both sulfonylurea-treated patients and control subjects. We also observed an inverse relationship between the frequency of reported hypo-

glycemia and tremor with a similar trend for sweating. Because symptomatic hypoglycemia was more frequent in those treated with insulin, these alterations in peripheral responses may relate to antecedent hypoglycemia in the period before the study (8). A differential impairment in the physiological response to hypoglycemia has been described previously and peripheral autonomic responses may be particularly sensitive to the effects of antecedent hypoglycemia (9).

Our data indicate that insulin-treated patients with type 2 diabetes do not have substantial impairments in counterregulatory responses, compared with sulfonylurea-treated patients, even when their glycemic control is good. The relatively small number of subjects limits the certainty of our conclusions. For example, although the glycemic thresholds for epinephrine release were not statistically different, the mean values were 3.9 and 3.4 mmol/l for those treated with sulfonylureas and insulin, respectively. However, although a true difference might have been established by studying more subjects, the control data indicate a high value for those on sulfonylureas rather than an impaired response for those taking insulin.

Previous studies examining the glucagon response in type 2 diabetes have found normal (10) and reduced responses (11). Glucagon responses were no different in either the 2 diabetes groups or control group. The somewhat attenuated glucagon response might be due to the high insulin levels, which were necessary to achieve hypoglycemia in insulin-resistant subjects. Insulin concentrations were ~200 mU/l, which have been associated with reduced glucagon response to hypoglycemia in other studies (4). The 2 diabetic groups were not perfectly matched in terms of BMI or HbA1, although we do not believe that this had a major effect on the data.

We conclude that the choice of treatment has no major impact on the physiological responses to hypoglycemia in patients with relatively well-controlled type 2 diabetes. Our data suggest that antecedent hypoglycemia might attenuate some physiological responses in patients with type 2 diabetes, although this hypothesis needs to be tested in a different study design.

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Hypoglycemia and Pulmonary Edema

A forgotten association

The association between hypoglycemia and pulmonary edema is well known, with a clear cause-and-effect relationship (1,2). However, only a few reports of this association have been published during the last few decades, so that the description of a new case may be relevant.

A 19-year-old woman was brought to the emergency room after an episode of generalized seizures. Diabetes had been diagnosed 4 years before. She was on a multiple insulin dose regimen, her metabolic control was poor (HbA_{1c} 8.1%, $n < 5.8$), and she had had an episode of severe hypoglycemia 3 years before; otherwise, the patient was in good health. On the day of admission, the patient had performed more physical activity than usual, had reduced the carbohydrate content of her dinner, but had not changed her insulin dose. At 2 A.M., her mother found her diaphoretic and comatose. A few seconds after being given some juice, the patient suffered a generalized seizure. Upon admission to the emergency room, the patient remained comatose, her plasma glucose level was 60 mg/dl, her blood pressure was 150/80 mmHg, her pulse was 160 bpm, and her respirations were 32/min. Physical examination revealed a bitten bleeding tongue, but the examination was otherwise unremarkable. The electrocardiogram showed a sinus rhythm. Arterial blood gases measurements were as follows: pH 7.36, PaO_2 46 mmHg, $PaCO_2$ 43.5 mmHg, and HCO_3 24.5 mEq/l with a saturation of 80% at room temperature. The chest X-ray showed typical signs of pulmonary edema and a normal heart shape and size. The patient was admitted to the intensive care unit where she improved steadily under oxygen therapy. There were neither symptoms nor clinical or analytical signs of infection. The patient left the inten-

sive care unit 24 h later with normal chest X-ray and blood gases.

The association of severe hypoglycemia and adult respiratory distress syndrome in a young woman with diabetes but otherwise in good health, in addition to the time course of the events, suggested a causal relationship. A search of the entire Medline database using the terms "hypoglycemia," "respiratory distress syndrome," and "pulmonary edema" provided 37 articles, of which only 6 were relevant and 5 were dated before 1976. However, these articles and some of their references revealed that pulmonary edema was a well-known complication of insulin shock therapy for psychiatric conditions, with a known presentation rate (~12%) and a mortality that was responsible for 16% of the deaths associated with this therapy (1,2). Pulmonary edema was an important item 34 years ago in the differential diagnosis of a patient with hypoglycemia and "a blood-pouring mouth" (3), but some years later, the association was described as "nearly forgotten" (4).

In this setting, pulmonary edema has been attributed to neurogenic mechanisms: sympathetic hyperstimulation induces lymphatic vasoconstriction and platelet aggregation, which causes microemboli (5,6). Both would lead to increased hydrostatic pressure in the pulmonary capillaries. In addition, because the integrity of the alveolocapillary membrane depends on glucose metabolism, in long-lasting hypoglycemias, its disruption could also contribute to the development of pulmonary edema (7).

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Hyperinsulinemia and Central Adiposity

Influence of chronic insulin therapy in type 1 diabetes

Obesity is well known to be associated with insulin resistance and consequential hyperinsulinemia (1,2). Moreover, further worsening of insulin resistance and greater degree of hyperinsulinemia are noted to be present in obese subjects with central fat distribution, as assessed by waist-to-hip ratio measurements >0.9 in men and >0.84 in women (1–3). The data in the literature suggest that central obesity may be responsible for hyperinsulinemia via the induction of insulin resistance (1–3). However, it is also conceivable that hyperinsulinemia itself may be responsible for central fat deposition, because insulin is known to inhibit lipolysis and facilitate lipogenesis. Therefore, to examine this hypothesis of the role of insulin in causing central fat deposition, we studied subjects with type 1 diabetes who have been receiving insulin exogenously for the management of their disease for several years.

The height, body weight, and waist and hip measurements were determined in 100 randomly chosen men with type 1 diabetes attending the Diabetes Clinic at the Veterans Affairs Medical Center in Phoenix, Arizona, during a 12-month period from July 1995 to June 1996. Their ages ranged between 22 and 70 years, and their duration of diabetes ranged between 2 and 37 years. The diagnosis of type 1 diabetes was established by documentation of several episodes of diabetic ketoacidosis during each subject's diabetes duration. All of the patients had received insulin throughout the duration of their diabetes since diagnosis, also confirming the presence of type 1 diabetes. There were 100 healthy nonobese male employees of the medical center who volunteered as control subjects, and they ranged in age from 20 to 60 years. They were nonobese, as established by a BMI of <27 kg/m^2. All waist, hip, and body weight measurements were conducted at the clinic by the same healthcare provider for all of the 200 subjects to reduce interpersonal technique variability and error. The waist circumference was measured at the level of the anterior superior iliac spine, and the hip circumference was measured at the widest part when viewed laterally, as described previously (1–3). Body weight and height were measured using a standard integrated portable scale. BMIs were determined in all of the subjects, and obesity was defined as a BMI >27 kg/m^2. Statistical analyses were conducted by Student's *t* test for comparison between subjects with type 1 diabetes and healthy volunteers. Simultaneously, relationships were assessed between obesity and central fat distribution on one hand and insulin therapy on the other by conducting a linear regression analyses. All data are expressed as means ± SEM.

Of the 100 subjects with type 1 diabetes, 43 were obese. Significantly higher ($P < 0.001$) waist-to-hip ratios were noted in subjects with type 1 diabetes (0.96 ± 0.012) in comparison with the accepted standard (<0.90) as well as the healthy volunteers (0.86 ± 0.007). Moreover, the waist-to-hip ratio was 0.98 ± 0.012 in obese subjects with a BMI of 34 ± 2 kg/m^2, whereas the waist-to-hip ratio was 0.93 ± 0.011 in lean subjects with a BMI of 24 ± 1 kg/m^2; both waist-to-hip ratio values were significantly greater ($P < 0.001$) in comparison with healthy volunteers. Thus, this pattern persisted in all type 1 diabetic patients irrespective of the presence or absence of obesity as reflected by BMIs. The daily insulin dose at the time of these measurements ranged between 15 and 70 U and was not significantly correlated with either BMIs or waist-to-hip ratios. However, a significant positive correlation was noted between BMIs and the duration of diabetes (i.e., duration of insulin therapy [$r = 0.46$, $P < 0.01$]). A similar, though even more significant relationship was noted between the waist-to-hip ratios

and duration of insulin therapy ($r = 0.61$, $P < 0.001$).

In conclusion, this study demonstrates that insulin therapy not only causes obesity in several subjects with type 1 diabetes, but also induces central fat deposition as reflected by rising waist-to-hip ratios even in the absence of obesity, as reflected by BMI. This finding is consistent with Diabetes Control and Complications Trial (4,5). A similar observation is also documented in subjects with type 2 diabetes receiving insulin therapy, especially in terms of weight gain, in the recently concluded U.K. Prospective Diabetes Study (6,7). Therefore, it is likely that insulin itself may be responsible not only for weight gain, but is also likely to induce central fat deposition in subjects with type 1 diabetes. Finally, it is likely that the central fat deposition after long-term insulin therapy may also be responsible for the insulin resistance noted in the later stages of the disease in subjects with type 1 diabetes.

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High Total Serum Renin Concentrations Are Associated With the Development of Background Retinopathy in Adolescents With Type 1 Diabetes

Serum total renin (1) and prorenin (2,3) have recently been proposed to be markers for identifying patients with type 1 diabetes who are at risk for developing diabetic nephropathy. However, the prognostic relevance of these factors for the development of diabetic retinopathy is still controversial (1,2,4). As part of the ongoing Berlin Retinopathy Study (5), we measured serum total renin concentrations in 21 adolescents and young adults with diabetes (mean age 20.1 ± 2.9 [SD] years; diabetes duration 12.3 ± 2.8 years) at the onset of early background retinopathy (11–50 microaneurysms or <25 leakages in fluoresceine angiography). Serum total renin represents both active renin and inactive prorenin, with the inactive prorenin component comprising at ~90% of total renin in normal subjects (6). We used a direct immunoradiometric assay (Nichols Institute Diagnostics; intra- and interassay coefficients of variation 2.8 and 5.8%, respectively), where total renin was measured after enhancement of immunoreactivity of prorenin by preincubation with the renin inhibitor remikren (7). Total renin concentrations were compared with those of 21 other patients without retinopathy, carefully matched one-by-one by age, sex, and diabetes duration (matched pairs). None of the patients had microalbuminuria (albumin excretion >20 µg/min). The annual means of HbA_{1c} (high-performance liquid chromotography, Diamat) and of blood pressure (measurements by DYNAMAP [Johnson & Johnson Medical,

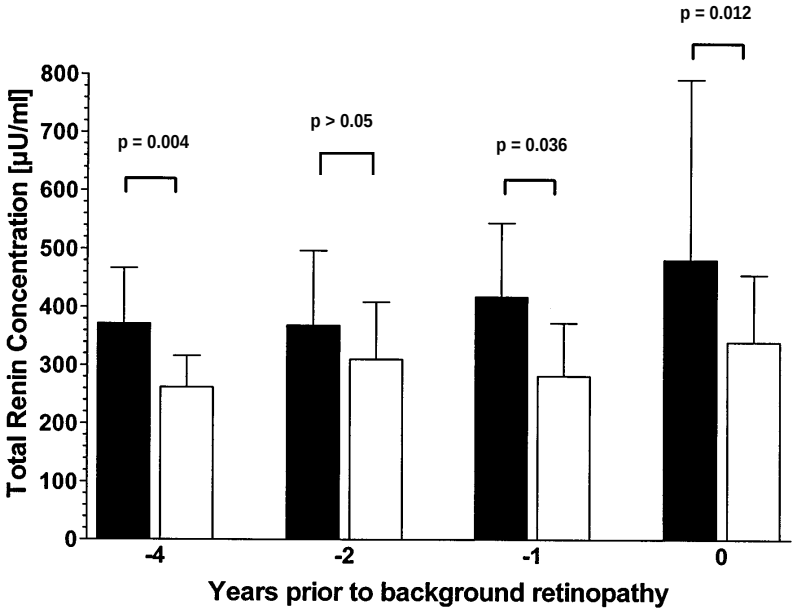

Figure 1—*Mean ± SD of total serum renin concentrations of 22 adolescents followed annually for the development of retinopathy. Patients developing background retinopathy (■) have significantly higher total serum renin concentrations than those remaining with normal retinal status (□) matched for age, sex, and diabetes duration up to 4 years before the detection of retinal changes.*

Norderstedt, Germany] in sitting position) were used for statistical evaluations. Serum total renin concentrations were significantly higher in patients developing early background retinopathy (482.6 ± 312.6 vs. 340.8 ± 115.9 μU/ml, $P = 0.012$, Wilcoxon's signed-rank test) compared with those without retinal changes. There was no correlation between serum total renin concentrations and age, duration of diabetes or glycemic control, or systolic and diastolic blood pressure before the development of retinopathy. Retrospective longitudinal follow-up was possible in 11 matched pairs. Total renin levels were significantly elevated up to 4 years before onset of retinopathy in patients developing this complication (Fig. 1).

