

## OBSERVATIONS

## Direct Comparison of Insulin Lispro and Aspart Shows Small Differences in Plasma Insulin Profiles After Subcutaneous Injection in Type 1 Diabetes

Two rapid-acting insulin analogs, lispro and aspart, are now available for clinical use (1).

The aim of our study was to compare the plasma-insulin profiles of these analogs after subcutaneous injection in patients with type 1 diabetes.

Fourteen patients with type 1 diabetes (six men and eight women, [mean  $\pm$  SEM] 35.4  $\pm$  3.3 years of age [range 22–59], HbA<sub>1c</sub> 7.3  $\pm$  0.3% [reference range 3.2–5.4], BMI 24.7  $\pm$  1.1 kg/m<sup>2</sup>, and diabetes duration 22.9  $\pm$  2.6 years) were recruited for the study. Only two patients had measurable C-peptide levels (0.04 and 0.11 nmol/l). All patients were on multiple-injection therapy with a breakfast insulin dose of 11.1  $\pm$  0.7 U (range 6–14) and no intermediate-acting insulin in the morning.

The study was designed as a single blind randomized crossover study. On the first day, seven patients were randomized to insulin lispro (Humalog, U-100; Eli Lilly, Indianapolis, IN), and the other seven were randomized to insulin aspart (NovoRapid, U-100; Novo-Nordisk, Bagsvaerd, Denmark). On the second study day (5–21 days later), the patients received the alternative insulin analog. The patients continued their usual insulin treatment between the study days.

All patients arrived to the clinic fasting. After an initial blood sampling, they were given 10 U s.c. of one of the insulins in the abdominal wall at 7:30 A.M. A standardized breakfast, which had an energy content of 418 kcal and a nutrient content of 21 g protein, 11 g fat, and 59 g carbohydrates, was served immediately thereafter. Plasma free insulin and

blood glucose concentrations were measured, as indicated in Fig. 1. If blood glucose was 3.5 mmol/l or lower, 20 ml glucose 30% was injected. One patient was excluded from the analysis because, by mistake, he took a large extra dose of insulin the night before the study.

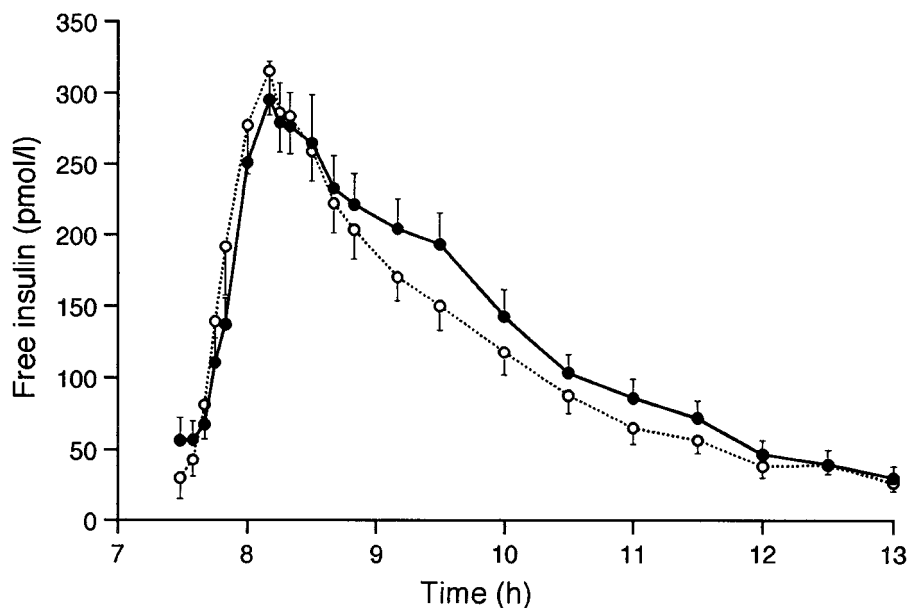
Free insulin was measured after polyethylene glycol precipitation by Mercodia Iso-Insulin (ELISA; Mercodia AB, Uppsala, Sweden), a two-site enzyme immunoassay containing two monoclonal antibodies against insulin. Identical results were obtained when equimolar concentrations of human insulin, insulin lispro, and insulin aspart were tested, indicating 100% cross-reactivity between lispro, aspart, and human insulin in this assay.

