OBSERVATIONS

Metformin Lowers Lipoprotein(a) Levels

Recently, a study of women with polycystic ovary disease showed a 42% decrease in levels of lipoprotein(a) [Lp(a)] with the use of metformin (1). However, in two further studies in which the primary goal was to study metformin's effect on fibrinolysis, Lp(a) levels were not decreased (2,3).

To assess the effect of metformin, we identified 23 patients who had lipid profiles performed before and after the initiation of metformin therapy. All patients were receiving a stable dose of anti-lipid medications during the period of assessment. Lp(a) levels were measured by an ultracentrifuge method, the VAP-II method, which has been validated against the Northwest Lipid Research Laboratories in Seattle, Washington (4).

Of the 23 study patients, 9 were female, 5 were African-American, and the remaining patients were white males. The mean age was 64.1 ± 6.1 years. In nine patients, C-peptide levels were available, and the mean level was 3.6 ± 1.8 ng/dl. The average metformin dose was 1.5 ± 0.5 g.

The average Lp(a) level before metformin therapy was 10.9 ± 3.2 mg/dl; after metformin therapy, it was 9.7 ± 5.4 mg/dl ($P = 0.03$). The Lp(a) level rose in seven patients, all of whom had demonstrated a pre-metformin level >10 mg/dl.

Thus, this retrospective nonrandomized noncontrolled study provides evidence that Lp(a) levels decrease with metformin. This finding is especially important because in our study and in one other study, the other available insulin sensitizer, troglitazone, has been shown to increase Lp(a) levels (F.O., D.S.H.B., unpublished observations; 5). Because most of our patients had Lp(a) levels within the normal range in both of our retrospective studies of metformin and troglitazone, a prospective randomized placebo-controlled study that includes only patients with elevated Lp(a) levels in relation to their racial group is needed. The Lp(a) levels should be measured by a single reference laboratory using a radioimmunoassay method.

In conclusion, we have shown in a nonrandomized non–placebo-controlled retrospective outcome study that metformin lowers Lp(a) levels.

DAVID S.H. BELL, MD
FERNANDO OVALLE, MD

From the University of Alabama Medical School, Birmingham, Alabama.
Address correspondence to David S.H. Bell, MB, University of Alabama Medical School, 2000 6th Ave. South, Birmingham, AL 35233.
D.S.H.B. is a member of an advisory panel for, and receives consulting fees, grant support, and research funding from, Bristol-Myers Squibb.

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Troglitazone Interferes With Gemfibrozil's Lipid-Lowering Action

Troglitazone lowers glucose by activating peroxisome proliferator-activated receptor (PPAR)-γ in the nuclei of myocytes and adipocytes (1). Gemfibrozil, by activating PPAR-α in the nucleus of the hepatocytes, results in a decrease in hepatic production of triglyceride and triglyceride-related lipoproteins and in a lowering of serum levels (2). There are no reports of a cross-reaction between gemfibrozil and troglitazone; that is, troglitazone does not stimulate PPAR-α, and gemfibrozil does not stimulate PPAR-γ (2). Therefore, gemfibrozil should have no direct effect on glycemia, and troglitazone should have no direct effect on dyslipidemia.

Recently, we have been conducting a retrospective outcome analysis of our patients who had already been receiving anti-lipid therapy with gemfibrozil and/or β-hydroxy-β-methylglutaryl (HMG) CoA reductase inhibitors (statins) when troglitazone therapy was started. To our surprise, we found that overall triglycerides were elevated with troglitazone use, and we undertook a further analysis of our data to investigate the effects of a combination of gemfibrozil and troglitazone on triglycerides and related lipoproteins.

Before starting treatment with troglitazone, all patients had been taking a statin, gemfibrozil, or both, and the dose of these drugs remained stable throughout the period of the study. The goal of starting troglitazone therapy was either to reduce insulin needs or to improve glycemic control, and with the use of troglitazone, either a significant improvement in glycemic control or a significant decrease in insulin dose was found in patients with those respective goals.

The patients were divided into two groups, those taking gemfibrozil and those not taking gemfibrozil. In the 18 patients taking gemfibrozil, there was an average increase in triglycerides from 197.3 to 250.2 mg/dl ($P = 0.04$) and an increase in VLDL cholesterol from 39.4 to 48.9 mg/dl ($P = 0.04$) despite a statistically significant improvement in glycemic control (HbA$_{1c}$ improving from 7.6 to 7.0%, $P = 0.002$). On the other hand, in the 14 patients not using gemfibrozil but using a statin, there was no change in levels of triglycerides (from 127.3 to 137.5 mg/dl, $P = 0.21$) or VLDL cholesterol (from 25.4 to 27.4 mg/dl, $P = 0.3$) despite an improvement in glycemic control (HbA$_{1c}$, from 8.3 to 7.8%).

These results suggest that gemfibrozil's effect of lowering triglycerides and triglyceride-related lipoproteins is lost when troglitazone is used concurrently. This loss could be the result of troglitazone attaching to PPAR-α as well as to PPAR-γ. Thus, troglitazone may occupy the receptor sites through which gemfibrozil exerts its effects, annulling the lipid-lowering effect. If this effect is shown to occur with all

fibrates, the use of other triglyceride-lowering drugs, such as nicotinamide, would be needed in the diabetic patient who uses troglitazone and has persistent hypertriglyceridemia despite adequate glycemic control. A prospective randomized study of the use of fibrates in the diabetic patient who uses thiazolidinediones is needed to confirm this finding.

DAVID S.H. BELL, MD
FERNANDO OVALLE, MD

From the University of Alabama Medical School, Birmingham, Alabama.

Address correspondence to David S.H. Bell, MB, University of Alabama Medical School, 2000 6th Ave. South, Birmingham, AL 35233.

D.S.H.B. and F.O. are members of an advisory panel for Sankyo Parke-Davis. In addition, D.S.H.B. has received consulting fees, grant support, and research funding from the company.

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Acarbose-Associated Hepatotoxicity

Acarbose, a complex oligosaccharide that acts by competitive and reversible inhibition of small intestine brush-border α-glucosidases thereby delaying absorption of carbohydrates in the gut, is increasingly being used for the treatment of NIDDM (1). Because acarbose is minimally absorbed in unchanged form after oral administration, the drug is widely believed to be safe, with only flatulence as a commonly reported complaint (2) and, rarely, severe gastrointestinal disturbances such as ileus (3,4). We have previously described a patient who developed acute hepatic injury while on acarbose (5). We describe an additional two cases of hepatotoxicity probably related to acarbose treatment. Both cases were submitted to a regional registry of hepatotoxicity in use in Andalusia, Spain, since 1994.

Case 1: A 45-year-old man had received a diagnosis of NIDDM 3 years before presentation and was prescribed acarbose (50 mg three times daily) in May 1994. The drug was well tolerated. In November 1995, a routine laboratory work-up revealed a serum aspartate aminotransferase (AST) concentration of 62 U/l and a serum alanine aminotransferase (ALT) concentration of 127 U/l. His total and direct bilirubin, alkaline phosphatase, and γ-glutamyltransferase were within the normal range. He had a history of nephrolithiasis, no toxic habits, and was not taking any other drugs. Screening for viral hepatitis (B, C cytomegalovirus, and Epstein-Barr virus) and autoimmune liver disorders (anti-nuclear antibodies, anti-smooth muscle antibodies, anti-mitochondrial antibodies, and anti–liver-kidney microsomal antibodies) was negative. Results of iron and copper studies were also negative. Abdominal ultrasonography was normal.

By February 1996, his ALT rose to 153 U/l. Suspecting this increase in liver enzymes could be drug-induced, acarbose was withdrawn in November 1996, and 4 months later the results of his liver test had returned to normal.

Case 2: A 54-year-old nonobese woman was prescribed acarbose (50 mg three times daily) for NIDDM in May 1997. She presented with fatigue and dark urine 5 months later. Her past medical history was unremarkable. She had no toxic habits, and she denied taking any other medications or herbal remedies. Physical examination was normal except for the presence of jaundice. Her AST level was 2436 U/l, her ALT level was 2,556 U/l, her total bilirubin level was 4.67 mg/dl (with a direct bilirubin level of 3.19 mg/dl), her γ-glutamyltransferase level was 601 U/l, and her alkaline phosphatase level was 174 U/l. Serology ruled out viral hepatitis A, B, and C (the latter by polymerase chain reaction), cytomegalovirus, and Epstein-Barr virus. No autoantibodies were present. Ultrasonography showed a normal liver and gallbladder and no expanded bile duct. Acarbose was stopped, and the results of her liver test were normal within 5 months.

The mechanism of drug-induced liver injury can be intrinsic (dose-dependent) or allergic (idiosyncratic), symptoms usually appearing soon after exposure (6). Unpredictable hepatotoxicity may also result from a metabolic aberration, rather than hypersensitivity, and usually develops after latency periods of 1 week to 12 months or longer, with no apparent signs of allergy (7). The temporal relation between institution of acarbose therapy and the onset of hepatic abnormalities, the resolution after stopping the drug, and the absence of alternative explanations strongly suggest the implication of acarbose.

As far as we know, only three cases of hepatotoxicity related to acarbose treatment have been reported (5,8,9). The clinical course of patient 2 was closely similar to that of patients previously described, with liver damage classified as acute hepatocellular injury according to international consensus criteria (10). In contrast, the profile of liver testing in patient 1 is consistent with chronic hepatocellular injury (10). As in the three other patients reported (5,8,9), the features of hypersensitivity (fever, rash, and eosinophilia) were absent. Taking this account together with the relatively long latency period between drug intake and symptom onset, the mechanism of acarbose-induced hepatotoxicity suggests a metabolic idiosyncrasy, caused by the original compound or its metabolites, mainly 4-methylpyrogallol derivatives (11).