In contrast to previous studies (1,2), we demonstrated that elevations in total renin concentrations could also precede the development of retinopathy in diabetes patients without microalbuminuria. These results support and extend the findings by Franken et al. (4), who reported an association between elevated prorenin levels and the development of proliferative retinopathy. Wilson and Luetscher (8) had also shown that elevated plasma prorenin levels precede the development of overt renal disease and retinopathy in adolescents, but they were not able to separate the development of renal or retinal disease in individual patients. The underlying explanation for the early rise of total serum renin, mostly without an elevation of active renin, in patients with diabetes developing late complications still remains unknown. Possibly it reflects a generalized alteration in the control of secretion and processing of renin in terms of a defect in intracellular processing with a consecutive increase of prorenin from the kidneys and other tissues expressing renin (9). Thus, serial measurements of serum total renin concentrations may serve as an additional noninvasive early marker for screening not only for nephropathy, but also for retinopathy in young patients with diabetes.

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Plasma Homocysteine and Its Determinants in Diabetic Retinopathy

Whether hyperhomocysteinemia contributes to the development of diabetic microangiopathy is still debated. Most of the older studies are difficult to interpret because of the insufficient characterization of the type of diabetes and status of complications. More recent works conducted on better-characterized patients are fairly consistent in showing a relation between hyperhomocysteinemia and diabetic nephropathy; however, it still remains unclear whether this association is causal (1–3). Furthermore, the relationship of plasma homocysteine with retinopathy is little explored to date. We contribute data on the relation of retinopathy with fasting plasma homocysteine and also explore some of the potential mechanisms of this association.

Sixty-nine patients with type 1 diabetes of ≥10 years' duration, consecutively seen on an outpatient basis, participated in the study. All of the patients were normotensive (blood pressure <140/90) and free from cardiovascular diseases as evaluated by the World Health Organization questionnaire, an electrocardiogram, and ankle/brachial pressure. To reduce to the minimum the confounding effect of renal injury, patients with macroalbuminuria and/or serum creatinine >1.3 mg/dl were excluded from the study. According to 45° fundus photography performed and evaluated following a standard protocol, participants were assigned to 1 of 3 groups: no retinopathy ($n = 34$), nonproliferative diabetic retinopathy (NPDR) ($n = 20$), or proliferative diabetic retinopathy (PDR) ($n = 10$). Plasma homocysteine was measured together with vitamin B_{12} and folate, the major environmentally determined factors influencing homocysteine metabolism. Furthermore, the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene, the most common genetic determinant of moderate hyperhomocysteinemia in the general population, was also studied (4).

Plasma homocysteine progressively increased with a significant linear trend ($P < 0.03$) from the stage of no retinopathy to the stage of PDR (7.3 ± 3.0 vs. 8.2 ± 2.6 vs. 9.5 ± 2.6 μmol/l, respectively). Post hoc Duncan's test indicated significantly ($P < 0.05$) higher levels of fasting plasma homocysteine in patients with PDR as compared with those without any sign of retinopathy. The 3 groups were not significantly different in terms of sex distribution, age, and smoking habits; blood pressure was also comparable (118 ± 14 vs. 118 ± 16 vs. 122 ± 11 mmHg, respectively) as were serum creatinine (0.8 ± 0.1 vs. 0.9 ± 0.1 vs. 0.9 ± 0.2 mg/dl) and creatinine clearance, evaluated by the Cockroft formula (98 ± 16 vs.

105 ± 21 vs. 91 ± 12). Furthermore, plasma concentrations of vitamin B_{12} and folate were also comparable in the 3 groups (405 ± 102 vs. 449 ± 141 vs. 430 ± 105 pmol/l and 14.5 ± 4.5 vs. 16.8 ± 5.6 vs. 13.8 ± 3.6 nmol/l, respectively).

The allelic frequency of the C667T mutation in MTHFR gene was similar in the group of patients with no retinopathy and NPDR (38 vs. 33%) but was significantly higher in the patients with PDR as compared with those with no retinopathy (75 vs. 38%, $P < 0.01$). Accordingly, the genotype distribution of the mutated gene was significantly different in the 2 groups with PDR or no retinopathy with a significantly higher frequency of homozygosity in patients with PDR (70 vs. 18%, odds ratio 10.3, 95% CI 1.7–69.8)

The data reported indicate a relationship between PDR and plasma homocysteine levels independent of some obvious confounders and coexisting conditions associated with the elevation of plasma homocysteine or retinopathy (i.e., cardiovascular disease, impaired renal function, vitamin status, and blood pressure). An association of diabetic retinopathy with the C677T mutation in the gene coding for the MTHFR, a key enzyme in the homocysteine catabolism, has been reported previously by Neugebauer et al. (5) in type 2 diabetic patients; in this study, however, plasma homocysteine was not measured. We subsequently reported moderate hyperhomocysteinemia in a small group of type 1 diabetic patients with retinopathy and normal serum creatinine, but the major determinants of homocysteine metabolism were not measured (6). To our knowledge, this is the first report that explores the relationship of plasma homocysteine and some of its major determinants with retinopathy.

Based on these data, a role for homocysteine in the development of PDR can be hypothesized, at least in selected groups of patients. This hypothesis is also supported by the results of in vitro experiments showing a synergistic effect of plasma homocysteine and hyperglycemia in inducing cell damage in the vascular endothelium (2). This study is also relevant inasmuch as it singles out a possible genetic marker for PDR. A genetic predisposition to retinopathy is not well documented; however, it is known that although almost the all of type 1 diabetic patients with longstanding diabetes develop some degree of retinopathy, relatively few progress toward PDR (7). To explain this finding, among others, genetic differences in response to hyperglycemia have been hypothesized, and according to emerging knowledge, homocysteine may also play a role. Of course, the cross-sectional nature of the study makes results compatible with the alternative hypothesis that higher plasma homocysteine levels may be a marker rather than a determinant of tissue damage in diabetic retinopathy, similar to what has been hypothesized in ischemia because of large vessels occlusions (8). However, it would be more difficult to explain on this basis the association of PDR with the C677T mutation in the MTHFR gene.

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Risk for Silent Celiac Disease Is Higher In Diabetic Children With a Diabetic Sibling Than in Sporadic Cases

Since the development of specific and sensitive markers of screening for celiac disease, such as IgA antiendomisium antibodies (EMA), increasing frequencies of typical and silent forms of the disorder have been reported in children and adolescents with type 1 diabetes (1,2). Recently, it has been estimated that the prevalence of celiac disease in diabetic Italian children is 5.5–6.7% (3,4), with respect to previous estimates of 2–3% based on the use of IgA-IgG antigliadin antibodies (AGA) (5,6). A possible explanation for the association between type 1 diabetes and celiac disease could be the involvement of the same susceptibility genotypes in the etiopathogenesis of these diseases. Indeed, it has been demonstrated that the genetic predisposition to increased immunity to dietary proteins is associated with HLA haplotype A1-B8-DR3 DQA1*0501/DQB1*0201 (2,6). Abnormal intestinal permeability in subjects with silent celiac disease could increase the absorption of dietary antigens, which could induce an autoimmune reaction in subjects with genetic susceptibility to diabetes. This hypothesis is consistent with the clinical observation that, in most patients, type 1 diabetes either precedes silent celiac disease or is diagnosed at the same time as celiac disease (1,7,8). If celiac disease and type 1 diabetes share a common genetic susceptibility, the prevalence of silent celiac disease

would be higher in diabetic siblings of type 1 diabetic patients than in nondiabetic siblings. To test this hypothesis, we examined a clinic-based cohort of 329 patients with childhood-onset type 1 diabetes (mean age 9.6 ± 5.2 years, range 1–17; diabetes duration 7.1 ± 4.3 years). All of the patients were cared for by the University of Turin Department of Pediatric, which treats >90% of childhood-onset diabetes patients in the area (9). A screening program of all patients with type 1 diabetes was performed using indirect immunofluorescence assay with a commercial kit (The Binding Site Ltd., Birmingham, U.K.). Three patients were diagnosed as celiac and treated with gluten-free diet for 3, 14, and 20 months before the onset of diabetes, respectively, whereas 22 were found positive at the EMA screening on 2 separate determinations; most of the patients were asymptomatic or complained only of mild gastrointestinal symptoms. The diagnosis of celiac disease was confirmed by jejunal biopsy in all but 1 adolescent patient who refused the test; in 2 patients, however, the results were doubtful. All patients reverted to antibody negativity after removal of gluten from the diet. Therefore, of this cohort of diabetic patients, celiac disease was diagnosed in 25 patients (6 boys, 19 girls), showing a prevalence of 7.6% (95% CI 4.8–10.4), which is consistent with previous estimates obtained in diabetic Italian children and adults (3,4,10). Consistent with previous data (1,2,5), the group of patients with both diseases had a higher female-to-male ratio and were of a younger age at onset than the group of patients with diabetes only (3:1 vs. 1:1.2, $P < 0.001$, and 5.3 ± 3.8 vs. 9.3 ± 4.5 years, $P < 0.001$, respectively).

We then assessed the prevalence of celiac disease in sporadic cases of type 1 diabetes (n = 229) and in patients with a diabetic sibling (n = 16), after excluding from the analysis 84 cases of only children. The prevalence of having both diabetes and celiac disease was 37.5% (95% CI 13.8–61.2) in patients with a diabetic sibling and 6.1% (3.1–9.1) in sporadic cases. The odds ratio for having both diseases was 9.21 (2.92–29.03), in diabetic patients with a diabetic sibling with respect to patients with nondiabetic siblings.

To our knowledge, this is the first report showing that the risk for silent celiac disease is higher in type 1 diabetic patients who have a diabetic sibling than in those with a nondiabetic sibling. It is likely that in families with multiple cases of type 1 diabetes, there is an increased prevalence of HLA-linked susceptibility genotypes and as non-HLA genes, which diabetes and celiac disease have in common. For instance, the prevalence of transglutaminase antibodies has been reported to be as high as 32% in type 1 diabetic patients with HLA DQA1*0501/DQB1*0201 or DQA1*0301/DQB1*0302, as compared with 2% in patients without these haplotypes (11). Environmental factors, however, could also be involved in this association. Screening for celiac disease in larger groups of families with multiple diabetic patients, coupled with the assessment of their HLA genetic susceptibility and nutritional habits might provide interesting clues on the etiopathogenesis of both celiac disease and type 1 diabetes.