Blood glucose was analyzed with the Hemocue method (Hemocue, Mission Viejo, CA). Serum IGF binding protein-1 was determined by an immuno-enzymometric assay with a kit from Medix Biochemica (Kauniainen, Finland). Differences among groups were tested with Wilcoxon's signed-rank test. Areas under the curve (AUC) were calculated with the trapezoidal method.

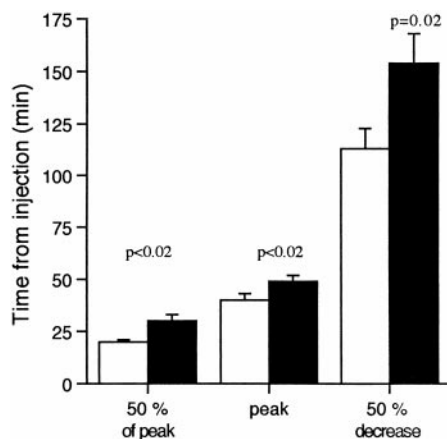
The fasting-free insulin concentration was 56  $\pm$  16 pmol/l before administration of insulin aspart and 30  $\pm$  15 pmol/l before administration of insulin lispro (NS) (Fig. 1). Both insulin analogs gave marked peaks of free insulin concentrations—

lispro at 40  $\pm$  3 min and aspart at 49  $\pm$  3 min after injection, respectively ( $P = 0.01$ ). The maximum insulin concentration was 316  $\pm$  31 pmol/l on insulin lispro and 295  $\pm$  27 pmol/l on insulin aspart (NS) (Fig. 1). The increase from 0 to 15 min after injection was 109  $\pm$  17 pmol/l after injection of insulin lispro and 53  $\pm$  11 pmol/l after injection of insulin aspart ( $P = 0.02$ ). Fasting insulin lispro levels reached 50% of peak concentration at 20  $\pm$  1 min and aspart at 30  $\pm$  3 min ( $P = 0.02$ ) (Fig. 2). The decrease of free insulin concentration from peak concentration to 50% of the maximum concentration was found at 113  $\pm$  10 min during insulin lispro and 154  $\pm$  14 min during insulin aspart ( $P = 0.02$ ) (Fig. 2). The fasting blood glucose concentration was 11.2  $\pm$  1.0 mmol/l before injection of insulin aspart and 13.7  $\pm$  1.4 mmol/l before injection of insulin lispro (NS). The course of the blood glucose profiles was similar, with peak concentrations after 40 min, and there was no difference between total AUC.

The concentration of fasting IGFBP-1, a liver-derived protein (2), was 12.3  $\pm$  3.4  $\mu$ g/l on insulin aspart and 16.8  $\pm$  3.9  $\mu$ g/l on insulin lispro. The concentrations fell to 8.3  $\pm$  1.4 and 13.5  $\pm$  4.5  $\mu$ g/l 2.5 h after injection and to 7.4  $\pm$  1.6 and 11.5  $\pm$  4.1  $\mu$ g/l after 5.5 h, respectively (all differ-



**Figure 1**—Plasma concentrations of free insulin in 13 patients with type 1 diabetes after a 10 U single subcutaneous injection of insulin lispro (○) and insulin aspart (●) at 7:30 A.M. immediately before breakfast. The values are means  $\pm$  SEM.



**Figure 2**—Time (minutes) from subcutaneous injection of 10 U of insulin lispro (□) and insulin aspart (■) from fasting levels to 50% of the peak free insulin concentration, peak concentration, and 50% decrease from peak concentration in 13 patients with type 1 diabetes. The values are means  $\pm$  SEM.

ences between the insulin analogs were nonsignificant).

The time period from injection to half the maximum value and to peak insulin concentration was significantly shorter during insulin lispro, indicating a faster absorption of this insulin.

The impression from previous studies is that insulin aspart gives a somewhat broader peak than lispro (3,4). We found a significantly more rapid lowering of the free insulin concentration to 50% of the peak concentration with lispro. The plasma profile of free insulin obtained is dependent on the rate of absorption of insulin from subcutaneous tissue and the elimination of insulin from the circulation. Because the metabolic clearance of insulin analogs is predominately receptor mediated (5) and because lispro and aspart have about the same affinity for the insulin receptor (1), it seems probable that they will have about the same clearance rate and that the lowering of free insulin after the peak is mainly due to the rate of absorption.

