

while the present study recruited diabetic and non-diabetic patients with angiographically documented IHD.

In agreement with a previous report [5], the present data show that endothelial function, as assessed by brachial scanning and EDD, is impaired to a similar extent in patients with diabetes and IHD compared with patients with IHD without DM (Table 1). Further, our data are in agreement with other studies, which have demonstrated endothelial dysfunction in patients with IHD and in patients with diabetes compared with healthy control subjects [6,13]. Thus one could assume that both diabetes and IHD impair endothelial function, but there is no additional endothelial dysfunction in patients with IHD who also have diabetes.

However, the present study does have limitations. Even though the data are very similar in the two groups, the small study population may give rise to a type 2 statistical error, which may explain the lack of difference between the two groups. The difference in cholesterol levels could be a confounder (Table 1), as previous studies have demonstrated that cholesterol-lowering medication improves endothelial function [14,15] and the CFR [16]. These effects would reduce endothelial function and the CFR in the control patients and perhaps mask an existing difference. However, the number of patients receiving statin treatment was similar in the two groups and the difference in total cholesterol and LDL-cholesterol, although significant, seems rather modest.

In conclusion, the present study has demonstrated that patients with Type 2 DM and IHD have similar CFR and peripheral artery endothelial function compared with non-diabetic patients with IHD. Although only a small number of patients were studied, the results appear to indicate that Type 2 DM *per se* does not result in further impairment of function in the coronary microcirculation and endothelial function over that caused by IHD alone.

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Insulin allergy decreased by Humulin S (Humulin R) and not by insulin aspart or Actrapid Penfill (Penfill R)

Symptoms of allergy to human insulin are found in less than 1% of diabetic patients treated *de novo*. Recently, human insulin analogs, such as lispro or aspart, have been well tolerated by individuals with allergy to other insulin preparations. However, we report a case of insulin allergy where the person did not tolerate insulin aspart but did not develop symptoms whilst using an alternative human regular insulin.

In December 2002 a 56-year-old Japanese man (body weight 50 kg, body mass index 18.9 kg/m²) with Type 2 diabetes for the past 20 years presented in diabetic ketoacidosis. He had discontinued his diabetic treatment several years previously and had never been treated with insulin. His admission plasma glucose and HbA_{1c} levels were 36.9 mmol/l and 17.0%, respectively, and his urine tested positive for ketone

Table 1 Additives used in insulin preparations that have been tested in the present study

Preparations (trade name)	Buffers	Preservatives	Isotonic agents	Retarding substances	Bacteria used in biosynthesis	Skin test
NovoRapid	—	Phenol and Metacresol	Glycerol	—	<i>Saccharomyces cerevisiae</i>	Positive
Penfill R	—	Metacresol	Glycerol	—	<i>Saccharomyces cerevisiae</i>	Positive
Humulin R (Humacart R)	—	Metacresol	Glycerol	—	<i>Escherichia coli</i>	Negative
Penfill N	Dibasic sodium phosphate	Phenol and Metacresol	Glycerol	Protamine sulphate	<i>Saccharomyces cerevisiae</i>	Positive
Humulin N	Dibasic sodium phosphate	Phenol and Metacresol	Glycerol	Protamine sulphate	<i>Escherichia coli</i>	Positive

NovoRapid, insulin aspart; Penfill R (Actrapid Penfill), unmodified soluble human insulin; Humulin R (Humacart R, Humulin S), unmodified soluble human insulin; Penfill N (Insulatard), NPH insulin; Humulin N (Humulin I), NPH insulin.

bodies. His arterial pH and bicarbonate levels were 7.160 and 6.0 mmol/l, respectively. Signs of infection and severe dehydration were observed and treatment with antibiotics, fluids, and human regular insulin [Humulin S (Humulin R); Lilly, Indianapolis, IN, USA] was commenced. Antibodies to GAD65, autoantibodies to insulin, and urinary C-peptide levels were estimated to be < 0.3 U/ml, 3.6% (normal < 7%), and 70.2 µg/day (normal 16–120 µg/day), respectively, suggesting Type 2 diabetes. The patient was discharged on oral hypoglycaemic agents. However, hyperglycaemia became difficult to control in April 2003, and treatment with premixed human insulin [Human Mixtard 30 (Penfill 30R); Novo Nordisk, Bagsværd, Denmark; 50 U/day) was started. His diabetic control improved during the 3 months following the administration of insulin injections, as shown by a reduction in HbA_{1c} from 12.2% to 7.0%. However, the patient noticed a skin rash and itching at the injection sites; therefore, his insulin was changed to human regular insulin, Actrapid (Penfill R) (Novo Nordisk; 36 U/day), in August 2003. Although the insulin dose decreased to 12 U/day, the local reactions continued, and the insulin injections were suspended in November 2003.

The patient had no previous history of any allergy. The percentage of eosinophils in his peripheral white blood cell count was 4.0%, and the total IgE level was 110 IU/ml (normal < 250 IU/ml). He showed a high level of human insulin-specific IgE (8.30 IU/ml; normal < 0.34 IU/ml), which was measured using the radioallergosorbent test, and anti-insulin antibodies (84%; normal < 7%). Skin tests were positive at 15 min for aspart, Actrapid (Penfill R) (Novo Nordisk), Insulatard (Penfill N) (Novo Nordisk), and Humulin I (Humulin N) (Lilly) as well as for an additive in insulin preparations—protamine. In contrast, a skin test for Humulin S (Humulin R) was negative at 15 min. All skin tests were negative at both 1 and 24 h. He was therefore thought to have an immediate-type insulin allergy.