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Laboratory Testing for Microalbuminuria in the General Community

Most authorities recommend routine screening for microalbuminuria (MA) to guide and monitor clinical efforts to delay the progression of nephropathy (1–2). For MA screening, the American Diabetes Association (ADA) recommends measuring urinary albumin excretion rates with timed and 24-h urine specimens or measuring albumin-to-creatinine ratios on random or spot urine specimens (1). The defined cutoff values for MA for each of these tests are ≥20 μg/min, ≥30 mg/24 h, and ≥30 μg/mg creatinine, respectively. Currently, no single method

Table 1—*Use of the 3 microalbuminuria screening tests by Montana laboratories in 1999 and their corresponding values recommended by the American Diabetes Association*

	Use of test	Use of units and cutoffs
ADA recommendations		
24-h collection (≥30 mg/24 h)	12 of 17 (71)	4 of 12 (33)
Timed collection (≥20 μg/min)	10 of 17 (59)	4 of 10 (40)
Albumin-to-creatinine ratio (≥30 μg/mg creatinine)	15 of 17 (88)	10 of 15 (67)

Data are *n* (%).

has emerged as the community standard. Although the recommendations for MA screening were first published by the ADA in 1996, few studies have examined which tests for MA are offered in community laboratories and how the results are reported in relation to the ADA's clinical recommendations. In 1999, the Montana Department of Public Health and Human Services surveyed laboratories in Montana to assess available forms of screening for MA and how the results were reported.

All 65 clinical and hospital-based laboratories in Montana were surveyed by mail in August 1999 to ascertain if their laboratory provided testing for MA and, if so, the methodology and units and cutoffs they used to report their results. Each laboratory was asked if it performed urine albumin testing on random or spot samples, timed collection, and 24-h collection and if they performed and reported albumin-to-creatinine ratios. They were also asked to indicate the units used to report results for each of these measures and the cutoff values used to report concentrations of albumin in the MA range. Laboratories that sent urine samples to a reference laboratory were asked to provide contact information; these reference laboratories were also surveyed. Responding laboratories were given the opportunity to verify their initial responses; 3 laboratories amended their responses.

Of the 65 clinical and hospital-based laboratories in Montana, 52 (80%) responded to the survey. Of the 52 responding laboratories, 13 (25%) provided quantitative testing for MA on site, 4 (8%) screened using qualitative reagent strips only, and 35 (67%) did not perform on-site quantitative assays. Of the 39 laboratories that did not test quantitatively, 30 sent specimens to a reference laboratory within or outside of Montana, and 9 laboratories neither tested nor referred specimens to a reference laboratory. In addition, 4 out-of-state reference laboratories were identified and completed the survey. These reference laboratories provided MA testing services to 17 of the 30 (57%) laboratories that sent specimens to outside laboratories. In total, 17 laboratories (13 in Montana and 4 out-of-state) performed at least one form of quantitative MA testing for Montanans with diabetes.

Table 1 displays the frequency with which the laboratories performed each of the 3 tests for MA by using the units and cutoffs recommended by the ADA. Overall, 10 of the 17 (59%) laboratories offered at least one of the tests recommended by the ADA and reported their results using units and cutoffs consistent with the ADA's recommendations. However, only 5 of the 17 (29%) laboratories offered these tests exclusively and reported the values using units and cutoffs recommended by the ADA.

Of the 17 laboratories that provided quantitative testing for MA, all 17 performed random or spot testing. Additionally, 13 reported results as milligrams per liter and used the following cutoffs for MA: >18.0 (n = 1), ≥18.9 (n = 2), ≥19.0 (n = 2), >20.0 (n = 3), ≥30 (n = 1), and ≥37.0 (n = 1); 3 laboratories did not report values for randomly collected specimens. Three laboratories reported the results of random tests as milligrams per decaliter with cutoffs of ≥1.9 (n = 1) and ≥2.0 (n = 1), and one laboratory stated that they did not report values for randomly collected specimens. One laboratory reported results for random tests in micrograms per milliliter and did not report a cutoff value.

Of the 17 laboratories, 15 performed albumin-to-creatinine ratios for MA. All 15 reported results as milligrams per gram of creatinine and reported the following cutoffs for MA: ≥13.2 (n = 3), >15.0 (n = 1), >16.0 (n = 1), and ≥30.0 (n = 10).

Of the 17 laboratories, 10 performed testing for MA from timed urine samples. Of those 10 laboratories, 5 reported results as micrograms per minute with the following cutoffs for MA: >20.0 (n = 1), ≥20.3 (n = 3), and ≥25.0 (n = 1). Two laboratories reported results as milligrams per liter and used cutoffs of ≥20.0 and ≥30.0. Three laboratories reported results using the following cutoffs and units: ≥11.2 mg/min, ≥20.0 μg/ml, and no cutoff with values reported as milligrams per decaliter.

Of the 17 laboratories, 12 performed testing for MA from a 24-h collection of urine samples. Eight of these laboratories reported results as milligrams per 24 h with the following cutoffs for MA: ≥11.2 (n = 1), ≥15.0 (n = 1), ≥25.0 (n = 1), >30.0 (n = 1), ≥30.0 (n = 4), ≥31.0 (n = 1), and ≥42.0 (n = 1). One laboratory reported results as milligrams per liter with a cutoff of ≥37.0, and 1 laboratory reported results as micrograms per gram with a cutoff of ≥30.0.

Our survey of laboratories in Montana indicates that MA testing is not yet provided in all laboratories and that a variety of units and cutoffs are reported. Thus, primary care physicians face challenges in obtaining and interpreting tests for MA. These findings suggest that strategies are needed to increase the availability of MA testing and to promote consistency in reporting of results and recommended cutoffs. Promoting the availability and consistency of MA testing through laboratory regulatory agencies, manufacturers, and laboratory associations can help primary care physicians and their patients benefit from MA screening, interpret the laboratory findings according to published recommendations, and implement appropriate measures to prevent the progression of diabetic nephropathy.

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Elevated Plasma Levels of Proinsulin in Adult Patients With Down's Syndrome

Previous studies have reported increased prevalence of diabetes in patients with Down's syndrome (1). However, these studies concentrated on young people with Down's syndrome. Recently, the mortality rate of Down's syndrome has declined and life expectancy has improved. It is important to examine pancreatic β-cell function and insulin resistance in adult patients with Down's syndrome to prevent diabetes.

In this study, we measured fasting plasma levels of glucose, insulin, and proinsulin, and calculated the proinsulin-to-insulin ratio (PI/I ratio) and insulin resistance index assessed by homeostasis model assessment (HOMA) (2) in adult patients with Down's syndrome. A total of 19 patients with Down's syndrome were studied. They were in a residential home for adult patients with mental and physical handicaps, and were identified as having Down's syndrome by chromosome analysis (15 patients with regular trisomy 21 and 4 patients with mosaic trisomy 21). As control subjects, 10 patients with mental retardation caused by other diseases including indistinct disorders were studied, all of whom were in the same residential home. None of the subjects was previously diagnosed with diabetes. The direct measurement of immunoreactive proinsulin was performed using a sensitive enzyme-linked immunosorbent assay (Yuka Medias, Ibaraki, Japan) (3). Data from control subjects and patients with Down's syndrome were expressed as mean ± SD, and compared by nonparametric Mann-Whitney *U* test. $P < 0.05$ was considered statistically significant.

Mean age of patients with Down's syndrome was 48.3 ± 4.2 years (range 45–59 years), and mean BMI of the patients was 21.1 ± 2.9 kg/m^2. Mean age of control subjects was 51.4 ± 9.3 years (range 44–73), and the mean BMI was 22.1 ± 2.6 kg/m^2. Mean fasting plasma levels of glucose (FPG) for the patients and the control subjects were 5.1 ± 0.4 mmol/l, and 5.1 ± 0.4 mmol/l, respectively. There were no significant differences in age and BMI, and in plasma levels of glucose between in the controls and the patients. FPG for each subject was no more than 6.0 mmol/l. Mean fasting plasma levels of insulin were not significantly different between the control subjects (38.6 ± 20.0 pmol/l) and the patients (35.7 ± 12.8 pmol/l). Mean fasting plasma levels of proinsulin were significantly higher in patients with Down's syndrome (10.85 ± 2.96 pmol/l) than in the control subjects (8.0 ± 3.51 pmol/l; $P < 0.05$). The PI/I ratio was also significantly higher in the patients (0.325 ± 0.097) than in the controls (0.236 ± 0.087; $P < 0.05$). There were no significant differences in HOMA between the control subjects (1.49 ± 0.87) and the experimental subjects (1.35 ± 0.51).

Elevated levels of plasma proinsulin and PI/I ratio have been reported to be one of indicators of β-cell dysfunction in type 2 diabetes and nonobese elderly subjects (4,5). The present study demonstrates that plasma levels of proinsulin and PI/I ratio were elevated in patients with Down's syndrome, whereas insulin resistance assessed by HOMA in the experimental subjects was not significantly different from that in the control subjects. These observations suggest that function of pancreatic β-cells in patients with Down's syndrome may be impaired, resulting in high incidence of diabetes. The elevated levels of plasma proinsulin and PI/I ratio in patients with Down's syndrome seem to be caused not by environmental factors, but by genetic factors (trisomy 21), because both the patients and control subjects live in the same residential home and share a similar lifestyle. Previous studies suggested that neurons of patients with Down's syndrome have a defect in the metabolism of reactive oxygen species that causes neuronal apoptosis, and that this defect may contribute to mental retardation and predispose to Alzheimer's disease (6). On the other hand, expression of antioxidant enzymes in the pancreatic islets is reportedly very low (7). These studies suspect that pancreatic β-cells of patients with Down's syndrome are oxidatively stressed, resulting in elevated levels of plasma proinsulin and PI/I ratio. In conclusion, the present study demonstrates that plasma levels of proinsulin and PI/I ratio were elevated in patients with Down's syndrome, suggesting that function of pancreatic β-cells may be impaired.

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Angiotensin II Blockade Is Associated With Decreased Plasma Leukocyte Adhesion Molecule Levels in Diabetic Nephropathy

The effects of blockade of the renin-angiotensin (RAS) system on levels of circulating adhesion molecules in type 1 diabetic patients with diabetic nephropathy were assessed. ACE-inhibition and angiotensin II receptor blockade reduced plasma concentrations of soluble (s) vascular cell adhesion molecule 1 (VCAM-1) and sE-selectin in type 1 diabetic patients with diabetic nephropathy, suggesting that interfering with the effects of angiotensin II decreases proatherogenic endothelial-leukocyte adhesion in diabetic nephropathy.

Type 1 diabetic patients with diabetic nephropathy are at extremely high risk of atherothrombotic disease. Increased adhesion of leukocytes to endothelial cells is an early feature of atherogenesis and is mediated by increased expression on cellular membranes of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), VCAM-1, and E-selectin. Soluble adhesion molecules have been detected in plasma and are thought to reflect shedding of membrane-bound forms. Increased plasma levels may thus indicate progressive atherogenesis, and they have in fact been shown to predict atherothrombotic events (1). Angiotensin II stimulates the synthesis of adhesion molecules and thus leukocyte-endothelium adhesion (2), but there are no data on the effects of interference with the RAS in diabetic nephropathy.

In view of these considerations, we performed a study on soluble adhesion molecules and renal function in type 1 diabetic patients with diabetic nephropathy during intervention in the RAS by blockade of the angiotensin II type 1 receptor (AT1) compared with the effect of ACE inhibition (renal data published elsewhere [3]).

The study was designed as a crossover trial with 5 treatment periods, each lasting 2 months. Sixteen patients were included. Clinical data during treatment with placebo were as follows: glomerular filtration rate (GFR) 90 ± 6 l ? min^{21} ? 1.73 m^{22} (means ± SEM), albuminuria 1,156 mg/24 h (643–2080, geometric mean; CI 95%), mean arterial blood pressure (MABP) 104 ± 2 mmHg (means ± SEM), and HbA_{1c} 8.8 ± 0.3% (mean ± SEM). The patients received the AT1 receptor antagonist losartan (50 and 100 mg), the ACE inhibitor enalapril (10 and 20 mg), and placebo in random order. Laboratory examinations were performed at the end of each treatment period and included assessment of plasma sVCAM-1; sICAM-1; sE-selection (adhesion molecules) by using an enzyme-linked immunosorbent assay (R&D Systems, Oxon, U.K.); von Willebrand factor (vWF), a general marker of endothelial function; and C-reactive protein (CRP), a marker of inflammatory activity. These markers, except sICAM-1, were also examined in a control group of 29 healthy subjects. The reduction in MABP and albuminuria ranged from 6 to 11 mmHg and 33 to 59%, respectively, during the different drug treatment periods compared with placebo, whereas GFR and metabolic control remained unchanged. There were no significant differences regarding the antihypertensive and antiproteinuric effects of the 2 drugs (3).