Interestingly, the three cases previously reported, as well as the two patients presented here, were Spanish. Moreover, three of them were detected in South Spain, which suggests that genetic variations in acarbose metabolism could account for the hepatotoxicity. In conclusion, hepatotoxicity, although a very rare adverse event of acarbose therapy, can be severe, and clinicians should be aware of this possibility when prescribing the drug.

RAÚL J. ANDRADE, PHD
MARIBEL LUCENA, PHD
JOSE L. VEGA, MD
MARINA TORRES, MD
FRANCISCO J. SALMERÓN, PHD
VICENTE BELLOT, MD
MARÍA D. GARCÍA-ESCAÑO, MD
PEDRO MORENO, MD

From the Liver Unit (R.J.A., M.L., M.D.G.-E., P.M.), Department of Gastroenterology, University Hospital, Malaga; the Gastroenterology Section (J.L.V., M.T.), Hospital Torrecardenas, Almeria; and the Gastroenterology Service (F.J.S., V.B.), University Hospital, Granada, Spain.

Address correspondence to Raúl J. Andrade, Unidad de Hepatología, Servicio de Aparato Digestivo, Hospital Universitario Virgen de la Victoria, Campus Universitario de Teatinos s/n, 29071-Maiaga, Spain. E-mail: andrade@uma.es.

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Maggot Therapy for the Treatment of Diabetic Foot Ulcers

The beneficial effect of fly larvae for wounds was first observed by Baron Larrey, physician-in-chief to Napoleon's armies and by Dr. Joseph Jones, a medical officer during the American Civil War. While working with soldiers who remained wounded for several days in the battlefield, they noticed that maggots developed at the wound destroyed only necrotic tissue (1). Maggot therapy (MT), the intentional treatment of suppurative skin infections with the larvae of calliphorid flies, was first introduced by Baer in 1931 (2). This method was used extensively in the 1930s and early 1940s in >300 hospitals in the U.S. alone (3), but it

was abandoned with the introduction of antibiotics and the use of aggressive surgical debridement. MT was reintroduced in the U.S. in 1989 (4–6) and has been used since the mid-1990s in the U.K. (7) and Israel (8). In the last 8 years, hundreds of patients have been treated by this method. In the present study, we report the treatment of diabetic patients having intractable severe skin lesions with the maggots of the green bottle fly, *Phaenicia (Lucilia) sericata*

A colony of *P. sericata* was maintained in the laboratory. Sterile eggs and maggots were obtained according to Sherman and My-Tien Tran (9). The wound was sealed completely, and surgical pads applied to the dressing to absorb wound exudate were held in place with tape or a bandage (5).

Since 1996, 27 foot ulcers of 22 diabetic patients (17 men and 5 women), aged 41–95 years (mean 64.8), were treated using MT. The patients, 12 of whom were hospitalized and 10 ambulatory, were treated in four departments of Hadassah Hospital, two geriatric hospitals, and one outpatient clinic in Jerusalem. The wounds had existed for 1–48 months (mean 10.3). MT was selected when all other surgical and nonsurgical treatment methods, including hydrogel and hydrocolloid dressings, antibiotics, hyperbaric oxygen, and disinfectants, had failed to heal the wounds. Informed consent was obtained from the patient or, when the patient was unable to give informed consent, from the legal guardian. Depending on the size and depth of the wound, 50–1,000 maggots, 24–48 h old, were applied to the skin two to five times weekly and were left on for 24–72 h. Thereafter, they were washed out of the wound with a jet of sterile physiological saline; if necessary, they were removed with forceps. The wound was photographed after each treatment or, at least, weekly. The wound quality (i.e., necrosis, drainage, and purulence) was recorded during each visit, and the odor of the wound and the degree of pain reported by the patient were noted.

Depending on the size of the wound, the number of treatments varied between 1 and 23 (mean 5.7), and the period of treatment varied between 1 and 45 days (mean 12.0). In 12 patients with superficial wounds, the lesions were debrided after only 1–4 treatments within 1–8 days. Debridement was complete in 18 wounds (66.7%), significant in 6 wounds (22.2%),

and partial in 2 wounds (7.4%); 1 wound (3.7%) remained unchanged. The three wounds that did not respond to MT were located under the sole or between the toes. Because these patients were walking, the maggots were squashed underfoot, and as a result the outcome was poor. In five cases in which diabetic patients were referred for amputation of the leg, the limb was salvaged. In an additional five patients with deep wounds, septicemia had been a serious threat but was prevented as a result of MT. As the therapy progressed, new layers of healthy tissue formed over the wounds. The offensive odor emanating from the necrotic tissue and the intense pain accompanying the wound decreased significantly. The majority of the patients did not complain of major discomfort during the treatment. Six patients with superficial, painful wounds complained of increased pain during treatment with maggots and were treated with analgesics. After maggot debridement therapy, patients were either sent for skin transplant or treated with hydrocolloid dressings or disinfectants.

Reports of successful MT in abscesses, burns, cellulitis, gangrene, ulcers, osteomyelitis and mastoiditis have been published (2,3,10,11). This method is used when antibiotic treatment, surgical debridement, and drainage do not halt progressive tissue destruction. It has been recommended especially for patients with diabetic foot and pressure ulcers.

In our study, maggots debrided the wounds in 12 of 22 patients within 1 week, and the average treatment period was 2 weeks. In a study that started in 1990 and used maggots to treat recalcitrant wounds, MT completely debrided most necrotic wounds within 1 week, demonstrating MT to be significantly more rapid than all other nonsurgical methods (4). Wound healing rates were faster in patients treated with MT than in patients receiving only conventional dressing. Of the 14 wounds that did not receive presurgical MT, 8 became infected postoperatively, whereas none of the 5 wounds treated presurgically with maggots became infected postoperatively.

The main disadvantages of MT are its esthetic and psychological aspects. Care should be taken to restrict the maggots to the area of the wound, using appropriate dressing. Analgesic treatment is recommended in cases in which the wound is too painful.

Different mechanisms of wound healing by maggots have been suggested, for example, *1*) liquefaction of necrotic tissue by secretion of proteolytic enzymes; *2*) digestion of necrotic tissue as food by larvae; *3*) mechanical washing out of bacteria by the serous exudate caused by the irritating effect of maggots in the wound; *4*) destruction of bacteria in the alimentary tract of the maggots, and in the wound, by their excretions, which contain antibacterial substances; *5*) change in the wound of an acidic pH to a beneficial alkaline pH as a result of the ammonia and calcium carbonate excreted by the maggots; *6*) secretion by the maggots of substances with healing properties, such as allantoin and urea; and *7*) formation of granulation tissue resulting from mechanical stimulation of viable tissue caused by the continuous crawling of the larvae and the excretion of growth-stimulating factors (3).

MT appears to be a valuable and cost-effective tool in the treatment of wounds and ulcers that are unresponsive to conventional treatment and surgical intervention.

KOSTA Y. MUMCUOGLU, PHD
ARIEH INGBER, MD
LEON GILEAD, MD
JOCHANAN STESSMAN, MD
REUVEN FRIEDMANN, MD
HAIM SCHULMAN, MD
HELLEN BICHUCHER, MD
INNA IOFFE-USPENSKY, PHD
JACQUELINE MILLER, PHD
RACHEL GALUN, PHD
ITAMAR RAZ, MD

From the Department of Parasitology (K.Y.M., I.I.-U., J.M., R.G.), Hebrew University–Hadassah Medical School; the Departments of Dermatology (A.I., L.G), Internal Medicine (Diabetes Unit) (I.R.), and Rehabilitation and Geriatrics (J.S.), Hadassah Medical Centre; and Hod Jerusalem (H.B.) and Neve Horim (R.F., H.S.), Jerusalem, Israel.
Address correspondence to Kosta Y. Mumcuoglu, PhD, Department of Parasitology, Hebrew University–Hadassah Medical School, P.O. Box 12272, 91120 Jerusalem, Israel. E-mail: kostam@cc.huji. ac.il.

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Magnesium Supplementation in Type 2 Diabetes

Hypomagnesemia is a common finding in diabetic patients with poor metabolic control and is associated with diabetic late complications in cross-sectional studies. However, it is not known whether hypomagnesemia represents an independent risk factor for diabetic complications. So far, the impact of hypomagnesemia on insulin resistance, the development of diabetes, and diabetic late complications is controversial. On the other hand, it is possible that hypomagnesemia is associated with the diabetic state per se as an epiphenomenon. A possible explanation could be increased urinary magnesium loss, especially in patients with poor metabolic control; however, improvement of metabolic control for 3 months did not lead to an increase of plasma magnesium levels (data for a longer period are required) (1). Although magnesium substitution seems to have beneficial effects in diabetic patients with hypomagnesemia, no guidelines concerning dosage and duration of treatment have been established so far. A consensus conference of the American Diabetes Association (2) concluded that prospective long-term studies are needed to determine whether magnesium deficiency increases the risk of complications such as cardiovascular disease, retinopathy, or nephropathy in patients with diabetes. The consensus statement suggests that serum magnesium levels in diabetic patients with risk for hypomagnesemia, e.g., congestive heart failure, coronary artery disease, ethanol abuse, long-term parenteral nutrition, or pregnancy, should be determined. Those patients should receive a replacement therapy if hypomagnesemia can be demonstrated.

Recently, a study by de Lourdes Lima et al. (3) has been published addressing the question of magnesium substitution in patients with type 2 diabetes, of which 47.7% had hypomagnesemia. The authors were comparing the effect of a dose-dependent magnesium substitution on metabolic control and on serum magnesium levels in patients with type 2 diabetes. One group was receiving a half-maximal dosage (20 mmol/day), the other group the maximal therapeutic dosage (40 mmol/day), and the patients were observed for 30 days. The patients were on relatively poor metabolic control on oral and dietary treatment (HbA$_{1c}$ 9.0 and 10.2%, respectively). Although there was no significant rise of plasma magnesium levels in either group, the group receiving the maximal dose could achieve plasma magnesium levels equal to those of healthy control subjects after 1 month of treatment, whereas in the other group, no such effect could be observed. In the group receiving the higher dose of magnesium, a significant fall of fructosamine was reported, whereas glycemia and HbA$_{1c}$ were not influenced.

