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Improvement of glycaemic control in type 2 diabetes: favourable changes in blood pressure, total cholesterol and triglycerides, but not in HDL cholesterol, fibrinogen, von Willebrand factor and (pro)insulin

A. Becker[1], F.E.E. van der Does[2], V.W.M. van Hinsbergh[3,4], R.J. Heine[1,5], L.M. Bouter[1,6], C.D.A. Stehouwer[1,4,7*]

[1]Institute for Research in Extramural Medicine, [4]Institute for Cardiovascular Research, Departments of [5]Endocrinology, [6]Epidemiology and Biostatistics, and [7]Internal Medicine, Free University Medical Centre, De Boelelaan 1117, 1081 HV Amsterdam, the Netherlands, tel.: +31 (0)20-444 43 09, fax: +31 (0)20-444 43 13, e-mail: cda.stehouwer@vumc.nl, [2]General Practitioner, Rotterdam, the Netherlands, [3]Gaubius Laboratory, TNO Prevention and Health, Leiden, the Netherlands, * corresponding author

ABSTRACT

Background: Diabetes mellitus causes a substantial increase in cardiovascular risk, which can only partly be reduced by antihyperglycaemic treatment. We were interested in whether improvement in glycaemic control is associated with improvement of other cardiovascular risk factors. Therefore, we studied among type 2 diabetic patients the association between on the one hand changes in glycaemic control and on the other hand within-subject changes of both classic cardiovascular risk factors and less conventional cardiovascular risk indicators that are typically associated with type 2 diabetes (proinsulin, insulin, fibrinogen, von Willebrand factor and the urinary albumin-creatinine ratio).

Methods: The 214 type 2 diabetic patients were randomly assigned to either a strict fasting capillary glucose target level (<6.5 mmol/l) or a less strict target (<8.5 mmol/l). Duration of follow-up was two years. Since the interventions did not yield statistically significant differences between the treatment arms, we reanalysed the data focusing on within-subject changes of cardiovascular risk factors and indicators across tertiles of average HbA_{1c}.

Results: Individuals in whom HbA_{1c} decreased had significant favourable concurrent changes in triglycerides, total cholesterol, blood pressure, and in the albumin-creatinine ratio in those who were normoalbuminuric at baseline. In contrast, these individuals had unfavourable, although not statistically significant, changes in HDL cholesterol, proinsulin, insulin, fibrinogen and von Willebrand factor. In the whole group, fibrinogen increased more than could be expected on the basis of the relationship between fibrinogen and age, namely from 3.5 ± 0.8 to 3.9 ± 0.9 g/l (p value <0.01).

Conclusions: Our results suggest that improvement in glycaemia in type 2 diabetes is associated with significant favourable changes in triglycerides, total cholesterol, blood pressure and, in normoalbuminuric individuals, albumin-creatinine ratio. In contrast, it is not consistently associated with favourable changes in some cardiovascular risk indicators typically associated with diabetes, which may in part explain why antihyperglycaemic treatment does not clearly lower atherothrombotic disease risk.

INTRODUCTION

Type 2 diabetes carries an increased risk of cardiovascular disease, which is not fully explained by several well-established risk factors (i.e., variables causally related to cardiovascular disease) associated with type 2 diabetes.[1,2] Hyperglycaemia, an important factor in causing micro-angiopathy in type 1 and type 2 diabetes,[3-6] is an additional possible explanation for the enhanced cardiovascular risk.[7-12] However, the United Kingdom Prospective Diabetes Study (UKPDS) showed that over the first ten years after diagnosis, intensive glucose-control treatment, when compared with conventional treatment, reduced the frequency of micro-vascular complications but not diabetes-related mortality or myocardial infarction.[13] Furthermore, the feasibility trial of the Veterans Affairs Cooperative Study Group (VA CSDM), which compared randomly allocated intensive and conventional glycaemic control, showed a trend towards an adverse effect of intensive glycaemic control on cardio-vascular mortality.[14]

Another explanation for the excess cardiovascular risk in type 2 diabetes may lie in less conventional cardiovascular risk indicators (i.e., variables whose association with cardiovascular disease may or may not be causal), such as proinsulin and insulin levels, and haemostatic and fibrinolytic abnormalities, which are typically associated with type 2 diabetes. For example, high levels of fibrinogen may partially explain the excess cardiovascular risk in type 2 diabetes.[15-17] In addition, cardiovascular risk may be increased by high von Willebrand factor levels (vWf) and microalbuminuria,[18-25] which may both reflect endothelial dysfunction.[19,21,26]