From the clinical point of view, the most important observation is that insulin analogs are absorbed much faster than human insulin after subcutaneous injection, with higher insulin peaks and shorter duration of action (3,4). Because high postprandial glucose and lipid levels have been emphasized as risk factors, especially in type 2 diabetes (6), this may be of importance. In this respect, the rela-

tively rapid rise of free insulin levels after injection of insulin lispro might be an advantage. The slightly slower decrease of free insulin concentrations after the insulin peak of insulin aspart might influence the need for daytime basal insulin and the need for a snack between the main meals of certain patients.

We previously found no difference in IGFBP-1 concentrations when using human regular insulin or insulin lispro (7), and in this study there was no difference between insulin aspart and lispro.

The main finding of our study is that the free insulin profiles of aspart and lispro resemble each other, but insulin lispro shows a more rapid uptake, reaches the maximum peak concentration earlier, and shows a more rapid decline than insulin aspart. We believe this finding may be of clinical importance.

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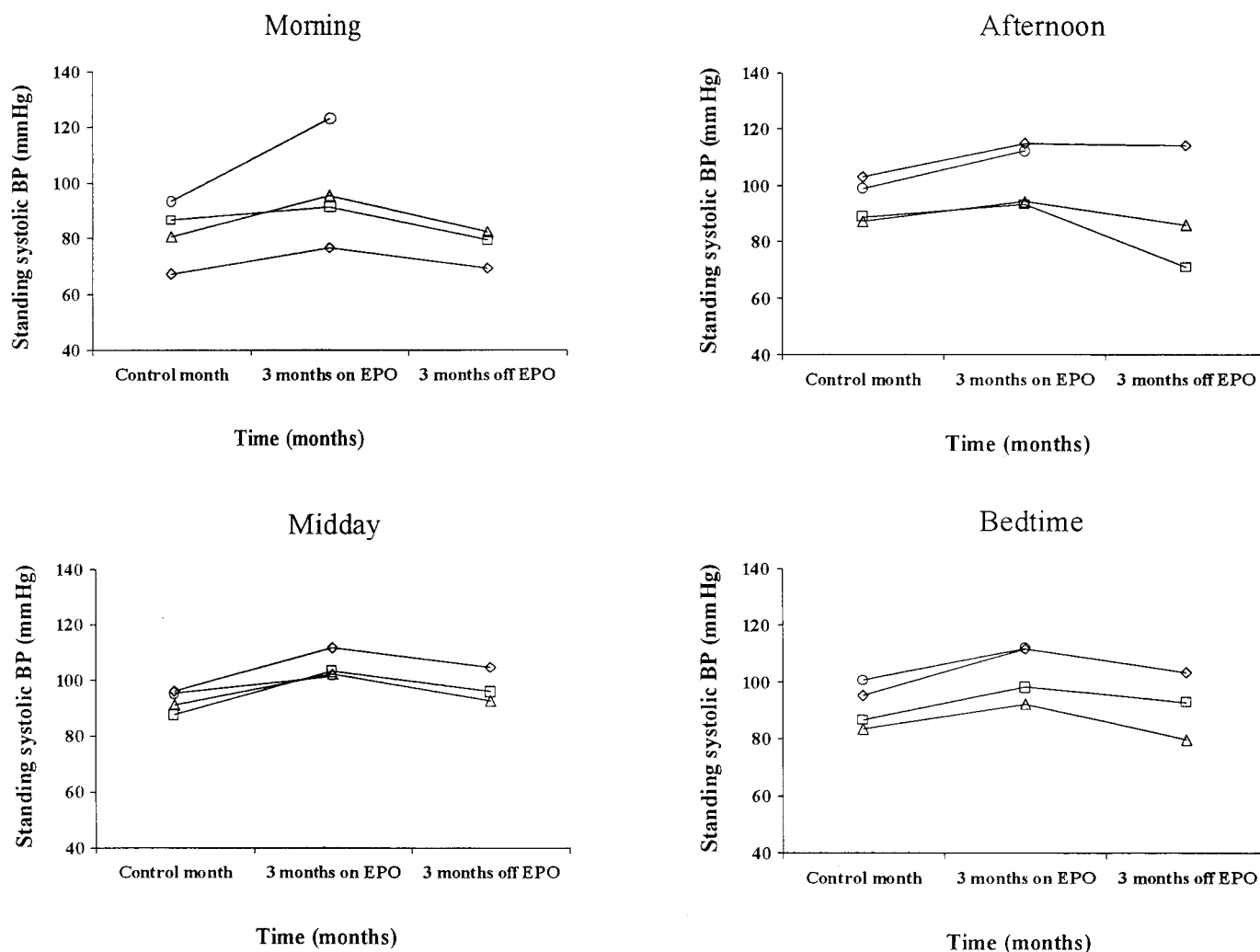
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## Erythropoietin Treatment of Postural Hypotension in Anemic Type 1 Diabetic Patients With Autonomic Neuropathy