Two years ago, we also investigated the effect of long-term (3-month) magnesium substitution in patients with type 2 diabetes and hypomagnesemia (4). The patients were receiving 30 mmol/day for 3 months. A significant rise in plasma magnesium levels could be demonstrated only after 3 months, whereas there was no change after 1 and 2 months of treatment. There was a decline of plasma magnesium to pretreatment levels 6 months after treatment. Metabolic control remained unchanged during the whole study. A very recent study was also not able to demonstrate a significant decline in HbA$_{1c}$ levels

or lipid parameters of insulin-requiring type 2 diabetic patients after an oral magnesium substitution for 3 months in a placebo controlled trial (5).

Taking the results of these three studies together, it seems reasonable to start with a high-dose replacement therapy initially for 1 month, or until plasma magnesium levels are normalized. Afterwards, a continuous substitution of magnesium should be performed at a lower dose. This substitution has to be applied continuously or magnesium levels will decline. It seems that the question of how to substitute magnesium in type 2 diabetic patients with hypomagnesemia has been resolved before long-term studies were able to demonstrate beneficial effects of a continuous magnesium substitution. Furthermore, magnesium supplementation evidently exerts no major effect on metabolic control. Studies evaluating the influence of magnesium supplementation on metabolic control are, therefore, no longer of high priority, in our opinion, in insufficiently treated patients, who could benefit from well-established therapies.

NICOLE EIBL, MD
CHRISTOPH SCHNACK, MD
GUNTRAM SCHERNTHANER, MD

From the Medical Department, Rudolfstiftung Hospital, Juchgasse 25, 1030 Vienna, Austria.
Address correspondence to Prof. Guntram Schernthaner, 1 Medical Department, Rudolfstiftung Hospital, Juchgasse 25, 1030 Vienna, Austria.

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Supplement to the Use of a Paired Value of Fasting Plasma Glucose and Glycated Hemoglobin in Predicting the Likelihood of Having Diabetes

We reported that in Hong Kong Chinese with various risk factors for glucose intolerance, the use of a paired value of fasting plasma glucose (FPG) of 5.6 mmol/l and an HbA_{1c} of 5.5% derived from multiple receiver operative characteristic curve (ROC) analysis helped to identify potential diabetic subjects (1). For paired values above these cutoff values, the likelihood ratios (LRs) of this occurring in diabetic subjects were 5.4 and 6.3, respectively, based on World Health Organization (WHO) or American Diabetes Association (ADA) criteria (2,3). Only subjects who had an FPG $\geq$5.6 mmol/l and <7.8 mmol/l (or <7.0 mmol/l, according to the new ADA criteria) and an HbA_{1c} $\geq$5.5% required an oral glucose tolerance test (OGTT) to confirm diabetes, thereby eliminating 77.7% (82.6% with ADA criteria) of the OGTTs performed (1).

With the same concept of using a paired value of FPG and HbA_{1c}, we reanalyzed the data using FPG 6.1 mmol/l (the cutoff for impaired fasting glucose [IFG]) (3) and HbA_{1c} 6.1% (the optimal value corresponding to 2-h plasma glucose $\geq$11.1 mmol/l using ROC analysis) (1) and found very interesting results, as shown in Table 1.

The paired values of FPG $\geq$6.1 mmol/l and HbA_{1c} $\geq$6.1% had 13–17 times increased likelihood to occur in diabetic subjects (depending on WHO or ADA criteria) than in nondiabetic subjects. This was compared with a likelihood of only 0.16 for diabetic subjects with an FPG <6.1 mmol/l and an HbA_{1c} <6.1%. Only those with an FPG $\geq$6.1 and <7.8 mmol/l and an HbA_{1c} $\geq$6.1% ($n = 328$) (or FPG $\geq$6.1 and <7.0 mmol/l and HbA_{1c} $\geq$6.1% if using ADA criteria [$n = 198$]) require an OGTT to confirm diabetes. This could potentially eliminate 88.6% [(2,877 − 328)/2,877] (or 93.1% using ADA criteria) of all OGTTs to be performed.

Those with FPG $\geq$6.1 mmol/l and HbA_{1c} <6.1% have, by definition, IFG (3), and if there are additional risk factors for glucose intolerance, they should have regular yearly screening using a single blood test of FPG and HbA_{1c}. According to our data, their LR of having diabetes is 2.8–3.7. For those with normal fasting glucose (NFG) (i.e., FPG <6.1 mmol/l) (3), regular yearly screening would also be indicated if they had risk factors for glucose intolerance, such as a history of gestational diabetes. For those having NFG and HbA_{1c} $\geq$6.1% or <6.1% and no major risk factors for diabetes, the recommendation for screening would need to be individualized. Longitudinal study is required to confirm the validity of the study.

We believe the idea of using paired values of FPG and HbA_{1c} is very useful in identifying potential diabetic subjects, especially in high-risk groups. However, the modified values of FPG $\geq$6.1 mmol/l and HbA_{1c} $\geq$6.1% would be more practical to use and correlate better with the newly proposed ADA diagnostic criteria for glucose intolerance.

GARY T.C. KO, MRCPI
JULIANA C.N. CHAN, FRCP
CLIVE S. COCKRAM, FRCP

Table 1—Analysis of glucose intolerance data using FPG of 6.1 mmol/l and HbA_{1c} of 6.1%

FPG (mmol/l)	HbA_{1c} (%)	n	ADA criteria		WHO criteria			LR	
			Nondiabetic	Diabetic	Normal	IGT	Diabetic	ADA	WHO
$\geq$6.1	$\geq$6.1	551	95	456	25	96	430	17.22	12.75
$\geq$6.1	<6.1	165	84	81	35	58	72	3.72	2.78
<6.1	$\geq$6.1	400	348	52	224	124	52	0.52	0.54
<6.1	<6.1	1,761	1,688	73	1,309	379	73	0.16	0.16
Total		2,877	2,215	662	1,593	657	627		

IGT, impaired glucose tolerance.

From the Department of Medicine (G.T.C.K.), Alice Ho Miu Ling Nethersole Hospital, Tai Po; and the Department of Medicine and Therapeutics (J.C.N.C., C.S.C.), Chinese University of Hong Kong, The Prince of Wales Hospital, Shatin, Hong Kong, China.

Address correspondence to Dr. Gary T.C. Ko, Department of Medicine, Alice Ho Miu Ling Nethersole Hospital, Tai Po, Hong Kong, China.

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Clinical and Metabolic Effects of Fasting in 41 Type 2 Diabetic Patients During Ramadan

Every year, millions of Muslims fast from dawn until dusk during the lunar month of Ramadan. A Muslim is required to abstain from any oral intake for an average time of 13 h daily during this month. We, therefore, conducted a study on 41 type 2 diabetic patients (30 women, 11 men, mean age 55 years [38–70]) to observe the clinical and metabolic effects of fasting. Nine of the patients were on diabetic diet only, 12 were on a single oral hypoglycemic agent (OHA), and 20 were on combined OHAs. Patients were recruited to the study during the 2 weeks before Ramadan and were asked to note any episodes that might indicate hypoglycemia, and the notes were later reviewed by an experienced physician in the presence of the patient.

Number of the symptomatic hypoglycemic periods, which were not biochemically verified, increased in eight of the patients (19.5%) during Ramadan. None of the patients, however, experienced severe hypoglycemia or neuroglycopenic symptoms (Table 1).

No statistically significant change was observed in mean body weight, BMI, total cholesterol level, or LDL cholesterol level. The mean HDL cholesterol level increased significantly during Ramadan, and it was still significantly higher 3 weeks after Ramadan than that before Ramadan. The mean triglyceride level was significantly lower during the 4th week of Ramadan than it was before Ramadan (Table 2).

Fasting in Ramadan can be considered a controlled partial type of fasting. The common practice is to eat two meals, one before dawn and one after sunset, instead of three. Generally, one would expect limitation of total food intake that might lead to weight loss during the holy month. This is not always the case, however, because a greater variety of foods are consumed in large amounts in dinners eaten at dusk. It is our common experience to see people several kilograms heavier by the end of every Ramadan. Our patients, on a fixed caloric intake, did not gain or lose weight

Table 1—*HbA$_{1c}$ levels and treatment used in eight patients who experienced a higher rate of hypoglycemic episodes during Ramadan fast*

Patient	Weekly hypoglycemic episodes before Ramadan	Weekly hypoglycemic episodes during Ramadan	Treatment received	HbA$_{1c}$ before Ramadan (%)	HbA$_{1c}$ 3 weeks after Ramadan (%)	HbA$_{1c}$ 8 weeks after Ramadan (%)
1	1	2	Diet only	6.6	6.7	6.6
2	1	4	Gliclazide	7.5	7.3	7.2
3	1	4	Gliclazide	7.6	7.2	7.4
4	1	3	Glybornuride	7.6	7.3	7.3
5	1	3	Gliclazide, MF	5.6	5.8	5.9
6	1	9	Glybornuride, MF	7.5	7.6	7.5
7	1	3	Glyburide, MF, AC	8.2	8.6	8.7
8	1	8	Glipizide, MF, AC	9.5	9.6	9.2

AC, acarbose; MF, metformin.