Plasma levels of sVCAM-1, sE-selectin, and vWF during the placebo period were higher than those in healthy control subjects (Table 1). The increased concentrations of sVCAM-1 and sE-selectin in the patients with diabetic nephropathy were significantly reduced by blockade of RAS, except for sE-selectin in the losartan-treated patients (P = 0.08) (Table 1). The relative reductions in sVCAM-1 and sE-selectin obtained during treatment with the AT1 receptor antagonist tended to be less pronounced than the reduction obtained with the ACE inhibitor (P . 0.09). The concentration of sICAM-1 remained unchanged in all 5 treatment periods. None of the drugs lowered the elevated level of vWF or the concentration of CRP.

Our study is the first to demonstrate that blockade of the activity of angiotensin II in diabetic nephropathy lowers the levels of some, but not all, adhesion molecules. These results suggest that interfering with the effects of angiotensin II decreases proatherogenic endothelial-leukocyte adhesion in diabetic nephropathy. Levels of vWF and CRP did not change, which suggests that a general improvement in endothelial function or a decrease in systemic inflammatory activity did not cause the decreases in sVCAM-1 and sE-selectin.

Table 1—*Concentrations of circulating adhesion molecules, von Willebrand factor, and C-reactive protein in healthy control subjects and during RAS blockade in diabetic nephropathy*

	Healthy control subjects (n = 29)	Losartan		Enalapril	
		Placebo	50 mg	100 mg	10 mg	20 mg
sVCAM-1 (ng/ml)	512 (484–541)	652 (599–709)*	629 (591–668)†	620 (570–674)‡	597 (553–645)§	595 (555–638)§
sICAM-1 (ng/ml)	‖	256 (225–292)	265 (228–307)	261 (229–297)	263 (231–300)	266 (235–301)
sE-selectin (ng/ml)	35 (30–41)	58 (47–71)*	55 (45–68)†	55 (44–68)¶	52 (43–64)§	53 (43–65)§
vWF (U/ml)	0.90 (0.74–1.11)	1.3 (1.04–1.47)*	1.30 (1.06–1.57)	1.29 (105–1.58)	1.18 (0.93–1.49)	1.15 (0.97–1.36)
CRP (ng/ml)	0.57 (0.40–0.81)	0.86 (0.48–1.54)	1.00 (0.54–1.82)	0.95 (0.48–1.87)	1.00 (0.50–2.00)	0.84 (0.45–1.55)

Data are geometric means (95% CI). *$P < 0.05$ vs. healthy control subjects; †P = 0.15 vs. placebo; ‡$P < 0.05$; §$P < 0.01$ vs. placebo; ‖no value from healthy control subjects; ¶P = 0.08 vs. placebo.

Our study cannot exclude the possibility that ACE inhibition implies a vasculoprotective effect in addition to those generated by angiotensin II blockade, e.g., through bradykinin accumulation.

Blockade of RAS has been described as exerting several nonhemodynamic vasculoprotective effects, such as an antiproliferative and antimigratory effect on smooth muscle cells and an improvement in endothelial function (4). Studies investigating the effect of blockade in RAS on expression of adhesion molecules in vivo are scanty. A reduction in abnormally elevated levels of adhesion molecules by ACE inhibitor treatment has recently been demonstrated in an open nonrandomized study in type 2 diabetic patients with microalbuminuria and borderline hypertension (5). Similar results have been found by Ferri et al. (6) in nondiabetic hypertensive patients.

The reduction in sVCAM-1 and sE-selectin in our study may reflect an antiatherogenic effect of blockade of the RAS. Therefore, further studies should be performed to investigate the pathophysiological significance of increased expression of adhesion molecules in diabetic nephropathy and the potentially beneficial effect of intervention in the RAS.

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Castration and Diabetes

Prostate cancer is the most common malignancy among American men, and the number of men with prostate cancer, as well as the number of men with diabetes, is progressively increasing in Japan. Various hormone therapies are performed to treat advanced prostate cancer. Recently, we examined 6 patients (mean age 78.5 ± 4.0 years) in whom diabetes control was remarkably worsened by either surgical (orchiectomy) or medical castration (administration of gonadotropin-releasing hormone analog), which was used in addition to antiandrogen drugs given to treat advanced prostate cancer.

Laboratory data relating to diabetes and sex hormones were as follows: levels of HbA_{1c} (normal 3.4–5.8%) before castration were 6.5, 6.5, 7.3, 6.0, 5.2, and 6.5% and several months after castration were 9.9, 13.0, 11.0, 8.3, 8.2, and 11.0%, the means of which were 6.3 ± 0.6 and 10.2 ± 1.7%, respectively ($P < 0.005$ by paired Student's *t* test). All but 1 case had increased insulin secretion. Fasting serum C-peptides were 1.4, 0.6, 1.5, 1.3, 1.5, and 1.2 nmol/l (normal 0.3–0.8), respectively. Patients who underwent surgical castration had high levels of luteinizing hormone (LH) and follicular stimulating hormone (FSH), in comparison with patients who underwent medical castration, in whom levels of LH and FSH were low. Serum levels of progesterone were within normal limits (<0.4 ng/ml), and testosterone decreased to undetectable levels (<0.2 ng/ml, normal 2.7–10.7) in all patients. All patients required more intensive antidiabetic therapy after castration and antiandrogen therapy. No significant changes occurred in diet, exercise, or BMI in any of the patients.

Previous reports concerning sex hormones and glucose metabolism have been controversial. In women who are in the luteal phase or are pregnant, progesterone is 1 of the sex hormones associated with insulin resistance. Some studies demonstrated that higher levels of testosterone are associated with insulin resistance in women, especially in patients with polycystic ovarian syndrome (1). In contrast, decreased testosterone and dehydroepiandrosterone sulfate were reported to be associated with insulin resistance and hyperinsulinemia in men (2). Serum concentrations of testosterone were also lower in men with type 2 diabetes than in normoglycemic men (3). Castrated male rats showed increased insulin resistance, which improved after administration of testosterone (4). A few studies demonstrated that antiandrogen therapy itself caused diabetic ketoacidosis in type 2 diabetic patients. Therefore, decreased levels of testosterone as a result of castration might play an important role in insulin resistance in our subjects.

Taken together, these findings contribute to the understanding of the relationship between sex hormones and glucose metabolism, and urologists and physicians should pay close attention to glucose metabolism when treating prostate cancer with castration and antiandrogen therapy.

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Decompensation of Leucine Nitrogen Kinetics in Gestational Diabetes Mellitus

Fetal and neonatal morbidity, specifically macrosomia or large-for-gestational-age infant, remains a persistent problem in pregnant women with gestational diabetes mellitus (GDM) (1,2). A number of studies have shown that rigorous management of maternal metabolism by diet or insulin therapy can normalize maternal plasma glucose concentrations, HbA_{1c} levels, and rates of glucose turnover (3,4). However, fetal macrosomia and related perinatal maternal and neonatal morbidity persist. Data from previous studies have shown an increase in maternal plasma concentrations of alpha amino nitrogen in women with GDM and a correlation between certain specific amino acid levels in the mother and fetal birth weight (6). In particular, there was a strong correlation between maternal levels of serine, proline, threonine, and ornithine, and the infant's birth weight. Because all of these are nonessential amino acids, we speculated that the increased levels of these amino acids may be related to changes in whole-body nitrogen turnover. Studies of whole-body protein turnover, as measured by [1-^{13}C]leucine or phenylalanine tracers, have not shown any significant impact of GDM in well-compensated subjects (6,7). Transamination of leucine and other branched-chain amino acids is an important nitrogen source for nonessential amino acids. In the present study, we have quantified the rate of leucine N turnover and its transamination in metabolically compensated women with GDM before any intervention. Our data show a higher rate of leucine N turnover in the presence of unchanged rate of urea synthesis in women with GDM.

Leucine and urea kinetics were quantified in 6 women with GDM between 21 and 32 weeks' gestation. Their data were compared with those of 8 normal healthy pregnant women studied during the third trimester using a similar protocol, which was reported previously (8). All subjects were healthy, had no other medical complications related to pregnancy, and were not receiving any medications other than vitamin supplements. Written informed consent was obtained from each subject after the procedure was fully explained. The protocol was approved by the institutional review board for investigation in humans.

Subjects were studied in the General Clinical Research Center during the morning after an overnight fast of 10 h. The details of the tracer isotope infusion protocol have been reported (8). After the basal studies, the response to a mixed-nutrient load was evaluated by giving oral Ensure Plus (Ross Laboratories, Columbus, OH) at a rate of 35 ml every 30 min (101 kcal and 3.7 g protein per hour) for the next 3 h.

The women with GDM were significantly older (38 ± 2.7 vs. 28 ± 3.4 years) and were studied earlier in gestation (28.5 ± 4.3 vs. 34.0 ± 2.0 weeks). There was no difference in weight, BMI, total body water (TBW) (TBW/wt: GDM, 58.8 ± 3.4%; normal, 55.8 ± 6.2%), weight gain during pregnancy, or the daily calorie intake between the normal and the GDM women. Their glycosylated hemoglobin levels were 5.36 ± 0.2%. The birth weight of infants born to mothers with GDM was not different from those of the infants born to normal mothers (normal, 3,183 ± 609 g; GDM, 3,426 ± 586 g).

The plasma glucose (GDM, 4.4 ± 0.5; normal, 4.1 ± 0.4 mmol · l^{-1}), urea nitrogen (GDM, 2.2 ± 0.7; normal, 2.9 ± 0.7 mmol · l^{-1}), and leucine (GDM, 89.5 ± 9.2: normal, 80.9 ± 18.7 μmol/l) concentrations during fasting were similar in the normal and GDM groups. In response to Ensure Plus feeding, the GDM group showed a significantly higher level of plasma glucose and leucine. There was no difference in the plasma insulin concentration during fasting or in response to feeding.

The rate of leucine nitrogen turnover (Q_N) was significantly higher during fasting in the GDM subjects (Table 1). The leucine carbon flux, fraction of leucine C-1 decarboxylated, and rate of urea synthesis during fasting was not significantly different in the GDM group when compared with the normal group. In response to feeding, there was a greater increase in leucine C flux in the GDM group ($P < 0.01$). The leucine Q_N during feeding, though higher in GDM subjects, was not significantly different between the 2 groups. Although the rates of deamination and reamination of leucine were higher in the GDM subjects, none of these differences were statistically significant. The fraction of leucine C oxidized and the fraction of leucine reaminated was unchanged in the GDM group. The rates of oxygen consumption, CO_2 production, and the respiratory exchange ratio were not significantly different between normal and GDM subjects.

Table 1—*Leucine and urea kinetics in pregnancy*

		Q_N (μmol · kg^{-1} · hr^{-1})		Q_C (μmol · kg^{-1} · hr^{-1})		C/Q_C (%)		S_U (μmol · kg^{-1} · min^{-1})	
	n	Fasting	Fed	Fasting	Fed	Fasting	Fed	Fasting	Fed
Subjects without GDM	8	135 ± 16	155 ± 34	109 ± 19	124 ± 17	15.7 ± 2.5	20.8 ± 4.7	2.59 ± 0.82	2.49 ± 0.76
Subjects with GDM	6	166 ± 8*	203 ± 39	124 ± 19	174 ± 31†	14.8 ± 2.6	21.3 ± 5.0	2.11 ± 0.87	2.35 ± 0.94

Data are *n* or means ± SD. Q_N, leucine nitrogen turnover ([1-^{13}C,^{15}N]leucine dilution); Q_C, leucine carbon turnover ([^{13}C]leucine dilution in ketoisocaproic acid); C/Q_C, fraction of leucine C-1 oxidized; S_U, rate of urea synthesis. Compared with normal group, *$P < 0.003$, †$P < 0.01$.