A case study of four patients

**P**ostural hypotension (PH) is a feature of autonomic failure that may result from various causes and can be very difficult to treat. Recently, we and others (1–3) have shown that some patients with severe symptomatic autonomic neuropathy from type 1 diabetes (type 1 DAN) have a normocytic anemia associated with erythropoietin (EPO) deficiency. The treatment of these patients with EPO rapidly corrects their anemia (1) and improves their overall well-being. Preliminary observations by Hoeldtke et al. (3) of the effect of a maximum of 9 weeks of EPO treatment on blood pressure (BP) demonstrated a positive response in four cases of PH in type 1 DAN patients, but evaluation of BP was limited to only two to three readings be-



**Figure 1**—Monthly average systolic BP on standing during the control month, the third month on EPO, and third month off EPO at the four different times of the day. ○, patient 1; ◇, patient 2; □, patient 3; △, patient 4. For statistical analysis, see text.

fore and after EPO treatment. These results stimulated the present study, in which we investigated the effect of EPO treatment for 3 months on supine and standing BP in four anemic EPO-deficient type 1 DAN patients.

The patients were treated with recombinant human EPO (25 IU/kg s.c. thrice weekly). All four patients were women (48, 41, 30, and 30 years of age with a duration of diabetes of 13, 24, 19, and 11 years, respectively) and each had normocytic anemia with inappropriately low serum EPO levels. The known causes of anemia were excluded. All of the patients included in the study had pronounced symptomatic PH and at least one other symptom of autonomic neuropathy. The autonomic function tests were severely abnormal. The patients were followed up for 7 months (1-month control phase, 3

months on treatment, and 3 months off treatment). The study was stopped in patient 1 after 3 months on EPO because of serious problems from foot sepsis. Three patients were already on fludrocortisone, the dose of which was kept constant during the study.

During the 7-month study, the patients performed regular BP readings themselves using an automatic sphygmomanometer (Omron 2000) after undergoing several training sessions according to a standardized protocol. The BP recordings were taken before meals and insulin injections at four different times (morning, midday, afternoon, and bedtime) thrice weekly. The supine BP reading was compared with the lowest value within 5 min of standing. Each patient took between 672 and 1,584 BP readings during the course of the study. The statistical sig-

nificance of the BP response to treatment with EPO was determined by fitting a nonlinear function of time to each patient's series of mean BP data (4). The applied function modelled a constant BP during the control phase, followed by a gradual increase after EPO administration and a gradual decrease after withdrawal of EPO. This model of assumed BP response was developed on the basis of observations made in patients with chronic renal failure during EPO treatment; it is specific in that it only detects BP responses attributable to the EPO stimulus and therefore excludes measurement errors.

Pronounced symptomatic PH was present in the four patients during the control month (monthly average systolic postural fall:  $44.9 \pm 3.5$ ,  $41.4 \pm 6.4$ ,  $52.3 \pm 5.1$ , and  $21.7 \pm 4.9$  mmHg, respectively). EPO treatment for 3 months