Table 2—*BMI, metabolic control, and lipid profiles of the patients before, during, and after Ramadan of 1997*

	Weight (kg)	BMI (kg/m²)	HbA$_{1c}$ (%)	Total cholesterol (mmol/l)	HDL cholesterol (mmol/l)	LDL cholesterol (mmol/l)	Triglycerides (mmol/l)
Before Ramadan	74.7 ± 11	30.4 ± 3	7.30 ± 1.6	5.36 ± 1.21	1.01 ± 0.20	3.00 ± 0.85	2.76 ± 2.40
During the last week of Ramadan	74.6 ± 11	30.4 ± 4	7.55 ± 1.7 (0.006*)	5.28 ± 1.29 (0.65)	1.11 ± 0.20 (0.004*)	3.08 ± 0.93 (0.40)	2.71 ± 2.30 (0.86)
3 weeks after Ramadan	74.9 ± 11	30.5 ± 4	7.32 ± 1.7 (0.84)	5.28 ± 1.26 (0.67)	1.09 ± 0.20 (0.030*)	3.08 ± 1.01 (0.41)	2.32 ± 1.79 (0.02*)
8 weeks after Ramadan	—	—	7.27 ± 1.6 (0.71)	—	—	—	—

Data are means ± SD (*P*). *P* values are the results of comparisons with values before Ramadan. *Denotes significant difference.

significantly during the Ramadan of 1997. Contrary to the common belief, nobody had reported severe hypoglycemia or neuroglycopenia in patients on OHA therapy during Ramadan (1–6). Eight of our patients had increased weekly incidence of symptoms attributable to low blood glucose that disappeared after Ramadan. Glycemic control in these patients was not affected by these episodes, and metabolic parameters measured in this subgroup were not significantly different from those of the rest of the group. In the whole group, we found a slight deterioration in HbA_{1c} that returned to the initial values early after Ramadan (Table 2). Laajam (3) reported an insignificant increase in the HbA_{1c} levels of his type 2 diabetic patients over the month of Ramadan. Others reported significant decreases in fructosamine and HbA_{1c} levels (2,5).

The available data about the effect of Ramadan on lipid profiles are inconclusive and contradictory. In agreement with our results, a favorable lipid profile has been reported in some of the studies done on healthy people (6,7) and type 2 diabetic patients (8,9) on partial fasting. This improvement is mainly explained by a reduction in weight and improved metabolic control.

The results of this study lead us to conclude that type 2 diabetes is not a contraindication to fasting in Ramadan. Type 2 diabetic patients on single or combination OHAs could observe Ramadan fasting with appropriate instruction about meals and OHA use. However, some patients may still experience an increased number of hypoglycemic episodes.

ALI RIZA UYSAL, MD
MURAT FAIK ERDOĞAN, MD
GÜNAY ŞAHIN, MD
NURI KAMEL, MD
GÜRBÜZ ERDOĞAN, MD

From the University of Ankara School of Medicine, Department of Endocrinology and Metabolism, Ibni Sina Hastanesi, Samanpazari, Ankara, Turkey.

Address correspondence to Gürbüz Erdoğan, MD, Meşrutiyet Cad. 29/3, 06420 Kizilay, Ankara, Turkey. E-mail: derdogan@escort.net.com.tr.

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Impaired Insulin Secretion in Japanese Diabetic Subjects With an A-to-G Mutation at Nucleotide 8296 of the Mitochondrial DNA in tRNA[Lys]

Because mitochondria play an important role in glucose-induced insulin secretion in pancreatic β-cells, mitochondrial DNA (mtDNA) may be involved in diabetes. Recently, we found a novel and heteroplasmic mtDNA mutation in tRNA[Lys] (8296A → G) (the 8296 mutation) that is associated with diabetes (1). We screened 1,216 diabetic subjects and 44 patients with sensorineural deafness and identified this mutation in 11 (0.9%) unrelated diabetic subjects and one (2.3%) patient with sensorineural deafness. The deafness subject was confirmed to have diabetes by the 75-g oral glucose tolerance test (OGTT). Of these 12 positive subjects, 7 had maternally inherited diabetes. Of 12 probands, 7 were deaf. All four family pedigrees we could evaluate had maternal inheritance of diabetes over two or three generations (1). Another mitochondrial point mutation associated with diabetes (2), an adenine to guanine mutation at position 3243 of mtDNA (the 3243 mutation), has been shown to be responsible for up to 1% of diabetes cases, and its clinical characteristics are well known (3,4). To investigate the pathogenesis of diabetic patients with the 8296 mutation, we assessed the insulin secretory capacities of 25 subjects with this mutation.

Insulin secretory capacity was assessed by measurement of plasma insulin and/or C-peptide concentrations at fasting, during 75-g OGTT, after intravenous administration of glucagon (1 mg), or by measurement of C-peptide levels in 24-h urine samples. We designated 12 families as DM1 to DM12 and subjects as DM1-1 to DM12-2. The insulin secretory capacities of the 25 subjects are shown in Table 1. Most of them had impaired insulin secretion, except for two subjects (DM5-1 and DM12-2) with hyperinsulinemia. Two (DM10-1 and DM11-1) subjects were diagnosed as having IDDM, but their insulin secretory capacities were not abolished.

Clinical characterizations of diabetic subjects with the 3243 mutation have been thoroughly evaluated. They had a higher frequency of lean body mass, a younger onset age, and a lower frequency of islet cell antibody (ICA) or GAD antibody (3). The 3243 mutation is believed to cause diabetes via impaired insulin secretion (3,4), and this insulin secretory failure is believed to be progressive. Many diabetic subjects carrying the 3243 mutation who are treated with insulin had been previously successfully treated with sulphonylurea for several years, indicating that they had undergone progressive insulin secretory failure (5).

Most diabetic subjects with the 8296 mutation also tend to exhibit impairment of insulin secretion similar to that of dia-

Table 1—*Insulin secretory capacity of subjects with the 8296 mutation*

Patient number	Age (years)	Onset age (years)	Urinary C-peptide* (nmol/day)	Fasting C-peptide* (nmol/l)	Fasting insulin* (pmol/l)	Glucose (nmol/l)		Insulin (pmol/l)		C-peptide (nmol/l)		Mode of Treatment
						A	B	A	B	C	D	
DM1-1	69	40	7.0	0.5	—	—	—	—	—	—	—	OHA
DM2-1	47	43	—	0.3	14.4	—	—	—	—	—	—	Diet
DM2-2	63	45	—	0.4	28.2	—	—	—	—	—	—	Diet
DM2-3	61	37	—	0.2	—	—	—	—	—	—	—	Insulin
DM2-4	58	40	—	1.0	46.2	—	—	—	—	—	—	OHA
DM2-5	55	50	—	0.6	19.2	—	—	—	—	—	—	OHA
DM2-6	50	40	—	0.8	57.6	—	—	—	—	—	—	Diet
DM3-1	57	41	21.8	0.7	37.2	7.7	17.7	24.6	91.8	—	—	OHA
DM4-1	69	60	—	—	24.0	9.1	16.2	18.6	108.0	—	—	OHA
DM5-1	61	47	24.5	0.7	76.8	—	—	—	—	—	—	OHA
DM6-1	73	61	21.7	0.9	—	—	—	—	—	0.9	1.3	OHA
DM7-1	64	54	11.4	0.3	—	—	—	—	—	0.2	0.6	Insulin
DM7-2	58	46	—	0.7	39.0	—	—	—	—	—	—	OHA
DM8-1	46	35	7.9	0.6	25.8	—	—	—	—	0.3	0.6	OHA
DM8-2	79	57	—	—	—	—	—	—	—	0.3	0.5	Insulin
DM8-3	49	44	—	—	—	—	—	—	—	0.2	0.4	Insulin
DM8-4	40	37	—	—	—	—	—	—	—	0.5	1.2	OHA
DM9-1	56	44	11.1	—	—	8.4	18.4	4.8	11.4	—	—	Insulin
DM10-1	58	31	1.8	0.1	—	—	—	—	—	0.1	0.4	Insulin
DM10-2	50	50	—	—	—	7.2	17.9	24.6	88.2	—	—	‡
DM11-1	75	45	1.1	—	—	—	—	—	—	0.3	0.5	Insulin
DM11-2	69	64	—	—	—	—	—	—	—	0.3	0.9	Insulin
DM 11-3	45	†	—	—	—	5.6	8.4	51.0	122.4	—	—	‡
DM12-1	47	47	—	—	—	5.6	14.4	39.0	132.0	—	—	‡
DM12-2	83	83	—	—	—	7.2	14.0	74.4	456.0	—	—	‡

*Average values (maximum of three measurements); †impaired glucose tolerance; ‡untreated. A, before OGTT; B, 120 min after OGTT; C, before glucagon injection; D, 6 min after glucagon injection.

betic patients with the 3243 mutation. All the subjects treated with insulin had been treated with oral hypoglycemic agents (OHA) initially for several years. Further, many of the subjects treated with OHA had previously also undergone diet therapy without other treatment. Two subjects (DM10-1 and DM11-1) were diagnosed as having IDDM. Their clinical features were not typical for acute-onset IDDM associated with islet autoimmunity. They both had a history of slowly progressive β-cell failure effectively treated by administration of sulphonylurea for >10 years. Autoantibodies to GAD were not detected in 25 subjects, including the two IDDM subjects with the 8296 mutation. In addition, the mean BMI was 21.4 kg/m² and mean onset age was 46 among the 12 probands with the 8296 mutation. These clinical findings resemble those for patients with the 3243 mutation. We suspect that progression of insulin secretion impairment might occur in diabetic subjects with the 8296 mutation in a manner identical to that seen in 3243 mutation subjects.

KEIICHI KAMEOKA, MD
HARUHIKO ISOTANI, MD
KOJI TANAKA, MD
HARUKO KITAOKA, MD
NAKAAKI OHSAWA, MD

From the Department of Internal Medicine (K.K., H.I.), Hirakata City Hospital; and the First Department of Internal Medicine (KT., H.K., N.O.), Osaka Medical College, Osaka, Japan.
Address correspondence to Dr. Keiichi Kameoka, Department of Internal Medicine, Hirakata City Hospital, 2-14-1 Kinya-Honmachi, Hirakata City, Osaka 573-1013, Japan.