We present data on a well-defined cohort of 214 type 2 diabetic patients followed for two years. After the baseline assessment, patients were randomly assigned to either a strict fasting capillary blood glucose target level (<6.5 mmol/l), or a less strict target (<8.5 mmol/l). This study design allowed us to study the effects of two different levels of treatment intensification and analyse the associations between within-subject changes of glycaemia, and changes in lipidaemia, blood pressure, proinsulin, insulinaemia, plasma fibrinogen, plasma vWf, and the urinary albumin-creatinine ratio (ACR). We were especially interested in whether improvement in glycaemic control was associated with improvement in these cardiovascular risk factors and indicators. Because the diabetic state is thought to be associated with an adverse cardiovascular risk profile, in part through the effects of hyperglycaemia (and insulin resistance), we reasoned that improvement of glycaemic control might be associated with favourable changes of cardiovascular risk factors and indicators. On the other hand, lack of any such associations might to some extent explain the inconsistent effects of improvement of glycaemic control on risk of atherothrombotic disease.[13,14]

MATERIALS AND METHODS

Design and measurements

Participants were treated by their own general practitioner (GP). A single physician (FEEvdD) and a diabetes educator performed three-monthly surveillance of treatment-related parameters at the study centre. Results were sent to the GP. The GP (and the patient) then decided whether a subsequent treatment step according to a standard step-up therapy regimen should be taken. Enrolment to the trial was between June 1992 and December 1993, and the trial ended in December 1995. The Ethical Review Committee of the Free University Medical Centre approved the study protocol. The study was approved and performed before results of the UKPDS were reported.

The regimen was a slightly modified version of the practice guidelines for type 2 diabetes of the Netherlands College of General Practitioners.[27] An additional feature of the regimen was a stepwise protocol for initiation of insulin therapy by the GP. The regimen had the usual build-up: tablets in increasing doses up to their usual maximum before other blood-glucose-lowering agents were added. In patients with a body mass index ≥27 kg/m^2, metformin was the first step. If the assigned target values for glycaemic control were not reached, a sulphonylurea (SU) – either glibenclamide, gliclazide or glipizide – was added. In patients with a body mass index <27 kg/m^2 SU was the first step. If the assigned target values were not reached on tablets alone, bedtime intermediate-acting insulin was added (and metformin, if any, discontinued). If target values were not reached with this combination therapy, SU was discontinued and twice-daily injections of a mixture of short- and intermediate-acting insulin were started. If glycaemic control remained poor, multiple insulin injection therapy was considered.

Patients were randomly allocated to one of two groups, which differed only in target values for fasting capillary glucose levels. In 'group 6', fasting target values for capillary blood glucose were near-normal glycaemia (<6.5 mmol/l). In the other arm ('group 8'), the fasting treatment target was <8.5 mmol/l, a value considered to be 'acceptable'.[27] Participating GPs were instructed to refrain from any further steps that might lower blood glucose as long as glucose levels were below the allocated target values. Further details of the study population have been described in detail elsewhere.[28] Briefly, 372 Caucasian subjects between 40 and 75 years, were invited. After exclusion of subjects with comorbidity and those who were probably nondiabetic, 232 gave informed consent and participated in the study. Three-monthly assessments included levels of glucose, HbA_{1c} and lipids, treatment modality, body mass index, blood pressure and early morning ACR. Six-monthly assessments included serum creatinine, proinsulin, insulin

and fibrinogen. VWf was measured at baseline, at one and at two years. Systolic and diastolic (Korotkoff V) blood pressure were measured on the right arm of the seated patient with a Hawksley random zero sphygmomanometer.

Laboratory assessments

All blood samples were taken in the fasting state. Serum and plasma were stored at -20°C for assessment of proinsulin, insulin and vWf, which took place after closure of the data collection. All other assessments were performed on the same day. Venous glucose was measured in sodium fluoride plasma by the glucose dehydrogenase method (Merck, Germany). HbA_{1c} was determined in EDTA plasma by ion exchange HPLC (reference range: 4.3 to 6.1%; Modular Diabetes Monitoring System, BioRad, the Netherlands). Immunospecific insulin and proinsulin were measured in serum by double-antibody radioimmunoassays (lot SP21, Linco Research, St Louis, USA for insulin, and Lilly Laboratory for Clinical Research, Indianapolis, USA for proinsulin).[29] Serum total cholesterol, HDL cholesterol and triglycerides were determined by enzymatic colorimetric techniques (CHOD-PAP and CPO-PAP, Boehringer Mannheim, Germany). LDL cholesterol was calculated with the Friedewald formula (not for patients with TG >8.0 mmol/l).[30] Fibrinogen was determined in citrated plasma by a spectrophotometric prothrombin time-derived method (ACL 1000, Instrumentation Laboratory, the Netherlands). VWf was determined in heparinised plasma by an ELISA and expressed as a percentage of normal pooled plasma (reference range: 50 to 150%).[18,31,32] Urinary and serum creatinine levels were measured by a modified Jaffé method (Boehringer Mannheim, Germany). Urinary albumin was measured by an immunonephelometric method (sensitivity limit: 6.2 mg/l; Beckman, Ireland). For calculation of urinary ACR, we only used early morning samples negative to dipstick tests for nitrite and leucocytes (86% of all samples).