Injections of human regular insulin, Humacart R (Lilly; 24 U/day), were restarted in December 2003, and the allergic reaction did not recur. In the 6 months following the recom-

mencement of insulin injections, his HbA_{1c} levels were between 6.3% and 7.5%, and the levels of human insulin-specific IgE and anti-insulin antibodies decreased to 0.35 IU/ml and 17%, respectively.

The human insulin analogues, aspart and lispro, are reported to be beneficial in reducing allergic reactions to insulin. Aspart, in particular, is less immunogenic for the development of antibodies against human insulin [1,2]. However, a case of allergy to both lispro and aspart has been reported recently [3]. Table 1 shows additives used in insulin preparations that have been tested in the present study. Human NPH insulin [Insulatard (Penfill N)] and Humulin I (Humulin N) might cause insulin allergy due to protamine. However, while both Actrapid (Penfill R) and Humulin S (Humulin R) are human regular insulins, the former caused insulin allergy, whereas the latter did not. Moreover, a skin test for aspart was positive, while that for lispro was not performed in this study. Proteins biosynthesized using recombinant DNA technology contain residual cellular components which may act as allergens even at a very low concentration [4]. Since the 1980s, recombinant human insulin has been produced using either the yeast *Saccharomyces cerevisiae* or the prokaryote *Escherichia coli*. Aspart is also produced by eukaryotic expression in the yeast *S. cerevisiae* [5]. While NovoNordisk Penfill preparations contain approximately 1.0 p.p.m. of polypeptide originating from yeast cells [6], Eli Lilly Humacart preparations contain < 0.026 p.p.m. of polypeptide originating from *E. coli* [7]. Alternatively, it is suggested that altered epitopes in the insulin molecule during manufacture could be involved in insulin allergy, which is a dose-dependent response [8]. However, in this study the allergic reaction did not subside with a decrease in the insulin dose. Based on these findings, differences in residual quantity and origin of cellular components used in the biosynthesis of insulin might be involved in the allergic reaction observed in this study.

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Strong association of C-reactive protein with 2-h-post-challenge glucose in non-diabetic subjects with hypertension

Recently, Hashimoto *et al.* identified BMI and OGTTT 2-h glucose as the most important predictors for plasma CRP in non-diabetic, non-smoking subjects without hypertension [1]. We have just finished a prospective multicentre trial on the diagnosis of impaired glucose tolerance in non-diabetic patients with hypertension (PreDiSc study) [2]. The objectives of the PreDiSc study were to correlate demographic and/or laboratory parameters that are clinically simple to determine with the results of the OGTT. After approval by the Institutional Review Board and signed informed consent, 267 patients older than 18 years, with treated or untreated hypertension with a suspicious fasting capillary glucose level between 5.55 and 7.22 mmol/l, were included in the study, provided they had at least one of the following additional inclusion criteria: BMI $\geq$ kg/m², history of impaired glucose tolerance or family history of diabetes mellitus. Chronic or acute inflammation was an exclusion criterion. After an overnight fast, a 75-g OGTT according to the World Health Organization (WHO) criteria was performed. High sensitivity (hs) CRP was determined by means of an immunonephelometric assay on a BN II Analyser (Dade Behring). Forty-nine per cent of the patients (127/260) had a normal glucose tolerance according to WHO criteria, 39% (101/260) had impaired glucose tolerance and 12% (32/260) had diabetes. In accordance with the findings of Hashimoto *et al.*, in subjects without hypertension we observed significantly lower plasma hs CRP levels in hypertensive patients with

Table 1 Stepwise multivariate regression analysis on log hs CRP (mg/l)

Variable	Partial regression coefficient	F	P-values
Body mass index (kg/m ²)	0.156	5.14	0.0242
OGTT 2-h glucose (mmol/l)	0.099	5.06	0.0253

normal glucose tolerance compared with impaired glucose tolerance [mean 0.42 mg/l (SEM 0.048 mg/l) vs. mean 0.66 mg/l (SEM 0.083 mg/l); $P = 0.017$]. There was a trend towards higher plasma hs CRP levels in patients with diabetes mellitus [mean 0.8 mg/l (SEM 0.234 mg/l)]. This difference, however, failed to reach statistical significance ($P = 0.171$), probably because of the small number of patients with diabetes ($n = 32$). The publication of Hashimoto *et al.* in subjects without hypertension prompted us to perform a comparable analysis in our hypertensive patients. In line with the approach of Hashimoto *et al.* we included only patients with normal OGTT results ($n = 127$) and impaired fasting glucose tolerance ($n = 101$) in this analysis. Log-transformed hs CRP significantly correlated with fasting glucose determined in capillary blood ($r = 0.200$, $P = 0.0026$). Univariate analysis also showed a positive association of BMI ($r = 0.156$, $P = 0.0184$) and OGTT 2-h glucose ($r = 0.099$, $P = 0.135$) with log-transformed hs CRP. Results of the stepwise multivariate regression on log hs CRP in our hypertensive patient population are shown in Table 1.

The partial regression coefficient of BMI with log hs CRP in our hypertensive study population was somewhat higher than in the non-hypertensive population of Hashimoto *et al.* (0.037). The same was true for the hs OGTT 2-h glucose level (0.054).

In conclusion, we found that BMI and OGTT 2-h glucose were associated with plasma hs CRP in non-diabetic hypertensive patients. The findings are in agreement with those described by Hashimoto *et al.* for a non-hypertensive population.

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