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Consequences of Metformin Intoxication

Although the association between lactic acidosis and biguanide antidiabetic agents is well established, this complication is seen only rarely in patients taking metformin (1,2). Accumulation of the drug has been implicated in the development of lactic acidosis, and since metformin is eliminated as the parent compound exclusively by the kidneys (3), such accumulation can only be caused by renal failure (1) or overdosage. These two mechanisms of accumulation are not analogous for several reasons: significant accumulation of metformin does not become apparent until some time after the development of renal failure in patients taking normal dosages of the drug because of the very high clearance in individuals with normal renal function (3), and renal failure is itself associated with the pathogenesis of acidosis because of the decrease in the ability of the kidneys to eliminate protons. In overdosage, because renal function is normal and stable, except in the presence of a preexisting renal abnormality or drugs that affect renal function or circulatory shock, drug accumulation is acute in nature by definition. Metformin intoxication has been reported in patients with psychiatric disorders but not necessarily with diabetes, a condition associated with an increased risk of cardiovascular disease or sepsis, both of which predispose to or may precipitate lactic acidosis.

Thanks to the measurement of plasma metformin concentrations, the present study was carried out to clarify the relationship between the degree of drug accumulation and acidotic complications in patients with metformin intoxication. Patients studied were those declared to the pharmacovigilance of the Lipha Company of France as having ingested a massive dose of metformin and for whom general characteristics, pH and lactate from arterial blood, and plasma metformin concentrations were available (Table 1). Doses ingested were estimated by interview or tablet counting. Hyperlactatemia, defined as an arterial lactate level above the normal range of 2 mmol/l, lactic acidosis, defined as an arterial blood lactate level >5 mol/l and an arterial pH ≤7.35, and plasma glucose abnormalities were analyzed. Major complications, including circulatory shock and death, were also recorded. All complications were assessed with consideration of the effects of intoxications with other agents and elevated plasma metformin concentrations. Plasma metformin concentrations were measured using high-performance liquid chromatography (4) and compared with those obtained from fasting patients with diabetes who were receiving long-term metformin treatment at the recommended dosage. Therapeutic values are 0.6 ± 0.5 mg/l (2).

Nine women and four men with a mean age of 46.9 (range 16–74) years were studied. Eight patients were known to have diabetes treated with metformin; the diagnosis was uncertain for one patient (no. 11). The estimated ingested dose of metformin ranged from 7.65 to 76.5 g. Most patients (10 of 13) had additionally ingested other drugs. Four patients were in clinical shock on admission; these individuals were among the five with the highest arterial lactate levels (note that patients are listed in decreasing order of lactate level in Table 1). Arterial lactate levels were elevated in all patients except two (nos. 12 and 13), and lactic acidosis was apparent in patients 1–7, of whom four were those in shock as described above. Moderate to severe renal impairment was present in patients 1 and 2 (both of whom were in shock), with serum creatinine levels of 342 and 187 μmol/l, respectively. Plasma glucose levels varied widely, ranging from abnormally low (1.1 and 1.9 mmol/l) through to normal, moderately raised (7.1–10.2 mmol/l) and considerably raised (13–27 mmol/l). One patient died (no. 1); this individual presented with clinical shock, lactic acidosis, and hypoglycemia.

Overall, complications (shock, lactic acidosis, hypoglycemia, and death) were observed only in patients who had ingested large quantities of more than one drug. For example, hypoglycemia was noted in patients who had taken a sulfonylurea (nos. 1 and 6) and lactic acidosis was not related

Table 1—*General characteristics of subjects with metformin intoxication*

Patient number	Age (years)	Sex	Diabetes	Dose ingested (g)	Overdosage with other drugs	Clinical shock	Arterial lactate (mmol/l)	pH	Serum creatinine (μmol/l)	Plasma glucose (mmol/l)	Plasma metformin (mg/l)	Treatment	Mortality
1	52	F	Yes	NA	Yes	Yes	35	6.73	342	1.1	50.9	G, A, D	Yes
2	64	M	No	12.75	Yes	Yes	15.6	7.22	187	7.7	13.8	—	No
3	67	F	Yes	76.5	Yes	Yes	12.9	7.30	141	9.9	53.6	D	No
4	38	M	Yes	25.5	Yes	No	12.0	7.34	152	7.1	140	D	No
5	53	F	Yes	NA	Yes	Yes	10.8	7.26	141	27	6.6	G	No
6	32	M	No	76.5	Yes	No	7.2	7.36	115	1.9	35.1	G, D	No
7	74	M	Yes	76.5	Yes	No	6.7	7.28	Normal	19.3	0.3	A	No
8	42	F	Yes	34	No	No	4.7	7.36	116	13	67.6	A	No
9	16	F	No	7.65	Yes	No	4.2	7.49	91	10.2	63.3	G	No
10	55	F	No	34	Yes	No	4.0	7.39	107	7.5	5.8	G	No
11	44	F	Yes (?)	12.75	Yes	No	3.1	7.36	Normal	—	40.1	G, D	No
12	32	F	No	51	No	No	1.5	7.40	68	5.3	1.4	G	No
13	41	F	Yes	25.5	No	No	1.4	7.34	Normal	7.1	1.2	G	No

A, alkalinization; D, dialysis; G, gastric lavage; NA, data not available.

to metformin intoxication in patient 7, who presented with a low plasma metformin concentration (0.3 mg/l). Three patients (8, 9, and 11) showed no signs of lactic acidosis, despite having plasma metformin concentrations that were among the highest in the series (40.1–67.6 mg/l; only one other patient had a plasma drug concentration above this range).

This is the first time that results of this nature, obtained in a series of patients with metformin overdosage, have been reported. The data show that intoxication with metformin is associated with hyperlactatemia in most patients (11 of 13), and that is not surprising, given the effects of metformin on lactate and glucose metabolism (5). Although hyperlactatemia occurred frequently, frank lactic acidosis was less common, noted only in patients who had ingested large amounts of more than one drug, and particularly in patients with shock (who presented with the highest arterial lactate values). Other complications (circulatory shock, hypoglycemia, death) were also noted only in patients with multiple overdosage. These observations are in accordance with those in patients with lactic acidosis after long-term metformin treatment (2): for either acute or chronic exposure to metformin, the prognosis of lactic acidosis is dependent not on the drug itself, but on the presence of underlying disease causing excessive lactate production from damaged tissues or defective lactate elimination or on certain other factors linked to the effects of drugs impairing pulmonary or circulatory function.

The present findings show how difficult it is to establish definitively the link between metformin and the onset of lactic acidosis, a condition that may be caused at least in part by shock syndrome and that may occur without accumulation of metformin (e.g., patient no. 7). Furthermore, metformin accumulation does not necessarily lead to lactic acidosis: patients 8, 9, and 11 had plasma metformin concentrations among the highest in the series and showed no sign of this condition. The term "metformin-associated lactic acidosis" may simply serve to confuse, since it implies that the accumulation of metformin is implicated in the pathophysiology of lactic acidosis, but it is evidently not clear that metformin is indeed the causative factor in these cases. The task, therefore, remains to determine the relative importance of the various factors that contribute to and determine the prognosis of hyperlactatemia. Because the highest arterial levels of lactate were not consistently associated with high plasma concentrations of metformin in this series of patients, intermediate plasma concentrations of the drug (e.g., as in patients 2 and 5; 13.8 and 6.6 mg/l) should be considered not to be significant factors in the pathogenesis of hyperlactatemia. Finally, the patient's underlying hemodynamic condition, not the degree of metformin accumulation, is the main determinant of marked hyperlactatemia. It should also be noted that metformin is a hyperglycemic, rather than a hypoglycemic, agent under conditions of either normal therapeutic use or massive accumulation; accordingly, hypoglycemia was recorded only in patients who had taken a sulfonylurea. In conclusion, in the light of these data and of data presented previously, metformin can no longer be considered to be in itself a toxic drug.

JEAN D. LALAU, MD
CATHERINE MOURLHON, MD
ANNE BERGERET, MD
CHRISTIAN LACROIX, MD

From the Service d'Endocrinologie-Nutrition (J.D.L., C.M.), Hôpital Universitaire, Amiens; the Service de Pharmacovigilance (A.B.), Lipha, Lyon; and the Laboratoire de Pharmacocinétique (C.L.), Hôpital Général, Le Havre, France.
Address correspondence to Jean D. Lalau, Hôpital Sud, 80054 Amiens Cédex 1, France.

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Three Cases of GAD65 Antibody–Positive Diabetes With Ketosis and Abrupt Onset Resulting in Non-Insulin-Dependent State

A new etiologic classification has been recently proposed for diabetes (1), with types 1 and 2 as the major forms. Type 1 diabetes is characterized by pancreatic β-cell destruction, and these patients are prone to ketoacidosis. This type usually leads to insulin dependence, although some patients could gradually change from non-insulin-dependent to insulin-dependent status. Most cases of type 1 diabetes are considered autoimmune and are characterized by autoantibodies such as islet cell antibody (ICA), GAD65 antibody, IA-2 antibody, or insulin autoantibody (IAA). Type 2 diabetes includes the most prevalent diabetes, resulting from insulin resistance with insulin secretory deficiency. In type 1 diabetes, ketosis seldom occurs spontaneously, but sometimes arises associated with infection, debilitating illness, or starvation. Autoantibodies against pancreatic β-cell antigens are usually undetected because β-cells are not destroyed.