Diabetes in pregnancy, both type 1 diabetes as well as GDM, is associated with fetal and neonatal morbidity, specifically related to macrosomia (1,2,9,10). In spite of the rigorous control of maternal glucose metabolism, as demonstrated by normal glycosylated hemoglobin and plasma glucose levels and glucose kinetics, the macrosomia remains a persistent problem (3,4,9,10). Therefore, it has been postulated that fetal macrosomia may be related to the excessive transfer of other nutrients (i.e., amino acids and fatty acids) from the mother to the fetus. However, an excessive transfer of nitrogen to the fetus has not been demonstrated.

Studies of urea synthesis and urea nitrogen excretion in well-compensated women with GDM, whether treated by dietary regulation or by insulin, have shown no change in urea kinetics when compared with normal subjects (4,6). In addition, estimates of leucine C and phenylalanine kinetics, even in the presence of mildly elevated glucose and insulin levels, were unchanged in GDM women when compared with normal women (4,7). Only in the insulin-treated GDM subjects, who also had higher HbA_{1c} levels, was there an evidence of higher rate of leucine C turnover and a higher rate of leucine C-1 decarboxylation (4).

The present data are the first to demonstrate an increase in leucine Q_N in GDM. Of significance, the increase in Q_N was not associated with an increase in urea synthesis. The calculated rates of deamination and reamination of leucine in the women with GDM, though higher, were not significantly different when compared with the rates in the normal subjects. Nevertheless, a higher rate of leucine N turnover would be expected to result in a higher rate of whole-body nitrogen turnover. Whether such an increase actually occurs or is a direct contributor to fetal macrosomia remains to be examined. We speculate that a higher rate of leucine N turnover in GDM could result in an increased rate of whole-body nitrogen turnover, and could contribute to fetal macrosomia. Such a hypothesis needs to be examined in further studies.

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COMMENTS AND RESPONSES

Response to Glasgow and Anderson

We were pleased by Glasgow and Anderson's recent letter (1), which responded to our earlier article on compliance and adherence (2). It is clear that we share the perspective that traditional usage of "compliance" as a concept for understanding patient behavior and accounting for failures in medical treatment is both unjust for patients and limiting. Despite this common perspective, however, our opinions diverge regarding how best to proceed from the current state of affairs. In the spirit of moving this important debate forward and identifying nuances to arguments that have been previously underacknowledged, we take this opportunity to identify some of the constraints we see as acting on Glasgow and Anderson's recommendations.

First, we wish to acknowledge the body of work done by Anderson and his colleagues (3–6), as well as other work that has addressed the limitations of "adherence" (6–8) and has proposed alternatives, such as "self-care" and "self-management" (9–11). At the same time, we find that Glasgow and Anderson's presentation of this literature as "a large body of work" is misleading when considered in the larger context of the totality of publications in this area. Even though this work is indeed critical, it is utterly dwarfed by the number of publications that orient to compliance and adherence as a problem with individual patient behavior. Given the dominance of this perspective, we continue to advocate the relatively simple move to the alternative term "adherence" as a maximally parsimonious strategy for striking the term "compliance" from therapeutic vocabulary and moving away from its pejorative implications. This point is intertwined with Glasgow and Anderson's criticism that our previous argument does "not go far enough." We agree that none of these proposals will go far enough until we

can enact truly collaborative and dialogic doctor-patient relationships. This must begin with the elimination of a "compliance" paradigm.

When we consider closely the litany of alternatives that have been suggested for "compliance" ("adherence," "self-care," "self-management," "empowerment," and "autonomy motivation"), we find underlying similarities that not only implicate constraints acting on the reformulation of the concept of "compliance" within the medical system, but that also may help account for the ongoing nature of this debate. To varying degrees, each of these terms ultimately focuses on shortcomings in patient behavior as an explanation for suboptimal treatment outcomes, thereby residing conceptually in the same camp as "compliance." Even "self-management" creates a situation in which poor self-care can be dismissed as noncompliance—words still heard almost daily in training programs and diabetes clinics.

Glasgow and Anderson's comments invite examination of the more fundamental issues underlying the compliance/adherence debates: In the context of our modern medical system, is it really possible to operationalize the phenomenon of patients not following treatment recommendations without implicating practitioners' authority? Do we overstate or romanticize the extent to which doctor-patient relationships can be equalized when we suggest alternatives to "compliance"? Perhaps Wagner's proposed term "collaborative management" (12) will indeed encourage us to move toward these goals by helping us to rethink the nature of these relationships. As we continue to consider these issues, it is important to remember that even though patients are indeed responsible for their diabetes management, practitioners are also inescapably invested in these processes in ways that will not disappear with changes in terminology. They are responsible for prescribing regimens that patients can safely execute, and, moreover, for overseeing this self-management process in a way that maximizes glucose control while protecting themselves and patients from liability and the negative consequences of uncontrolled diabetes. Shifting to a terminological focus on patients' autonomy may only mask the underlying causes of practitioners' concerns with compliance and some of the ways in which the phenomenon is inherently authoritative. To the extent that these issues give shape to patient-practitioner relationships, then, we continue to share Glasgow and Anderson's concern with moving away from "compliance." We propose beginning with the simpler step of replacing "compliance" with "adherence" as an approach that is more sensitive to the current organization of medical care in the U.S.

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Increased Plasma Plasminogen Activator Inhibitor 1 in Relatives of Type 2 Diabetic Patients

In a recent study published in *Diabetes Care*, Gürlek et al. (1) presented data showing increased plasminogen activator inhibitor 1 (PAI-1) levels in the offspring of patients with type 2 diabetes, which is predicted by the clustering of anthropometric and metabolic features in patients with insulin resistance. In fact, increased levels of PAI-1 in the first-degree relatives of type 2 diabetic patients have been described previously (2,3). We published data from 132 first-degree relatives (5 parents, 81 offspring, and 46 siblings [unrelated to each other]) of type 2 diabetic patients showing increased levels of PAI-1 compared with age-matched control subjects (geometric mean values; 12.7 vs. 7.7 ng/ml). This finding was not accounted for by adjustment for other markers of insulin resistance (2).

Unlike the data from Gürlek et al. (1), but in common with many other large cross-sectional studies, we found levels of PAI-1 to show a strong correlation with fasting plasma insulin levels (2), as would be expected given the strong association of both with dynamically measured insulin resistance (4,5). It is possible that Gürlek et al. failed to demonstrate this association because of a smaller sample size and greater coefficient of variability of measures of PAI-1 and insulin. It is of interest that the PAI-1 antigen values cited by Gürlek et al. are ~20-fold higher than those that we and others have found in similar subjects. This may reflect the release of the platelet pool of PAI-1 into plasma during processing. In this context, the absence of correlation may not justify the subtitle "Lack of association with plasma insulin levels."

Suppressed fibrinolysis due to increased plasma levels of PAI-1 is now an established feature of the syndrome of insulin resistance (6). The mechanism linking insulin resistance to increased PAI-1 remains unclear, although it may be medi-

ated by more than one of the metabolic consequences of insulin resistance. Just as both insulin and VLDL triglyceride stimulate PAI-1 expression in vitro (7,8), so simultaneously elevated levels of insulin, glucose, and triglyceride have been shown to increase circulating PAI-1 levels in human subjects (9).

We agree with Gürlek et al. that caution is still required in attributing a causal role for elevated PAI-1 levels in the atherogenic process.

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Increased Plasma PAI-1 in Relatives of Type 2 Diabetic Patients

It is obvious that the observations of Mansfield et al. (1) concerning the increased PAI-1 activity in first-degree relatives of type 2 diabetes patients are in accordance with our results (2). Indeed, our reason for not citing their study in our article is our lack of awareness of its presence according to our Medline search results. This may be caused by the discrepancy between the keywords assigned to their article and the keywords we have used while searching for the related articles in this field. In fact, in the discussion section of our article (2), we have used the phrase "to the best of our knowledge," which shows that we consider our study to be the first one about increased PAI-1 activity in first-degree relatives of diabetic subjects.

In this issue, Mansfield (3) claims that our failure to demonstrate the correlation between PAI-1 activity and fasting plasma insulin concentration might be due to the small sample size and greater coefficient of variation concerning PAI-1 and insulin. We think that this argument is not valid based on the Spearman's rank correlation analyses we have performed. Although not mentioned in detail in our article, the Spearman's correlation coefficient (r value) between PAI-1 activity and fasting plasma insulin concentration was -0.14, corresponding to a P value of 0.44. So, even if we had a greater sample size, we would have failed to demonstrate the expected positive correlation between these 2 parameters. In our opinion, the phrase "lack of association with plasma insulin levels" as a subtitle for our article is suitable because of the lack of any correlation between PAI-1 activity and insulin. As stressed by Mansfield (3), there are studies that have demonstrated a positive correlation between PAI-1 and insulin levels. However, it should also be noted that neither insulin nor proinsulin levels are predictive of PAI-1 activity in healthy subjects as assessed in multivariate linear regression analyses (4).

Mansfield (3) has stressed that our absolute PAI-1 antigen concentrations are relatively higher than cited in the study by Mansfield et al. (1). We would like to emphasize that we strictly followed the instructions of the manufacturer of PAI-1 assays (Diagnostica Stago, Asnières-Sur-Seine, France). The discrepancy regarding the absolute values of plasma PAI-1 antigen concentrations may be multifactorial. First, it may be related to ethnic differences. For instance, in a previous study in a Turkish population (5), the median PAI-1 antigen level has been cited as 41 ng/ml, and this value seems considerably higher than the value cited (7.7 ng/ml) by Mansfield et al. (1). Also, the contribution of platelets to PAI-1 concentration is a well-defined phenomenon. For instance, elevated plasma PAI-1 antigen concentrations have been reported in patients with primary and secondary thrombocytosis (6). So, the platelet counts of our study population might have accounted for, at least in part, the aforementioned discrepancy.

Unfortunately, we have not assessed the platelet counts of our subjects. Even if we assume that, as suggested by Mansfield, the activation of platelets and the resultant release of intraplatelet pool of PAI-1 during sampling have contributed to our plasma PAI-1 antigen concentrations, the plasma PAI-1 activity has not been affected by this situation. It has previously been shown that plasma mainly contains active PAI-1, whereas the platelet PAI-1 occurs as an inactive form (7). As reflected by the title of our study (2), we have primarily evaluated the plasma PAI-1 activity in our subjects.

We agree with Mansfield (3) concerning the causal role of PAI-1 in the atherogenic process in the prediabetic period. This issue can be solved by prospective large-scale studies conducted in both normal and impaired glucose tolerant first-degree relatives of diabetic subjects.

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Homeostasis Model Assessment and Related Simplified Evaluations of Insulin Sensitivity From Fasting Insulin and Glucose

No need for log transformation but beware of limits of validity

In recent issues of *Diabetes Care*, 2 articles confirm the concordance between the homeostasis model assessment insulin resistance index (HOMA-IR) and insulin sensitivity (SI) measured with either the glucose clamp (1) or the minimal model (2). In addition, both indicate that the relationship between HOMA-IR and SI is nonlinear and fits better with an exponential curve (1,2). Accordingly, Fukushima et al. (2) propose to use ln(HOMA-IR) rather than HOMA itself as a measurement of insulin resistance. Evidence supporting the accuracy of these alternative evaluations of SI from baseline insulin (I) and glucose (G) levels (1–4) appears to be more and more convincing. However, a recent large-scale study shows that such methods are not precise enough to be recommended for the clinical assessment of SI in individual subjects (5). In addition, it is very surprising that, besides $G \times I$ expressed either as a HOMA-IR equal to $G \times I/22.5$ (3) or a fasting insulin resistance index, which is equal to $G \times I/25$ and thus almost equivalent (4), other indexes based on the ratio G:I are also reported to fairly correlate with SI (6). The physiological basis for these indexes is the feedback homeostatic loop between SI and I (7) that is described by the relationship: $SI \times I = a$ (constant). This implies that, unless this homeostatic loop is broken, there is a simple hyperbolic relationship between SI and I as follows: $SI = a/I$. Therefore, SI is proportional to I^{-1}. It is logical to assume that G should also be included in the formula for predicting SI, but whether the best predictor of SI is I/G, $I \times G$, or another formula with the general form $SI = aI^bG^c$ is not clear. After testing different empiric relationships (general form $SI = aI^bG^c$) in 7 distinct samples of subjects in comparison with the minimal model, we found that an index SI = a/I based on the concept of $Si \times I$ = constant (with a = 40 if SI units are $min^{-1}/(\mu U/ml) \times 10^{-4}$) was actually the best predictor of SI (8). Thus, we proposed SI = 40/I as a simplified evaluation of SI (9).