resulted in an increase in standing systolic and diastolic BP in all four patients at all four times throughout the day (Fig. 1). The BP decreased again after EPO withdrawal (Fig. 1). After 3 months of EPO treatment, standing systolic BP in the four patients increased from 5 to 30, 6 to 15, 5 to 13, and 9 to 17 mmHg in the morning, midday, afternoon, and at bedtime, respectively. The standing mean BP in the morning increased significantly in all patients ( $P = 0.001-0.036$ ). There was also a significant increase in standing mean BP in patients 2-4 ( $P = 0.001$ ), patients 3 and 4 ( $P = 0.001$  and  $0.003$ ), and patients 1, 2, and 4 ( $P = 0.001-0.025$ ) at midday, in the afternoon, and at bedtime, respectively. There was usually a small and highly variable increase in supine BP, so that the effect of EPO treatment on the actual BP drop was also highly variable. EPO treatment corrected the anemia in all four patients, and hemoglobin increased from 116, 99, 100, and 115 g/l to 140, 131, 124, and 132 g/l, respectively. Hemoglobin decreased to the baseline level by the end of the third month off treatment.

PH in diabetic autonomic neuropathy is extremely variable and difficult to assess. The study of treatment is thus rendered exceptionally difficult. We believe that by using numerous objective measurements of BP (up to 1,584 readings), we have reduced potential bias and present valid results. The greatest benefit to our patients was the increase in standing BP, which was significant at almost all times throughout the day. This was accompanied by a decrease in dizziness on standing, though we appreciate that this was an open study and therefore prone to bias. Of course, some of the improvement in overall well-being might be attributed to the improvement of the anemia in all the patients.

The effect of EPO in raising standing BP might be mediated through a number of different mechanisms. Vasoconstriction might occur after increased norepinephrine levels after injection of EPO (5) or as a result of increased binding of nitric oxide by the greater concentration of hemoglobin, thus reducing its capacity to vasodilate (6). Furthermore, elevated levels of hemoglobin may increase blood viscosity and thereby augment peripheral vascular resistance (7). EPO may also increase vascular sensitivity to angiotensin II, thus further promoting vasoconstriction (5). BP is very sensitive to blood-volume

changes, although EPO-increasing red cell mass does not normally increase blood volume (8). Despite the above-mentioned indirect effects of EPO, some authors postulate that EPO exhibits direct pressor effects on vascular smooth muscle cells (9) or enhances DNA synthesis in smooth muscle cells, thereby contributing to vascular hypertrophy (10). EPO might also have a direct trophic effect on the nervous system, as shown recently in neuroblastoma cells (11). These interesting observations need to be developed, and their relevance to effects of EPO on BP in our patients remains speculative.

In summary, we have demonstrated that treatment with EPO increases the standing BP in diabetic patients with severe autonomic neuropathy. This effect, combined with the correction of the anemia, can lead to considerable improvement in the well-being of these severely disadvantaged patients and may provide an additional treatment for seriously affected patients when other measures have failed.

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## COMMENTS AND RESPONSES

### Hyperhomocysteinemia and Macroangiopathy in Type 2 Diabetes

Elevated homocysteine is widely regarded as an independent risk factor for macrovascular disease, particularly in patients with type 2 diabetes (1). The increased prevalence of macroangi-



opathy in type 2 diabetes is further demonstrated by Buyschaert et al. (2). The authors found a significant increase in the prevalence of macroangiopathy in type 2 diabetic patients with hyperhomocysteinemia. Their data, however, should not be overinterpreted because the etiological role of homocysteine in atherosclerosis in the presence of macroangiopathy is far from clear (3).

First, the metabolism of homocysteine is altered for a period of several days following acute ischemic events, such as myocardial infarction (4) and stroke (5), as a result of an increased release from damaged tissues. This is caused by methylation of DNA, RNA, and various proteins, leading to an increase in S-adenosylhomocysteine and subsequently to increased homocysteine production (3). Furthermore, nephropathy may reduce homocysteine clearance in type 2 diabetic patients. To our knowledge, there are no published studies examining the time course of homocysteine concentrations following acute ischemia, which may be silent. Hence, assessing homocysteine levels in patients with preexistent macroangiopathy may produce misleading results. This may in part account for the conflicting results regarding the association of homocysteine levels with insulin resistance that have been reported in various studies (6).