We recently treated three cases of GAD65 antibody–positive diabetes with an insulin-requiring state at disease onset. As shown in Table 1, the three were 33, 56, and 36 years old (BMI 45.2, 24.0, and 22.1 kg/m^2) at onset. They were hospitalized because of thirst, polyuria, general fatigue, and weight loss. They had no family history of diabetes, past history of hyperglycemia, or glycosuria. On admission, plasma glucose concentrations were 56.4, 36.6, and 27.3 mmol/l. All had ketonuria. Insulin therapy was immediately started, and plasma glucose and HbA$_{1c}$ levels were well controlled within the normal range. They discontinued insulin therapy within 2–6 years after diabetes onset. Plasma glucose and HbA$_{1c}$ levels had been well controlled with caloric restriction for another 2–5 years (until the present) after insulin therapy was discontinued, during which time BMI decreased (41.4, 22.8, and 20.6 kg/m^2). They had apparently not changed

Table 1—Clinical features of three cases at onset

Case number	Age (years)	Sex	BMI (kg/m²)	Plasma glucose (mmol/l)	HbA₁c* (%)	Urine ketone body	HLA DR	DQ
1	33	M	45.2	56.4	15.1	+	DRB1*0802	DQA1*0401 DQB1*0303
							DRB1*1101	DQA1*0501 DQB1*0402
2	56	M	24.0	36.6	13.7	+	DRB1*15 or 16	DQA1*0101 DQB1*0602
							DRB1*1001	DQA1*0101 DQB1*0602
3	36	M	22.1	27.3	14.2	+	DRB1*13	DQA1*03 DQB1*0604
							DRB1*0901	DQA1*03 DQB1*0301

*Normal range 4.0–6.0%.

daily physical activity. In all three patients, insulin secretion was preserved (measured by fasting plasma C-peptide [normal mean ± SD; 0.50 ± 0.10 nmol/l]: 1.56, 0.79, and 0.53 nmol/l, all measured in 1997) after insulin therapy was discontinued.

Although GAD65 antibodies were detected at onset, titers in all cases decreased to the normal range within 4 years after onset (Fig. 1). Neither IAA nor IA-2 antibodies were detected at onset. None had other autoantibodies or clinical evidence for other autoimmune diseases, such as autoimmune thyroid disease or adrenal insufficiency. GAD65 and IA-2 antibodies were detected using the radioligand binding assay (2). The upper level of the normal range for GAD65 antibody assay was estimated to be 0.020 (mean SD of 115 healthy control subjects is ± 0.006 and +3 SD is 0.012) (2). We participated in GADA proficiency testing (Immunology of Diabetes Society), and our assay

showed the highest sensitivity and specificity (100% validity, 100% consistency, 100% sensitivity, and 100% specificity, lab ID No. 190). IAA was measured using the modified method of Vardi et al. (3).

We sometimes experience a temporary fall in insulin requirements in some patients with type 1 diabetes after initial treatment with insulin, known as the "honeymoon period." This inevitably returns to insulin dependence within several months. Thus, the three patients should be distinguished from the honeymoon period.

In our patients, positive GAD65 antibody status became negative during follow-up. Although it is unclear why the GAD65 antibody status changed, we speculate possibilities as follows. First, after the autoimmune reaction to islets started, for some reason it stopped. In our patients, intensive insulin therapy may be one of the reasons. It has been reported that prophylactic insulin treatment may prevent diabetes in

rodent models of autoimmune diabetes. In a human pilot trial, prophylactic insulin treatment may decrease the onset rate (4). These findings may be explained by induction of immunologic tolerance to insulin. If the change of GAD65 antibody status in our patients were explained by modulation of the immune system by insulin administration, we propose that these cases be considered type 1 diabetes with a non-insulin-dependent state at present.

Second, GAD65 antibodies could be transiently positive without autoimmune destruction of pancreatic β-cells. It has been reported that expression of GAD appears to be glucose-dependent (5). It may be that in patients with a tendency to generate the GAD65 antibody, high plasma glucose levels could cause GAD65 antibody positivity, and GAD65 antibody status may be changed by normalizing plasma glucose levels. If the change of GAD65 antibody status in our patients were explained without immune modulation, these cases should be considered type 1 diabetes with non-insulin-dependent state at present.

Although we would like to classify these patients as type 1 diabetes with non-insulin-dependent state at present, more discussion is needed because we cannot neglect the possibility of type 2 diabetes. Recognition of these cases is important in the proper classification of diabetes and for understanding type 1 diabetes pathophysiology.
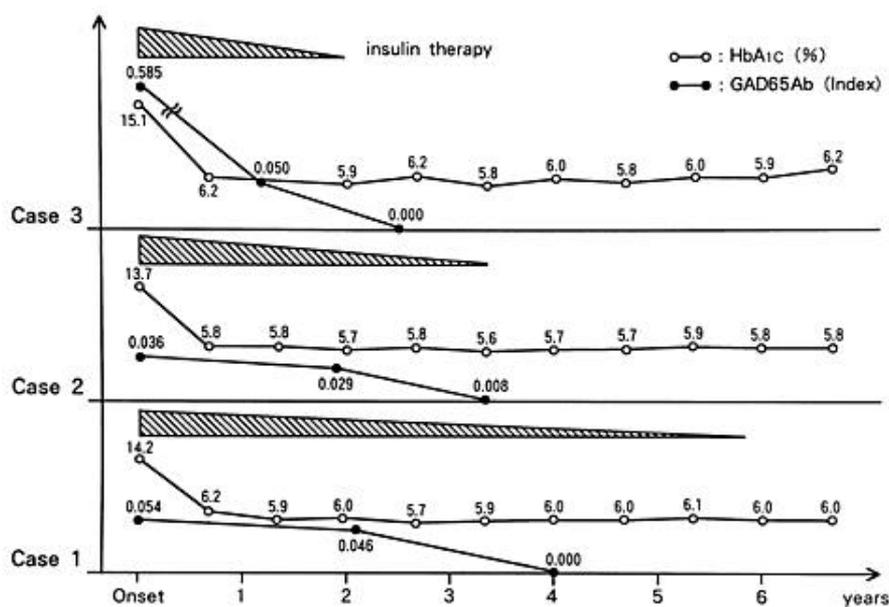
JIRO MORIMOTO, MD
TARO MARUYAMA, MD
AKIRA KASUGA, MD
YUKAKO OZAWA, MD
AKIRA KOBAYASHI, MD
SHINSUKE FUNAKOSHI, MD
RYOJI IWASAKI, MD
YUTAKA SUZUKI, MD
AKIRA SHIMADA, MD
TAKAO SARUTA, MD

Figure 1—Clinical course of three patients ○, HbA₁c; ●, titers of GAD65 antibody.

From the Department of Internal Medicine (J.M., Y.O., A.S., T.S.), Keio University School of Medicine; Tokyo Denryoku Hospital (A.Ka.); Chugai Diagnostics Science (A.Ko.), Tokyo; and the Social Insurance Saitama Chuo Hospital (T.M., S.F., R.I., Y.S.), Saitama, Japan.

Address correspondence to Jiro Morimoto, MD, Department of Internal Medicine, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. E-mail: akirasmd@wa2.so-net.ne.jp.

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COMMENTS AND RESPONSES

Ceiling Effect Reduces the Validity of the Diabetes Treatment Satisfaction Questionnaire

The Diabetes Treatment Satisfaction Questionnaire (DTSQ) is considered to be a suitable instrument for intervention studies that compare treatment effects (1,2). However, an important characteristic of this measure has not yet been discussed in the literature. The six-item treatment satisfaction scale of the DTSQ can range from 0 (very dissatisfied) to 36 (very satisfied) (2). Petterson et al. (1) recently described severely skewed treatment satisfaction scores (median [interquartile range]) for 126 patients treated with diet (35 [31–36]), 404 patients treated with tablets (35 [32–36]), and 204 patients treated with insulin (34 [30–36]). Almost half of the tablet-treated and diet-treated patients (48 and 43%) and about one-third (36%) of the insulin-treated patients had a maximum treatment satisfaction score of 36. For the patients treated with insulin, this percentage was 31. The following negatively skewed DTSQ scores have been reported in our recent study (3): 30.4 ± 4.9 (mean ± SD) and −1.1 (skewness). Reanalyzing these data yielded that two-thirds (66.0%) of the patients had a total treatment satisfaction score of ≥30. Comparable DTSQ scores have also been reported in other studies (4,5). The consequence of a very negative skew is a so-called ceiling effect, i.e., it is almost impossible to measure improvement or to distinguish among various grades of excellence (6). In this letter, we describe two methods that can be used to counteract skew. First, Ware and Hays (7) compared a six-point satisfaction scale (ranging from "very satisfied" to "very dissatisfied") with a five-point evaluation scale (ranging from "excellent" to "poor"). They concluded that the latter showed greater response variability, reliability, and validity than the former (7). A second method has been described by Streiner and Norman (6). Researchers can use seven value labels, of which only two are used to differentiate among various degrees of treatment dissatisfaction. For the DTSQ items 1, 6, and 8, for example, this can be accomplished by the use of the following value labels: 6 (extremely satisfied), 5 (very satisfied), 4 (quite satisfied), 3 (satisfied), 2 (a bit satisfied), 1 (dissatisfied), and 0 (very dissatisfied).

FRANS POUWER, MSC
FRANK J. SNOEK, PHD
ROBERT J. HEINE, MD, PHD

From the Departments of Medical Psychology (F.P., F.J.S.) and Endocrinology (R.J.H.), Research Institute for Endocrinology, Reproduction and Metabolism, Vrije Universiteit Hospital, Amsterdam, the Netherlands.

Address correspondence to Frans Pouwer, MSC, Department of Medical Psychology, Van der Boechorststraat 7, 1081 BT, Amsterdam, the Netherlands. E-mail: f.pouwer.psychol@med.vu.nl.

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Micronized Fenofibrate in the Management of Dyslipidemia

In a recent American Diabetes Association position statement (1), recommendations were made for the management of dyslipidemia in adults with diabetes. In those requiring drugs for the treatment of elevated levels of LDL cholesterol, HMG CoA reductase inhibitors (Stating) were listed as the first choice for treatment. In those with hypertriglyceridemia, after optimizing glycemic control, gemfibrozil was recommended. In those with hypertriglyceridemia plus elevated levels of LDL cholesterol, statins were indicated to have a moderate effect. In those with combined hyperlipidemia (elevation of both triglyceride and LDL cholesterol levels), after

optimizing glycemic control, high-dose statins were recommended as first choice, and a combination of statins plus gemfibrozil were recommended as second choice.