Two things remain unclear: *1*) which index (HOMA-IR, ln[HOMA-IR], G/I, or 40/I) fits better with minimal model SI, and *2*) what are the limits of validity of this alternative measurement of SI?

We measured SI with the minimal model in 68 obese patients (36.25 ± 1.66 years, BMI 34.8 ± 0.7); 44 with type 2 diabetes (53.7 ± 1.8 years, BMI 28.2 ± 0.87); 27 patients explored for reactive hypoglycemia (37.1 ± 3.3 years, BMI 23.1 ± 1.3); 57 athletes (28.6 ± 1.6 years, BMI 22.5 ± 0.28); and 20 lean control subjects (25.73 ± 2.6 years, BMI 20.9 ± 0.6). Correlations of SI with these indexes are shown on Table 1. A step-wise regression analysis chose 40/I as the best correlate of SI in obese and type 2 diabetic patients. Mean differences between SI and 40/I were as follows: 1.8 ± 0.12 $min^{-1} \times 10^{-4}$ (μU/ml) (obese), 2.15 ± 0.34 (type 2 diabetic patients), 6.9 ± 0.97 (athletes), and 8.38 ± 3.3 (hypoglycemic patients). These results show that *1*) log-transformed HOMA-IR correlates well to SI, but not better than the simpler indexes 40/I or G/I; *2*) these simple indexes calculated from I and G poorly correlate with SI in type 2 diabetic patients and do not correlate at all in hypoglycemic patients and athletes.

Therefore, we agree with Bonora et al. (1) and Fukushima et al. (2) that HOMA-IR may provide a good prediction of SI, but we want to point out that log transformation is not necessary because the exponential-like shape of the relationship between SI and I is likely to reflect the homeostatic relationship ($SI = a/I$) rather than an until-now-unreported exponential law. As shown on Table 1, 1/(HOMA-IR) correlates at least as well as ln(HOMA-IR). In addition, in all of the series we have studied, G does not improve the prediction of SI, so that we suggest the index SI = 40/SI as a simple and accurate prediction of SI. It is also very important to emphasize that all of these indexes lose their validity when the feedback loop between SI and I is disturbed (i.e., in major β-cell defects such as overt

Table 1—*Correlation coefficients between SI (minimal model) and alternative indexes of insulin sensitivity*

	40/I	HOMA-IR	I/G	G/I	ln(HOMA)	1/HOMA	1/ln(HOMA)
Obese	0.668*	−0.435*	−0.510*	0.639*	−0.580*	0.659*	0.251
Obese + lean	0.343*	−0.277*	−0.342*	0.346*	−0.491*	0.516*	0.189
Type 2 diabetic patients	0.363†	−0.13 (NS)	−0.16 (NS)	0.24 (NS)	−0.177 (NS)	0.213 (NS)	0.083 (NS)
Hypoglycemic patients	−0.02 (NS)	−0.13 (NS)	−0.03 (NS)	0.07 (NS)	0.074 (NS)	−0.069 (NS)	0.075 (NS)
Athletes	0.11 (NS)	−0.20 (NS)	−0.08 (NS)	0.07 (NS)	−0.218 (NS)	0.221 (NS)	−0.257 (NS)

*$P < 0.001$; †$P < 0.05$.

diabetes or when SI values are high [athletes and reactive hypoglycemia]). Thus, these indexes should be used only in populations in whom their validity has been demonstrated (e.g., nondiabetic obese patients). Outside of these conditions, caution is surely required (5) and there remains a need for other simple validated indexes.

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Assessment of Insulin Sensitivity

Comparison between simplified evaluations and minimal model analysis

There is a need to have a simple index of insulin sensitivity that provides a good correlation with the standard methods as glucose clamp or minimal model analysis (MINMOD) for diabetic patient clinics and large population studies. Fasting insulin (I) and insulin-resistance index assessed by homeostasis model assessment (HOMA-IR), defined as the product of fasting plasma insulin and glucose divided by 22.5, are simplified tools to estimate insulin sensitivity (1–3). Raynaud et al. (4) clarified 40/I as a good evaluation. Emoto et al. (5) and Bonora et al. (6) demonstrated that HOMA-IR and log-transformed HOMA (ln[HOMA]) provided good correlations with the insulin sensitivity index in recent clamp studies. In the present study, we applied MINMOD to compare the estimates of insulin sensitivity (SI) with various simplified evaluations (7,8). The statistical analysis was performed with the StatView 5 system (Berkeley, CA).

We examined 71 Japanese subjects with normal glucose tolerance (NGT) and type 2 diabetic subjects to assess insulin sensitivity (33.5 ± 1.6 years of age, BMI 20.3 ± 0.33 kg/m^2). There were 46 subjects with normal glucose tolerance (27.7 ± 1.3 years of age, BMI 19.9 ± 0.42 kg/m^2) and 25 patients with type 2 diabetes (44.4 ± 2.6 years of age, BMI 21.1 ± 0.49). Correlation coefficients and *P* values of the simplified evaluations with MINMOD-derived SI are shown in Table 1. There was a significant correlation between SI in 40/I, HOMA-IR, ln(HOMA), I, 1/HOMA, the ratio of fasting insulin to glucose (I/G), and the ratio of fasting glucose to insulin (G/I). Among them, 40/I, HOMA-IR, ln(HOMA), and I correlated well with MINMOD SI in both NGT and type 2 diabetic subjects. These simple indexes are considered good surrogates for insulin sensitivity estimation. Correlation coefficients of HOMA-IR and ln(HOMA) were higher than 40/I and I in NGT, type 2 diabetes, and all subjects in this study (Table 1). Raynaud et al. (4) demonstrated that 40/I is the best evaluation compared with I, I/G, and HOMA-IR. The reason for the difference between the studies is not known, but it may be in the ethnic differences or clinical characteristics of the subjects examined. Banerji and Lebovitz (9) described 2 subpopulations of type 2 diabetic patients: one with normal insulin sensitivity, and the other with insulin resistance. Arner et al. (10) reported that type 2 diabetic patients with abdominal obesity displayed peripheral insulin resistance, whereas nonobese diabetic patients

Table 1—*Comparison of simple indexes with MINMOD-derived SI*

	40/I	HOMA-IR	ln(HOMA)	I	I/HOMA	I/G	G/I
All							
r	0.509	0.529	0.577	0.487	0.558	0.311	0.283
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.01	<0.05
NGT							
r	0.518	0.531	0.541	0.507	0.523	0.449	0.471
P	<0.0005	<0.0001	<0.0001	<0.0005	<0.0005	<0.005	<0.005
Type 2 diabetes							
r	0.516	0.557	0.547	0.523	0.483	0.391	0.412
P	<0.01	<0.005	<0.005	<0.01	<0.05	0.053 (NS)	<0.05

Data are correlation coefficients and *P* values.

showed only a secretory defect. We previously described a population of normal insulin sensitivity in nonobese type 2 diabetic patients (7). In the present study, the BMIs of NGT and type 2 diabetic subjects were 19.9 ± 0.42 and 21.1 ± 0.49 kg/m^2, respectively. Haffner et al. (11) described the insulin-sensitive and insulin-resistant type 2 diabetic populations and the importance of dyslipidemia on insulin resistance. The racial differences of insulin sensitivity are well documented in the Insulin Resistance Atherosclerosis Study (12). Further studies are necessary to characterize the validity of simplified evaluations in terms of the factors responsible for insulin sensitivity.

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Lack of Evidence for Bromocriptine Effect on Glucose Tolerance, Insulin Resistance, and Body Fat Stores in Obese Type 2 Diabetic Patients

Bromocriptine, a potent dopamine D_2 receptor agonist, has been shown to reduce glucose intolerance, insulin resistance, and body fat stores in an obese rodent model of type 2 diabetes (1). These effects of bromocriptine were associated with strong inhibition of basal lipolysis, hepatic lipogenesis, and hepatic glucose output, because bromocriptine also acts as an α_1-adrenergic receptor antagonist in liver and adipose tissue (1–3). However, such promising effects were reported in only a few human studies by the same group of investigators (4–6). To confirm this hypothesis, we examined the effect of bromocriptine in 13 (9 men and 4 women) obese (BMI 30.5 ± 1.6 kg/m^2, mean ± SEM) type 2 diabetic patients aged 51.0 ± 4.9 years, using a relatively higher dosage (5.0 mg/day) and longer duration (6–8 months) of treatment than reported previously (4–6). This study was approved by the institutional review board, and all participants gave their informed consent. They were instructed not to alter their usual eating patterns and exercise habits. Their preexisting medications (if any) remained unchanged during the treatment period. Bromocriptine (2.5 mg) was administered orally once daily at dinner for 1 month and then was increased to twice daily at breakfast and dinner for the remaining period (in 1 patient the dosage was further increased to 3 times daily). No adverse effects of the drug were noted except mild and transient nausea and nasal congestion, which occurred in 3 patients. Percent body fat was measured using bioelectrical impedance analysis. Abdominal visceral and subcutaneous fat mass were measured at the height of umbilicus by planimeter-assisted computed tomography. The responses of plasma glucose and insulin were determined during a 3-h standardized meal tolerance test before and after bromocriptine therapy. Basal plasma concentrations of leptin, insulin-like growth factor binding protein-1 as an index of hepatic insulin resistance (7,8), and tumor necrosis factor-α were measured using respective immunoassays. Drug compliance was considered good because periodically determined plasma concentrations of prolactin were suppressed to <1 ng/ml ($P < 0.001$ vs. baseline) in all cases. Compared with baseline values, bromocriptine neither induced any reduction in mean percent body fat (32.4 ± 3.3 vs. 30.9 ± 3.2%, before vs. after treatment, respectively), visceral fat mass (225.4 ± 18.9 vs. 224.4 ± 24.7 cm^2), nor subcutaneous fat mass (251.9 ± 36.6 vs. 283.1 ± 34.4 cm^2). Moreover, bromocriptine did not improve metabolic variables relating to insulin resistance, such as the homeostasis model assessment (9) index (7.8 ± 3.2 vs. 5.9 ± 1.3), the recently pro-

posed composite insulin sensitivity index (10) obtained from our standard meal tolerance test (4.8 ± 1.3 vs. 3.8 ± 0.7), plasma concentrations of insulin-like growth factor binding protein-1 (3.5 ± 1.3 vs. 2.3 ± 0.7 ng/ml), tumor necrosis factor-α (0.9 ± 0.2 vs. 1.2 ± 0.2 pg/ml), and leptin (9.8 ± 2.2 vs. 10.3 ± 2.4 ng/ml). In addition, plasma levels of epinephrine, norepinephrine, dopamine, triglyceride, and HDL cholesterol remained unaltered (data not shown). However, it should be noted that 1 woman showed a considerable improvement in insulin resistance (21.6% reduction in area under the glucose curve in the face of 40.5% reduction in area under the curve of insulin), and 1 man reduced his visceral fat mass by ~25%. From these results, we conclude that bromocriptine has no consistent beneficial effects on adiposity or insulin resistance in obese type 2 diabetic patients. The reason for the discrepancy between previous studies and our study remains to be clarified, but it may depend, in part, on the differences in the drug formulation of bromocriptine.

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Are the Results Really Different?