Second, in their study, Buyschaert et al. (2) have rightly taken into account the prevalence of potential confounders of plasma homocysteine concentrations, namely fibrate and metformin therapy (2,7). However, the authors failed to comment on the use of hormone replacement therapy (HRT) in their cohort of patients (mean age 63 ± 10 years), the majority of whom were women (82 of 122). Combined estrogen-progesterone HRT has been shown to lower homocysteine concentrations in postmenopausal women (8,9), with the greatest benefit being seen in women with high levels of homocysteine (8). Estrogens may lower homocysteine through several mechanisms that include changes in the transamination pathway of methionine catabolism (10) and an increased activity of renal methionine synthase (11), the enzyme responsible for the remethylation of homocysteine to methionine. Additionally, estrogens may increase LDL-receptor expression, which facilitates LDL binding to homocysteine and its subsequent clearance

(12). While the debate goes on regarding the potential cardiovascular benefit of HRT in general use, its selective use in subjects with high homocysteine concentrations may be justified. It would therefore be important for Buyschaert et al. to clarify the distribution of homocysteine and folate concentrations in relation to the prevalence of HRT usage in their study.

In summary, there is little doubt that homocysteine is implicated in the pathogenesis of diabetic macroangiopathy. The correlation of its level would be most valuable in primary prevention studies because its concentration in patients with macroangiopathy is difficult to interpret.

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**Response to Chan et al.: hyperhomocysteinemia and macroangiopathy in type 2 diabetes**

**W**e thank Chan et al. (1) for their interest in our work (2) and their relevant comments. We agree that there is still much to be learned about the relationship between homocysteine and atherosclerosis, as was recently stressed by Christen et al. (3). We also agree that homocysteine metabolism is altered for a few days after acute ischemic events, as reported by Egerton et al. (4) and Lindgren et al. (5). Therefore, patients with acute cardiovascular events were excluded from our analysis and could not interfere with the interpretation of our results.

Chan et al. (1) pertinently mention the role of fibrates and metformin as potential confounders of homocysteine concentrations. Our data in type 2 diabetic patients confirm that fibrates significantly increased homocysteine levels, whereas

there was no influence from metformin treatment.

Chan et al. (1) also comment aptly on postmenopausal use of estrogen-progesterone hormone replacement therapy (HRT). Recent reports show that homocysteine levels increase after menopause (6,7) and that HRT lowers homocysteine concentrations (8,9).

In our cohort, 84% of women ( $n = 68$ ) had reached menopause, and reliable information on both menopause and HRT status was available for 46 of them. A total of 20 (43%) of the latter patients were treated with HRT (HRT<sup>+</sup> group), whereas 26 had no hormonal substitution (HRT<sup>-</sup> group). Age, diabetes duration, BMI, smoking prevalence, HbA<sub>1c</sub>, and creatinine clearance were comparable between the two groups. In contrast, menopause duration was significantly shorter in HRT<sup>+</sup> group than in the HRT<sup>-</sup> group ( $16 \pm 4$  vs.  $22 \pm 6$  years,  $P = 0.002$ ). There was a nonsignificant trend for lower homocysteine levels in patients with HRT ( $13.2 \pm 5.3$  vs.  $17.4 \pm 9.9$   $\mu\text{mol/L}$ ,  $P = 0.07$ ). Folic acid and vitamin B<sub>12</sub> concentrations were comparable ( $7.4 \pm 3.9$  vs.  $7.2 \pm 3.6$  ng/ml and  $398 \pm 167$  vs.  $521 \pm 298$  pg/ml) in the HRT<sup>+</sup> and the HRT<sup>-</sup> groups, respectively. Plasma cholesterol (C), LDL-C, and triglycerides were also similar in the HRT<sup>+</sup> and the HRT<sup>-</sup> groups, whereas HDL-C and C/HDL ratio were  $58 \pm 17$  vs.  $49 \pm 13$  mg/dl ( $P = 0.058$ ) and  $4.0 \pm 0.9$  vs.  $5.0 \pm 2.5$  ( $P = 0.068$ ) in patients with and without HRT, respectively. It is of interest to mention that macroangiopathy was significantly less frequent in HRT<sup>+</sup> than in HRT<sup>-</sup> patients (10 vs. 39%,  $P = 0.04$ ), as also observed in nondiabetic subjects (10).

In view of the trend in association between the use of HRT and lower homocysteine levels, as well as the reduced prevalence of macroangiopathy in HRT<sup>+</sup> women, we agree with Chan et al. (1) that it could be of interest to measure homocysteine in postmenopausal type 2 diabetic patients as another incentive for selective use of HRT when high homocysteine is found.