At the time when the review was written, these were the drugs available in the U.S. Recently, however, another fibric acid derivative, micronized fenofibrate, has also been approved by the Food and Drug Administration and is now marketed. Because it has been approved for several years in a large number of other countries, there is wide experience with it in the treatment of both those with and those without diabetes. The drug is potent in reducing triglycerides and increasing HDL cholesterol. As well, it has a greater effect in lowering LDL cholesterol than does gemfibrozil. Hence, in future recommendations, it should be considered as first-line not only in the treatment of hypertriglyceridemia that persists after glycemic control is improved, but also as first choice in the treatment of combined hyperlipidemia. Its use as a single agent in the latter condition would avoid the combination of a statin and gemfibrozil, thereby reducing the possibility of myositis.

GEORGE STEINER, MD, FRCP(C)

From the Department of Medicine, The Toronto Hospital and The University of Toronto, Toronto, Ontario, Canada.

Address correspondence and reprint requests to Dr. George Steiner, Department of Medicine, Room NUW9-112, The Toronto Hospital (General Division), 200 Elizabeth St., Toronto, Ontario, Canada MSG 2C4.

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Association Between Fibrinogen Levels and Insulin Resistance

Imperatore et al. (1) have recently published an impressive cross-sectional study on the relationship between fibrinogen and the metabolic syndrome usu-ally termed "syndrome X." This study clearly demonstrated in a large and relatively young population that high fibrinogen levels were associated with some symptoms of the metabolic syndrome (high plasma glucose, triglycerides, blood pressure, and low HDL cholesterol) or with their combination, independent of various confounders such as age, smoking, coexisting inflammatory diseases, or use of drugs. Thus, there is now strong evidence that hyperfibrinogenemia is a characteristic of syndrome X, as previously suggested (2). In fact, disturbances of the hemostatic system, i.e., excess of plasma fibrinogen and defective fibrinolysis, have been recently added by Reaven to the revised definition of the metabolic syndrome (3).

As mentioned by Imperatore et al., the link between hyperfibrinogenemia, hyperinsulinemia, and insulin resistance is not fully elucidated. Aside from a single study by Landin et al. (4), there do not appear to be any studies in which direct measurements of insulin sensitivity, such as glucose clamp or minimal model procedure, have been used to evaluate the relationship between fibrinogen and insulin resistance. Landin et al. reported a negative correlation between fibrinogen levels and the rate of glucose disposal during a euglycemic clamp technique ($r = -0.35$, $P < 0.05$; 22 men, 11 with mild untreated hypertension). Surprisingly, no correlation was observed between fibrinogen and fasting insulin ($r = 0.06$, NS).

We have investigated the association between plasma fibrinogen levels, basal insulin, and insulin sensitivity in a random sample of 36-year-old nondiabetic nonhypertensive patients, 20 men and 30 women, with BMIs ranging from 18.6 to 35.9 kg/m². Insulin sensitivity was assessed with the minimal model procedure over a 180-min intravenous glucose tolerance test with iterative sampling, as previously described (5). Plasma insulin was determined by radioimmunoassay and fibrinogen by the method of Clauss. The two major findings of our study were a highly significant negative correlation between fibrinogen and insulin sensitivity ($r = -0.76$, $P < 0.0001$) (Fig. 1) and a positive correlation between fibrinogen and basal insulin ($r = 0.59$, $P < 0.0001$) (Fig. 2). We performed a partial correlation analysis to assess the influence of BMI on these data: the negative relationship between fibrinogen and insulin sensitivity
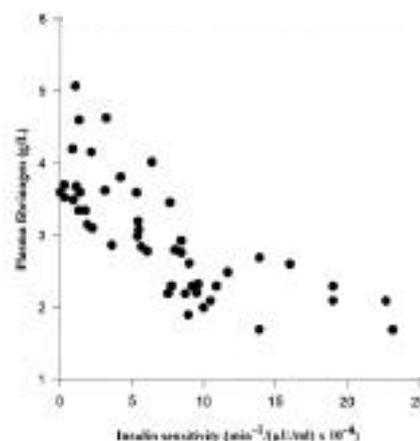

Figure 1—*Negative correlation between plasma fibrinogen and insulin sensitivity measured with the minimal model procedure (n = 50, r = −0.76, P < 0.0001).*

remained significant after adjustment for BMI ($r = -0.64$, $P < 0.0001$). These results provide further evidence for the connection between insulin resistance and hemostatic disorders in the pathogenesis of the metabolic syndrome. Nevertheless, they do not allow analysis in terms of causality.

A role for free fatty acids (FFA) has been proposed to explain the association between fibrinogen and insulin resistance, since hepatic fibrinogen synthesis should be stimulated by FFA (6). A defective fibrinolysis with high plasminogen activator inhibitor-1 (PAI-1) levels may be found in type 2 diabetic patients, in relation to the amount of visceral fat (7). Finally, there is a large body of literature emphasizing the
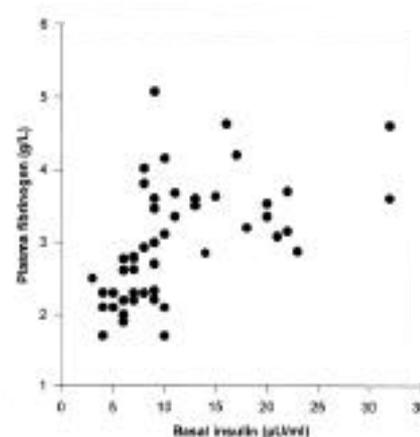

Figure 2—*Positive corelation between plasma fibrinogen and basal insulin (n = 50, r = 0.59, P < 0.0001).*

pathogenetic role of tumor necrosis factor-α (TNF-α) in the insulin resistance states, and it is now well known that inflammation is associated with both hypercoagulability (increased fibrinogen) and hypofibrinolysis (increased expression of PAI-1) (8).

Large prospective studies involving quantification of insulin sensitivity and measurement of various metabolic and hemostatic parameters, especially FFA and PAI-1 or cytokines such as TNF-α, are needed to clarify these points.

ERIC RAYNAUD, PHD
JEAN-FRÉDÉRIC BRUN, MD, PHD
ANTONIA PEREZ-MARTIN, MD
JACQUES MERCIER, MD, PHD

From the Centre d'Exploration et de Réadaptation des Anomalies Métaboliques et Musculaires (CER-AMM), Lapeyronie University Hospital, Montpellier, France.
Address correspondence to E. Raynaud, PhD, CERAMM, Unité d'Exploration Métabolique, CHU Lapeyronie, 34295 Montpellier cedex 5, France.

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Response to Raynaud et al.

We thank Raynaud et al. (1) for their thoughtful comment on the association between insulin resistance and fibrinogen levels. In our work, a large unselected group of normoglycemic men was studied by measuring fasting plasma insulin along with the classical components of the metabolic syndrome (i.e., blood pressure, fasting plasma triglycerides, HDL cholesterol, and glucose); we conclude that it is very likely the condition of insulin resistance rather than hyperinsulinemia per se that is related to hyperfibrinogenemia (2). Raynaud et al., by directly measuring insulin resistance with the minimal model procedure, provide an important and missing piece of information nicely elucidating the up to now poorly investigated issue of the relationships among hyperfibrinogenemia, hyperinsulinemia, and insulin resistance.

We fully agree with Raynaud et al. that these findings should not be interpreted in terms of causality, since cause-effect relationships cannot be established by cross-sectional studies. Furthermore, we would like to expand the interpretation of our own data and that obtained by Raynaud et al. Tough evidence exists supporting a possible role for insulin resistance in the pathogenesis of hyperfibrinogenemia; however, based on current knowledge, other concurrent or alternative interpretations should be explored as well. In particular, because the metabolic syndrome may influence the development of cardiovascular disease, it cannot be ignored that hyperfibrinogenemia may be an epiphenomenon rather that a causative factor in the process of atherogenesis. Plasma fibrinogen is known to rise acutely in response to a number of conditions including endothelial damage and inflammation, both involved in the pathogenesis of atherosclerosis (3). In support of this hypothesis is the independent relation—found in our study after careful exclusion of individuals with clinically evident cardiovascular disease—between fibrinogen levels and some well-established cardiovascular risk factors, such as LDL cholesterol and smoking, which do not cluster with the components of the metabolic syndrome.

In conclusion, a clear association exists between the metabolic syndrome and hyperfibrinogenemia; this association is likely mediated by insulin resistance. Further studies, in particular intervention studies, are needed before either insulin resistance or hyperfibrinogenemia are firmly established as cardiovascular risk factors (4).

OLGA VACCARO, MD
GIUSEPINA IMPERATORE, MD
GABRIELE RICCARDI, MD

From the Department of Clinical and Experimental Medicine, Federico II University Medical School, Naples, Italy.
Address correspondence to Olga Vaccaro, Dipartimento di Medicina Clinica e Sperimentale, Universita Federico II, Via S. Pansini 5-80131, Napoli, Italy.

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Autoimmunity and Intraperitoneal Insulin Treatment by Programmable Pumps

Lack of relationship

Continuous intraperitoneal insulin infusion (CIPII) by programmable pumps is a promising therapy for patients with type 1 (insulin-dependent) diabetes, since it improves metabolic control and decreases the frequency of severe hypoglycemia (1). But this treatment leads to an increase of anti-insulin immunogenicity (2–4), as shown by sustained elevated levels of anti-insulin antibodies. The report of five cases of hyperthyroidism in type 1 diabetic patients treated with intraperitoneal (IP) insulin infusion (5) has raised the question of a more general stimulation by CIPII of autoimmunity in type 1

diabetic patients, this population being genetically more susceptible to autoimmune disorders. This study prompted us to examine the evolution of organ-specific and non–organ-specific antibodies before implantation and then during long-term CIPII treatment.

Informed consent was given by 28 patients with type 1 diabetes who were C-peptide negative. Their mean age (± SD) was 40 ± 9 years, and their mean duration of diabetes was 21 ± 9 years. Of these patients, 13 were women. One of the women was known to have chronic thyroiditis with hypothyroidism (treated with 0.125 mg/day Levothyroxine).