I'm somewhat surprised to see that, in this issue, Wasada et al. (1) cited the findings of our study (2) as being discrepant to their recent findings concerning bromocriptine use in obese male type 2 diabetic patients. Indeed, given the fact that the patient populations could hardly have been more different (we studied the effects of bromocriptine in obese female nondiabetic volunteers), the results of the 2 studies were much more similar than implied by Wasada et al. For example, by using nonspecific methods to measure insulin resistance, Wasada et al. concluded that bromocriptine did not improve insulin sensitivity in their obese male patients with type 2 diabetes. We used a specific method for assessing insulin-mediated glucose disposal in our population of obese female nondiabetic subjects. We also found no change in insulin action. Furthermore, we demonstrated that plasma insulin concentrations measured at hourly intervals for 24 h did not change with bromocriptine therapy. Finally, body weight was constant in both studies. Where, then, is the conflict?

Additionally, we found fasting concentrations of LDL and HDL cholesterol to be similar before and after bromocriptine treatment. Wasada et al. (1) also stated that HDL cholesterol concentrations did not change with bromocriptine treatment. The only significant metabolic changes we observed in our population of glucose-tolerant individuals in association with bromocriptine treatment were in plasma triglyceride and free fatty acid concentrations measured hourly for 24 h. Because Wasada et al. did not make these measurements, it is particularly confusing to read that their results were considered to be in conflict with ours.

In fact, the only potential disparity between the results of the 2 studies was our finding of somewhat lower values for plasma glucose concentrations in bromocriptine-treated patients after eating lunch. For inexplicable reasons, Wasada et al. (1) did not present data on changes in either fasting glucose or glycated hemoglobin levels after 6–8 months of treatment with bromocriptine in patients with type 2 diabetes. Moreover, they did not report the plasma glucose concentrations observed during the oral glucose tolerance tests.

We have no argument with the conclusion of Wasada et al. (1) that bromocriptine treatment has no effect on insulin resistance in obese type 2 diabetic patients. However, we are disappointed by their decision to not describe the results of treatment with bromocriptine on glycemic control; to not do so, though, was their prerogative. Nevertheless, I think their decision to explicitly state that their results are in conflict with ours, with the further implication that our results are misleading, was inappropriate.

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Homocysteine and Insulin Levels in Type 2 Diabetic Patients

Drzewoski et al. (1) recently reported on the inverse relationship between plasma insulin levels and homocysteine concentrations in type 2 diabetic patients. They found that plasma homocysteine concentrations were significantly higher in poorly controlled (HbA_{1c} 9.8 ± 1.6%) type 2 diabetic patients on "maximum doses of oral hypoglycemic agents" compared with well-controlled (6.6 ± 0.7%) type 2 diabetic patients and nondiabetic subjects (1).

However, their results should be interpreted with caution, because several factors that could potentially affect plasma homocysteine concentrations in type 2 diabetes were not considered (2). Therapeutic agents frequently used in the management of the insulin resistance syndrome, such as fibrates and biguanide, have been implicated as a cause of elevated plasma homocysteine concentrations. Both bezafibrate and fenofibrate have been shown to be associated with raised plasma homocysteine concentrations, which may occur as early as 6–12 weeks after drug initiation (3,4). Although the mechanism whereby short-term fibrate therapy causes hyperhomocysteinemia is unclear, there is evidence that long-term metformin therapy may cause hyperhomocysteinemia in type 2 diabetes through the reduction of vitamin B_{12} and folate concentrations (not measured in their study) (5). Both vitamin B_{12} and folate are important coenzymes for homocysteine remethylation to methionine. Therefore, deficiency of these coenzymes may lead to reduced remethylation resulting in homocysteine accumulation. Hence, inclusion of types of oral hypoglycemic agents as well as lipid-lowering drugs in each patient group is essential. It is possible that more poorly controlled type 2 diabetic patients were taking metformin than the well-controlled group, which may have contributed to higher plasma homocysteine concentrations.

No information was given regarding the prevalence of diabetic complications, such as nephropathy, in their patient groups. It has been shown that an increased urinary albumin excretion rate is associated with raised plasma homocysteine concentrations in type 2 diabetes (5). If diabetic nephropathy was more prevalent in the patients' poor glycemic control, this may also act as a contributory factor for the raised plasma homocysteine concentrations.

Lastly, the authors concluded from their study that elevation of plasma homocysteine concentrations is inversely correlated with endogenous insulin levels (1). This conclusion may not hold true without assessing the degree of insulin resistance. In type 2 diabetes, the relationship between insulin levels and plasma homocysteine concentrations is unclear because the limited studies performed were on nondiabetic subjects. In healthy nondiabetic subjects, acute (exogenous) hyperinsulinemia using a hyperinsulinemic-euglycemic clamp decreases plasma homocysteine concentrations (6,7), whereas insulin resistance (and thus endogenous hyperinsulinemia) has been shown to be associated with elevated plasma homocysteine concentrations (8). Further studies are required to determine the complex relationship between insulin levels, insulin resistance, and plasma homocysteine concentrations in type 2 diabetes.

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Homocysteine and Insulin Levels in Type 2 Diabetic Patients

Response to Chan

The interest of Chan (1) in our observation of the inverse correlation between plasma insulin and homocysteine concentrations in type 2 diabetic patients (2) is highly appreciated. He makes 3 points. The first is that oral antidiabetic medication, particularly metformin, and concomitant lipid-lowering medication, with special reference to fibrates, may unfavorably influence homocysteine levels in our study group of patients with poorly controlled type 2 diabetes (2). It has been suggested that fibrate therapy may lead to the elevation of plasma homocysteine concentrations (3). However, in the group of 26 poorly controlled type 2 diabetic patients we studied, only 1 patient was treated with fenofibrate (200 mg once a day) and his plasma homocysteine was 13.6 nmol/l, which was not the highest value found in this group. Therefore, it is unlikely that fibrate administration will cause hyperhomocysteinemia in our patients.

The impact of metformin treatment on homocysteine levels has been previously studied by Hoogeveen et al. (4), who found that high doses of metformin had no impact on blood homocysteine levels. Moreover, the proportions of metformin users in both groups of poorly and well-controlled patients were similar: 20 of 26 (77%) and 13 of 18 (72%) patients, respectively. It is highly unlikely that the metformin treatment used in our patients may corroborate an interpretation of our study results.

The second point is that diabetic nephropathy, which may be expected to be more prevalent in the poor-control group, is a contributing factor for elevated plasma homocysteine concentrations. Because we could not agree more with this opinion, we assumed the following exclusion criteria for this study: hypertension and any other overt cardiovascular disease, any cardiovascular or cerebrovascular event in the past, microalbuminuria (albumin excretion rate >30 mg/24 h) and macroalbuminuria, proliferative retinopathy, any symptoms of malabsorption, malnutrition or other gastrointestinal dysfunction, autonomic neuropathy, and smoking. To our reckoning, such strict criteria were necessary to exclude most, if not all, of the factors that are believed to be implicated in homocysteine metabolism impairment.

Lastly, Chan (1) rightly raises the issue of the yet unclear role of insulin in the homocysteine metabolism in diabetic and nondiabetic subjects. He argues that insulin resistance, which is associated with endogenous hyperinsulinemia, is also associated with elevated plasma homocysteine concentrations (5). However, hyperinsulinemia found in insulin resistance is the result of the ineffective insulin action (i.e., constitutes a sign of a relative insulin deficiency). This seems to be in agreement with our findings (2).

Recently published animal studies confirm that the role insulin plays in amino acid metabolism is directly involved in the regulation of homocysteine metabolism (6). Therefore, absolute or relative insulin deficiency may be implicated in plasma homocysteine elevation, and, although the metabolism of homocysteine seems to be altered in diabetes, insulin may play a significant yet unclear role.

Finally, we fully agree with Chan's conclusion that more interventional and cohort studies are needed to elucidate the role of insulin and insulin supplementation on plasma homocysteine level in humans.

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Comparison Between 2 Insulin Sensitivity Indexes in Obese Patients

Matsuda and De Fronzo (1) have recently described a new insulin sensitivity index (ISI) calculated from blood glucose and insulin after an oral glucose load, which is highly correlated to the glucose clearance rate during euglycemic-hyperinsulinemic clamp and to the homeostasis model assessment insulin resistance index (HOMA-IR). The authors claim that ISI, which takes into account both hepatic and peripheral insulin sensitivity, should represent a more accurate measure of insulin resistance than HOMA-IR.

We evaluated the correlation between HOMA-IR (2) and ISI in a consecutive series of 767 (141 men, 626 women) obese (BMI >30 kg/m^2) outpatients, with an age of 46.4 ± 13.9 years, a BMI of 36.3 ± 7.1, and a waist circumference of 118.6 ± 15.9 in men and 108.2 ± 12.5 cm in women, with no known history of diabetes or treatments for hyperlipidemia. However, 146 (19%) of them were currently treated for hypertension. Of the patients studied, 82 (10.7%) had fasting plasma glucose (FPG) >7 mmol/l and 153 (20%) had FPG between 6.1 and 7 mmol/l; 162 (21.1%) had diabetes, and 179 (23.3%) had impaired glucose tolerance (IGT) following proposed World Health Organization diagnostic criteria (3). Mean total cholesterol was 5.6 ± 1.2 mmol/l, HDL cholesterol 1.2 ± 0.3 mmol/l, tryglicerides (median [25th–75th percentile]) 1.93 (1.14–2.12) mmol/l, and uric acid 254.8 ± 119.4 µmol/l. Insulin was measured with an enzymatic immunoassay (Roche Diagnostics, Milan, Italy) in the fasting state and 30, 60, 90, and 120 min after a 75-g oral glucose load.

Median values (25th−75th percentile) for HOMA-IR were 4.42 (2.65–5.30), and for ISI 3.11 (2.04–4.90). Patients with IGT or diabetes showed significantly higher HOMA-IR and lower ISI than normotolerant subjects, in both sexes (data not shown). The Spearman's correlation between HOMA-IR and ISI was r = 0.88 in women and 0.87 in men. Both indexes showed significant correlations with triglycerides (r = 0.30 and −0.30 in women, and 0.43 and −0.44 in men, for HOMA-IR and ISI, respectively), HDL cholesterol (r = −0.30 and 0.30 in women, and −0.25 and 0.23 in men, respectively), and uric acid (r = 0.31 and −0.34 in women, and 0.36 and −0.39 in men, respectively).

The newly proposed ISI had been validated in a small population of subjects with different degrees of obesity and glucose tolerance (1). In a much wider sample of obese subjects, the correlation of ISI with clinical parameters related to insulin resistance, such as low HDL cholesterol, hypertriglyceridemia, and hyperuricemia, is similar to that of HOMA-IR, suggesting that the 2 indexes could be similarly useful for the identification of subjects with metabolic syndrome.

Considering its high correlation with clamp-derived measures, the new index has been proposed as a method for assessment of insulin sensitivity in epidemiological studies (1). Although glucose tolerance is often studied in epidemiological studies, standard OGTT requires only 2 venous blood samples (at 0 and 120 min),

and HOMA-IR is calculated from fasting glucose and insulin only. On the other hand, ISI requires the determination of both glucose and insulin in 5 venous blood samples, implying much higher costs. For this reason, further evidence about the sensitivity and specificity of this index should be collected before its use can be recommended in large-scale epidemiological studies.

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Type 1 Diabetes in Sardinia Is Not Linked to Nitrate Levels in Drinking Water

Several environmental factors, mainly dietary and viral (1,2), have been associated with the etiopathogenesis of type 1 diabetes. Although 2 ecological investigations found a positive association between the risk of type 1 diabetes and the intake of nitrates from drinking water (3,4), no correlation between nitrite and nitrate intakes from food and drinking water and the disease was reported in Finland (5). Similarly, no association was found between nitrate content in drinking water and risk for type 1 diabetes in the Netherlands (6). In view of these conflicting data, we examined nitrate intake in Sardinia, the Mediterranean island with a risk for type 1 diabetes that approximates that of Finland (7), by studying variations in levels of nitrates in tap water and bottled water in relation to the incidence of the disease.