In summary, our findings demonstrate the current importance of homocysteine determination in routine initial evaluations of all patients at risk for macroangiopathy. Additional caution is needed when interpreting such data be-

cause of various confounding factors, including the use of HRT.

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## The Role of Hepatitis C Virus Genotypes in Development of Autoimmune Diseases

In the study by Betterle et al. (1), it was stated that there was no association between the occurrence of organ-specific/nonorgan-specific autoimmune diseases and chronic hepatitis C virus (HCV) infection. It was evident in previous studies (2,3) that there was a clear association among the antigenic subtypes of the HCV, prognosis of disease, autoimmune disease development, and response to the interferon- $\alpha$  (IFN- $\alpha$ ) treatment. Also, these studies stress that some specific subtypes, e.g., HCV-1b, may have worse prognosis and poor response to the treatment (4). This result may be due to the fact that the various genotypes of the HCV have various immunological responses in the host. So, it seems logical that the development of the HCV subtype-specific immune response may stimulate the formation of antibodies directed to some organs or may be generalized, which are named organ- and/or nonorgan-specific autoimmune diseases. Therefore, various types of viral antigens may play an important role during the development of the various types of autoimmune diseases via hosts' immune responses. In the study (1), there were some autoantibody positivity results before IFN- $\alpha$  treatment (one patient with low-titer classic islet cell antibody pattern, one with high levels of anti-GAD antibodies, two with anti-glucagon cell antibody, four with microsomal antibodies, two with thyroglobulin autoantibodies, five with parietal cell antibodies, and—from nonorgan-specific antibody group—two patients with anti-nuclear antibody [ANA] positivity); but, it is not clear which HCV subtypes were infecting antibody-positive patients. Like this situation, the HCV subtype-specific host-immune response may be responsible, as with the autoimmune diseases that developed during IFN- $\alpha$  treatment. Therefore, it would have been useful in this study to assess whether a clinical linkage was

present between the viral subtype and autoimmunity.

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HCV genotypes	Patients	Autoantibodies	Thyroid antibodies	PCA	ICA	ANA
1b	23 (32.8)	4 (17)	0	1	2	1
1a	6 (8.6)	2 (33.3)	0	1	1	0
2c	8 (11.4)	3 (37.5)	0	2	0	1
3a	29 (41.4)	6 (20.7)	4	1	1	0
4	4 (5.7)	0 (0)	0	0	0	0

Data are n and n (%). ANA, anti-nuclear antibodies; ICA, islet-cell antibodies; PCA, parietal cell antibodies.

## Response to Sanver et al.

In this issue of *Diabetes Care*, the letter by Sanver et al. (1) reported previous studies that demonstrated evidence of a correlation among the hepatitis C virus (HCV) antigenic subtypes, prognosis of disease, autoimmune disease development, response to the interferon (IFN)- $\alpha$  treatment, and various immunological responses in the host. In our 70 patients, we carried out the type of HCV genotypes as published (2). The correlations between HCV genotypes and autoimmunity are reported in Table 1.

From our data, the autoimmunity varies from 37.5% in 2c to 0% in 4 HCV-genotype groups. Interestingly, all of the patients who tested positive for thyroid autoantibodies are in the 3a HCV genotype group, but it is important to consider that in our group, 29 (41.4%) patients show 3a genotype. All patients who tested positive for autoantibodies, except one, showed the positivity before IFN- $\alpha$  therapy. In the majority of the cases, autoantibodies increased under IFN therapy and the clinical autoimmune disease rarely appeared. It is accepted that the autoimmune diseases are correlated with a particular genetic pattern of susceptibility and that the environmental factors may act as trigger factors, but direct evidence of this phenomenon remains circumstantial. In our patients, the presence of auto-

immune phenomena has not been significantly increased with respect to the normal population. These data are in conflict with the fact that HCV chronic infection may act as a trigger factor in autoimmunity. So, our data are not sufficient to confirm the hypothesis that a specific genotype may be correlated with the autoimmune response. We think that it will be necessary to carry out further studies, before and after IFN therapy in humans, to clarify the specific role of genetic HCV subtypes and the genetic susceptibility in inducing autoimmunity.

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