Before implantation, all the patients had been treated by continuous subcutaneous insulin infusion (100 U Velosuline; Novo Nordisk, Copenhagen) for at least 6 months. Then, they were implanted with an IP insulin delivery pump (Minimed MIP 2001, Sylmar, CA, $n = 16$; Siemens Promedos ID 3, Stockholm, $n = 7$; Infusaid Model 1000, Norwood, MA, $n = 5$) using Hoechst 21 PH semisynthetic genapol stabilized insulin (Frankfurt, Germany) with a concentration of 100 or 400 U/ml, according to the device. Duration of IP insulin treatment was at least 24 months (range 24–60 months) and represented a cumulative follow-up of 83 patient-years.

Before implantation, and then every year after implantation, the following parameters were determined for each patient: anti-insulin (AIA), anti-thyroglobulin (ATG), anti-thyroperoxidase (ATPO), gastric parietal cell (PCA), smooth muscle (SMA), mitochondrial (AMA), liver-kidney microsome (LKM), anti-nuclear (ANA), anti-endomysium (EmA), and anti-gliadin (AGA) antibodies.

AIA was measured by radioimmunoassay according to a previously described method (4). Results are expressed as the percentage of total radioactivity that can be precipitated. ATG and ATPO were determined by a two-step immunoluminometric assay and a competitive luminescence immunoassay, respectively (BRAHMS; Henning, Berlin, Germany). ANA, EmA, SMA, AMA, PCA, and LKM antibodies were detected by a standard indirect immunofluorescence technique with appropriate substrates: Hep2 cell line for ANA (Sanofi Pasteur, Marnes la Coquette, France), monkey esophagus for EmA, and rat tissues (kidney, stomach, and liver) for the other anti-

bodies (Biomedical Diagnostics, Marne la Vallée, France). An anti-human IgA conjugate was used for EmA and a polyvalent conjugate for the others. Dilution limits were 1:5 for EmA and 1:50 for the other antibodies. IgG and IgA antibodies to gliadin were detected by enzyme-linked immunosorbent assay; cut-off values were 20 U (Biomedical Diagnostics). Comparison of antibody levels was made by nonparametric tests for paired values.

The results show that before implantation, the sera of 19 of 28 patients were negative for all the tested antibodies. Nine patients (six women, three men) presented at least one positive antibody. Eight patients (28%) had organ-specific antibodies (five patients were positive for ATPO, three patients for PCA), two patients had ANA, and one had SMA.

After implantation and during IP insulin treatment, no newly positive serum appeared: the sera of the 19 of 28 patients that were negative before implantation remained negative for all the tested antibodies.

Among the nine patients with at least one positive antibody, no variation was observed in the titer of PCA, ANA, SMA, AMA, or LKM antibodies or in AGA IgG and IgA or EmA antibodies. The titer of ATPO antibodies from the five already positive sera varied during IP insulin treatment. After 2 years of IP insulin treatment, an increase was observed in two patients (before IP insulin treatment: 269 and 725 U/ml; after 2 years: 1,268 and 1,797 U/ml), and a decrease was observed in three patients (before IP insulin treatment: 2,801, 2,962, and 1,585 U/ml; after 2 years: 2,098, 1,643, and 1,007 U/ml) (normal values are <100 U/ml). After 4 years of IP insulin treatment, ATPO titers were again fluctuating. One of the patients developed clinical hyperthyroidism.

Mean insulin antibody level increased during treatment by CIPII, as previously reported (4) (before implantation: 21.4 ± 4%; 2 years after implantation: 31 ± 7%, $P < 0.01$). But no significant difference in insulin antibody level was observed either before or after implantation between the group of 9 patients with at least one positive antibody and the group of 19 patients with negative sera (before IP insulin treatment: 15.9 ± 5 and 23.8 ± 4%; after 2 years: 27 ± 8 and 36.3 ± 6%, respectively).

Type 1 diabetes, especially in women, is often associated with other clinical or subclinical organ-specific autoimmune

manifestations (6). Thyroid diseases are the most common of these. Non–organ-specific autoantibodies are also frequently observed (7). In our population, before implantation, eight patients had at least one positive organ-specific antibody, and one of them was already known to have Hashimoto's hypothyroidism. This represents a 28% prevalence of clinical or subclinical autoimmune manifestations. These results are similar to those found by Betterle et al. (8), showing a 21% prevalence of clinical or latent autoimmune manifestation, and to those of Perros et al. (9), reporting a 17% prevalence of thyroid disease in type 1 diabetic women. The 10% prevalence of non–organ-specific autoimmunity is also comparable to the results of the literature (7).

After implantation and throughout the 2 years of follow-up during treatment by CIPII, the prevalence of autoimmune manifestations did not change, as shown by the fact that no newly positive serum appears. One patient who already had thyroid autoantibodies developed clinical hyperthyroidism with ophthalmopathy. This represents an incidence of 2%, which is not different from the 2.4% incidence of new thyroid disease appearing in type 1 diabetic patients described by Perros et al. (9). Conversely, our results are in disagreement with those of Jeandidier et al. (5), who reported five cases of hyperthyroidism in 62 diabetic patients treated by IP insulin infusion. These last findings have not been confirmed in any other French implantation center of the EVADIAC group, and a center effect is probably involved.

As reported before (2–4), anti-insulin antibody increased during IP insulin treatment, but no correlation was observed between anti-insulin antibody level and autoimmune manifestations.

Thus, our findings confirm that IP insulin treatment by implantable pumps induces an increase of anti-insulin antibody levels but do not demonstrate any stimulation of organ-specific or non–organ-specific autoimmune manifestation. This seems reassuring for the future of this treatment.

VÉRONIQUE LASSMANN-VAGUE, MD
MARIELLE SANMARCO, MD
PIERRE-JEAN LEJEUNE, PHD
CHRISTIAN ALESSIS, MD
PHILIPPE VAGUE, MD, PHD
PAULINE BELICAR, MD

From the Service d'Endocrinologie (V.L.-V., C.A., P.V., P.B.), Diabétologie, Centre Hospitalier Universitaire Timone; and the Laboratoire d'Immunologie (M.S.) and the Laboratoire de Biochimie Endocrinienne et Métabolique (P.J.L.), Faculté de Médecine, Marseille, France.

Address correspondence to V. Lassmann-Vague, CHU Timone, 13385 Marseille cedex 5, France. E-mail: pvague@ap-hm.fr.

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Response to Lassmann-Vague et al.

Autoimmunity and intraperitoneal insulin

Type 1 diabetes is an autoimmune disorder that can be associated with a variety of organ-specific autoimmune disorders. The thyroid gland is a common autoimmune target in individuals without other clinical autoimmune diseases, and in diabetes, clinical thyroid autoimmunity has an increased frequency (1). A report by Jeandidier et al. (2) described, 3 years ago, an 8% incidence of hyperthyroidism in a group of 62 type 1 diabetic patients receiving continuous intraperitoneal insulin infusion (CIPII) using programmable pumps. These authors appropriately suggested, based on their observations, that there was a potential for CIPII to induce autoimmunity. This form of insulin delivery triggers insulin antibody responsiveness in 20–30% of patients so treated (3–5). The mechanism of insulin responsiveness is remarkably similar to autoimmune activation in that HLA genes control responsiveness to an autoantigen or cross-reactive antigen. How autoimmune responsiveness is controlled by HLA response or susceptibility genes in the context of cross-reactive antigen is elegantly described in molecular detail in the cow's milk model for human type 1 diabetes (6). In some individuals, multiple autoimmune responses can occur directed at a variety of target organs, e.g., thyroid and islets, that result in disorders, e.g., Graves' disease and type 1 diabetes.

The question is whether insulin immune responsiveness by CIPII in patients with type 1 diabetes can activate immune responsiveness to other organs. Because immune responsiveness is determined by ligand-receptor binding in a communication system that is highly interconnected between T- and B-cells, in which there are $\sim10^{22}$ and $\sim10^8$ possible epitope binding sites, respectively, virtually any possible result is conceivable given the correct genotype (7,8). Thus, the concept of whether CIPII is associated with hyperthyroidism is reasonable to pursue.

In their letter, Lassmann-Vague et al. (9) evaluate nine anti-insulin autoantibodies against a variety of tissues before and during CIPII treatment. During their 83 patient-year evaluation, no changes were observed, yet their patients had a significant increase in insulin antibody responsiveness. These data argue against organ-specific autoimmune activation by CIPII. The most clinically relevant answer can be approached by examining whether thyroid disease prevalence is greater in diabetic patients using CIPII than in those not using CIPII. Lassmann-Vague et al. (9) report an argument against such cross-reactive autoimmune activation. Their 28 patients did not show the 8% rate of hyperthyroidism, but rather the expected rate of ~2% observed in non–CIPII-using diabetic subjects (10). The two reports by Jeandidier et al. (2) and Lassmann-Vague et al. (9) are not statistically compelling, since only two fewer patients with hyperthyroidism, as in the case of Jeandidier et al., or one more case with hyperthyroidism, in the case of Lassmann-Vague et al., would result in opposite conclusions to the question posed above. Although neither paper has the statistical power to refute or confirm the thyroid-CIPII question, data from the EVADIAC (a well-organized group of French CIPII clinical centers), alluded to in the Lassmann-Vague et al. letter, includes >300 CIPII-treated patients in whom no increased thyroid disease was apparently observed at the centers. If these data are formalized from the Lassmann-Vague personal communications, then the autoimmune CIPII issue would appear to be resolved.

M. ARTHUR CHARLES, MD, PHD

From the Diabetes Research Program and the Department of Medicine, University of California, Irvine, California.

Address correspondence to M. Arthur Charles, MD, PhD, University of California at Irvine, Medical Science Bldg. I, Room C250, Irvine, CA 92697. E-mail: macharle@uci.edu.

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