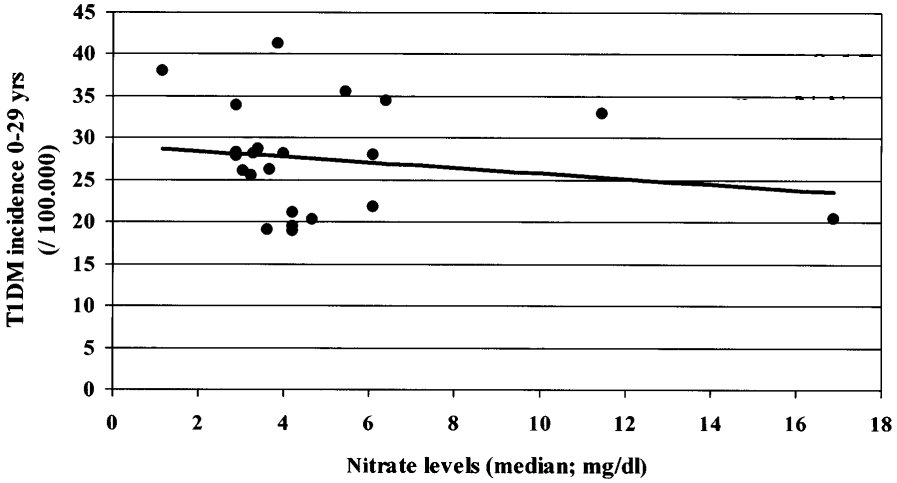

Figure 1—*Correlation between type 1 diabetes incidences and nitrate levels in tap water among the 22 local health authorities.* $r_P = -0.17$; P = *NS.*

Data about the nitrate concentration of tap water in Sardinia during 1993 were obtained from the Environmental Laboratory of the Hygiene Department of Cagliari University and from the databases of the 22 local health authorities. The samples (5,541) of tap water from 353 of 375 municipalities across Sardinia were analyzed. The median value of nitrate level in the water was calculated for each of the 22 local health authorities. Data regarding sales (at the provincial level) of the 11 Sardinian bottled waters in 1994 were made available by Sarda Acque Minerali SpA, the major company supplying the local market. These data cover 75% of local sales. Data on nitrate concentrations of bottled water in 1993–1994 have been published (8). We aggregated the 11 brands in 2 groups according to the nitrate concentration as follows: <10 mg/l (8 brands <3 mg/l) and ≥10 mg/l (3 brands).

The incidences of type 1 diabetes for the age-groups 0–29 and 0–14 years of age were obtained from the Sardinian EURODIAB Register. A description of the registry's method has been reported previously (7). During the period 1989–1998, a total of 1,975 newly diagnosed patients 0–29 years of age were identified, of whom 1,142 were <15 years of age. The male-to-female ratio was 1.55 in both age-groups. The register is estimated to be 87% complete. The crude incidence ratios were calculated using the demographic data of the 1991 census obtained from the National Institute of Statistics and were pooled for the 22 local health authorities and for each of the 4 Sardinian provinces.

The simple correlation (r_p) between type 1 diabetes incidences and the nitrate levels in tap water of the 22 local health authorities shows no effect of increased nitrate concentrations in these waters and the incidence of type 1 diabetes either in the 0–14 years of age (r_p −0.06, *P* = N.S.) or in the group 0–29 years of age (r_p −0.17, *P* = NS) (Fig. 1). There was no effect from sex in the same age-groups (males 0–14: r_p −0.20, *P* = NS; males 0–29: r_p −0.20, *P* = NS; females 0–14: r_p 0.13, *P* = NS; females 0–29: r_p −0.10, *P* = NS). A negative trend between nitrate levels and type 1 diabetes was noted, in contrast with previous reports (3,4).

Similarly, no correlation was found at the provincial level between the consumption of bottled water and the incidence of type 1 diabetes. In fact, the Oristano province, with the highest risk (diabetes incidence 0–14 years of age: 35 of 100,000), has a consumption of bottled

waters with nitrate level >10 mg/l of 60% (expressed as percentage of the total consumption). In the other provinces, listed by decreasing risk for type 1 diabetes, the consumption of nitrate level >10 mg/l was 73% in the Cagliari province (incidence 0–14 years of age: 28 of 100,000), 61% in the Nuoro province (incidence 0–14 years of age: 25 of 100,000) and 82% in the Sassari province (incidence 0–14 years of age: 21 of 100,000).

From our data, it can be noted that nitrate levels for both tap and bottled waters in Sardinia are well within the acceptable maximal concentration of 50 mg/l established by the European Community and also under the recommended levels of 25 mg/l.

We are the first to realize that the exposure estimates used in this study are limited to the content of nitrates in drinking water and that an ideal assessment of whole nitrate exposure would record measurements of individual nitrate intake, including nitrate content in foods and the relevant timing of exposure. For these reasons, even though we cannot exclude a possible role of nitrates in the etiopathogenesis of type 1 diabetes, in Sardinia as in Finland, this role seems not to be related to the nitrate content in the drinking water.

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Puberty as a Risk Factor for Diabetic Neuropathy

In a recent study by Massin et al. (1), cardiac autonomic neuropathy was assessed by heart rate variability (HRV) using 24-h Holter recordings in a cohort of diabetic children and adolescents. It was demonstrated that despite good metabolic control, HRV was reduced in diabetic patients and that abnormalities of the Holter parameters were common findings. Poor long-term metabolic control, diabetes duration, puberty, and microalbuminuria were risk factors for reduced HRV. This is the first study to demonstrate that puberty is an independent risk factor for cardiac autonomic neuropathy.

Recently, we investigated 112 children and adolescents with type 1 diabetes for peripheral and autonomic nerve function. We used the Neurometer (Neurotron, Baltimore, MD) measuring the current perception threshold (CPT) to perform quantitative sensory nerve testing. CPT was determined for 5, 250, and 2,000 Hz frequencies on the left index finger and the left great toe (median and peroneal nerves, respectively). Autonomic nerve function was assessed by cardiovascular reflex tests measuring resting heart rate, heart rate variation to deep breathing, heart rate, and blood pressure responses to standing and to sustained handgrip. Pediatric reference ranges for these methods were previously established in our laboratory (2,3). Abnormal CPT results were observed in 21.3% of the patients and abnormal cardiovascular tests were found in 22.0% of the patients. To assess which factors were associated with peripheral and autonomic dysfunction, multiple logistic regression analysis was performed using the presence of abnormal nerve test results as dependent variables. The initial model included diabetes duration, mean HbA_{1c} level over 1 year, pubertal stages, sex, daily dose of insulin, cholesterol level, triglyceride level, systolic and diastolic blood pressure, and height as independent variables. Diabetes duration, HbA_{1c} level, and puberty remained in a model that was highly predictive of both peripheral and autonomic dysfunction ($P = 0.001$). In this multivariate analysis, late puberty (Tanner stages 4–5) represented an independent risk of peripheral sensory dysfunction (odds ratio 2.5, 95% CI 1.2–5.0, $P = 0.02$), but not for cardiovascular autonomic abnormality.

In the study by Massin et al. (1), Holter assessments were used to detect autonomic dysfunction, which could be more sensitive than cardiovascular reflex tests to demonstrate subtle abnormalities. Their findings extend our knowledge on the effect of puberty on diabetic complications and provide further evidence that pubertal changes may contribute to the development of both peripheral and autonomic neuropathy. In conclusion, puberty should be taken into account as a risk factor for diabetic neuropathy, and screening for nerve dysfunction should be performed in adolescent patients.

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No Relationship Between Antibodies to GAD and Microangiopathic Complications in Young Chinese Diabetic Patients

A higher level of antibodies to GAD has been reported in type 1 diabetic patients with peripheral neuropathy than in those without it (1). However, other researchers (2,3) have not confirmed this finding. We have previously reported the preponderance of type 2 diabetes among Chinese patients with early onset of the disease (4). We have also found a low prevalence of antibodies to GAD, even in patients with acute onset of diabetes (5). In this study, we examined the relationship between antibodies to GAD and diabetic microangiopathic complications in 150 young Chinese diabetic patients.

The prevalence of microangiopathic complications in these 150 diabetic patients has been reported previously (6). All patients were <40 years of age and experienced onset of diabetes before 35 years of age. They were recruited irrespective of their modes of presentation during an 18-month period between 1995 and 1996 from the Prince of Wales Hospital Diabetes Centre in Hong Kong. There were 65 men (mean age ± SD: 30.5 ± 6.1 years) and 85 women (30.8 ± 6.1 years, $P = 0.709$). The Clinical Research Ethics Committee of the Chinese University of Hong Kong approved the study design.

Peripheral sensory neuropathy was assessed using both monofilament and graduated tuning forks. Fundoscopic examination was performed by a diabetologist through dilated pupils. Albuminuria was assessed using both a random spot urine sample for measurement of albumin-to-creatinine ratio (ACR) and a 4-h timed urine collection for measurement of albumin excretion rate (AER). Urinary tract infection was excluded by a midstream urine sample. Albuminuria was defined as a random spot urine ACR ≥3.5 mg/mmol and a 4-h AER ≥20 μg/min in sterile urine. Peripheral neuropathy was considered to be present if 2 of the following were positive: reduced sensation to monofilament examination in any part of the sole with normal skin, a score ≤7/8 by graduated tuning fork, or symptoms of numbness over both lower limbs. Retinopathy was defined as hemorrhages, exudates, laser marks, or history of vitrectomy. Fasting blood was taken for antibodies to GAD. It was measured by a radioimmunoprecipitation (RIP) assay as previously described (7). The normal upper limit for antibodies to GAD using the RIP assay was 18 U in both healthy Caucasian and Asian subjects (4). A level of antibodies to GAD >18 U was considered to be positive.

Of these 150 patients, 50 (33.3%) had microangiopathic complications, 11 (7.3%) had peripheral neuropathy, 34 (22.7%) had albuminuria, and 21 (14%) had retinopathy (6). The anti-GAD–positive ($n = 18$) and anti-GAD–negative patients ($n = 132$) had similar prevalence of microangiopathic complications (peripheral neuropathy: 5.6 vs. 7.6%; albuminuria: 11.2 vs. 24.2%; retinopathy: 16.7 vs. 13.6%, respectively; $P > 0.05$ for all). Patients with or without diabetic complications had similar levels of antibodies to GAD and prevalence of anti-GAD positivity (with neuropathy vs. without: 8.5 ± 4.9 vs. 14.5 ± 22.2 U and 9.1 vs. 12.2%; with albuminuria vs. without: 12.2 ± 20.1 vs. 14.6 ± 21.9 U and 5.9 vs. 13.8%; with retinopathy vs. without: 15.4 ± 24.5 vs. 13.8 ± 21.0 U and 14.3 vs. 11.6%, respectively; $P > 0.05$ for all).

Some studies have recently shown an increased level of antibodies to GAD in type 1 diabetic patients with sensory neuropathy (1). GAD synthesizes the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and it appears that both the pancreatic β-cells and GABA-secreting neurones share a protein that is unusually susceptible to becoming an autoantigen (8). Some researchers have suggested that diabetic neuropathy might allow the leakage of GAD from the damaged peripheral nerves that helped to sustain or reactivate the antibody response (9). However, this finding has not been confirmed by other researchers (2,3).

We have previously reported that in young Chinese diabetic patients, nearly 50% of patients were insulin deficient, but antibodies to GAD were present in only 12% (4). These findings suggest that causes other than autoimmunity might account for the insulin deficiency in these patients. Furthermore, we were unable to demonstrate a difference in the prevalence of anti-GAD positivity between patients with and without neuropathy. There was also no association between the prevalence of anti-GAD positivity or its level and retinopathy or albuminuria. In conclusion, there was no association between microangiopathic complications and antibodies to GAD in young Chinese diabetic patients.

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