Statistical analysis

We studied the effects of the random assignment by comparison of groups 6 and 8 at the last available measurements, using t tests, χ^2 tests or Mann-Whitney U tests. Comparison of baseline and follow-up measurements were carried out using paired t tests, McNemar's χ^2 tests or Wilcoxon's signed-rank tests.

Since the interventions did not yield statistically significant differences between the treatment arms we reanalysed the data focusing on within-subject changes in cardiovascular risk factors and indicators in the entire cohort. Cohort analysis of a randomised clinical trial (RCT) is legitimate, because an RCT is a cohort, with intervention as a determinant. Adjusting the analysis for the intervention (high versus low target) eliminates possible bias caused by this determinant.[33] The cardiovascular risk factors and indicators considered were proinsulin, insulin, lipids, blood pressure, fibrinogen, vWf and ACR. Normoalbuminuria was distinguished from microalbuminuria and albuminurea using 3.5 mg/mmol as the cut-off.[34]

To study within-subject changes, change rates (slopes) were calculated for each patient separately by linear regression analysis based on all the available measurements. Preliminary analyses showed that patients with less than the full two years of follow-up were likely to confound analyses. Therefore, we chose to confine all analyses using these change rates to those patients who completed two years of follow-up (n=166). Relations between change rates of glycaemic parameters and change rates of outcome measures were studied by univariate scatter diagrams, and by partial correlations after adjustment for sex, diabetes duration, and group 6 or 8. We also addressed these associations by using analysis of variance (ANOVA) and trend analysis, comparing the change rates of the outcome measures across tertiles of HbA_{1c} change rates. All multivariate analyses were performed with and without additional adjustment for change in body weight.

RESULTS

During the first year, ten patients found participation too much of a burden, six moved and one died (7%). One outlier (a woman with a BMI of 59) was excluded from the analyses. Thus, 106 patients in group 6 and 108 patients in group 8 were included in the analyses. During the second year, 12 of these remaining 214 patients found further participation too burdensome and dropped out, eight were lost to follow-up and two died. At closure of the data collection, 26 patients had not yet reached the two-year visit. Mean duration of follow-up at the time of the analysis was 22 months. *Table 1* shows the characteristics of the study population. Fasting plasma glucose was significantly lower in group 6 as compared with group 8, but no clinically meaningful contrast in HbA_{1c} had been achieved at a mean follow-up of 22 months. Furthermore, the difference in treatment intensity did not yield statistically significant differences between groups 6 and 8 at follow-up in any of the other outcome measures. In the total population, glycaemic control improved slightly from baseline to follow-up, as reflected by lowering of the fasting glucose levels and lower variability of mean HbA_{1c}. Furthermore, total and LDL cholesterol, and blood pressure decreased. In contrast, body mass index, fibrinogen and ACR (increase), and HDL cholesterol (decrease) deteriorated during follow-up. Fasting serum insulin increased significantly due to 23 patients starting insulin injections during follow-up. At baseline 16.5% of the participants were on antihypertensive medication and 13.5% lipid-lowering medication, versus 24.1 and 16.7% at follow-up.

Table 1

Characteristics at baseline and at follow-up in group 6 (n=106), group 8 (n=108) and the total population (n=214)

	AT BASELINE		AT FOLLOW-UP*		TOTAL POPULATION	
	GROUP 6	GROUP 8	GROUP 6	GROUP 8	AT BASELINE	AT FOLLOW-UP*
Sex (% male)	53	44			49	
Age (years)	63.3 ± 8.4	63.3 ± 8.3			63.3 ± 8.3	
Diabetes duration	3.4 (0.7-14.2)	3.2 (0.3-12.7)			3.3 (0.5-13.3)	
Cardiovascular history†	21	23	26[c]	29[c]	22	28[d]
Hypertension‡ (% yes)	59	56	59	59	57	59
Smoker (%)	19	22	17	21	20	19
Treatment modality (%)			[d]	[a,d]		[d]
Diet alone	32	37	8	30	35	19
Metformin (M)	4	2	7	5	3	6
Sulphonylurea (SU)	43	38	37	27	40	33
SU + M	8	7	21	14	8	17
Insulin (with or without SU)	14	17	26	25	15	26
Body mass index (kg/m²)						
Men	27.9 ± 3.7	28.2 ± 3.7	28.4 ± 3.9[d]	28.3 ± 3.5	28.0 ± 3.7	28.4 ± 3.7[c]
Women	28.2 ± 5.9	29.8 ± 4.7	28.5 ± 5.6	30.2 ± 5.3	29.0 ± 5.4	29.4 ± 5.4[c]
Fasting plasma glucose (mmol/l)	9.4 ± 2.8	9.7 ± 3.3	8.8 ± 2.3	9.5 ± 3.3[a]	9.6 ± 3.1	9.2 ± 2.9
HbA_{1c} (%)	7.2 ± 1.6	7.6 ± 1.9	7.2 ± 1.2	7.4 ± 1.4	7.4 ± 1.7	7.3 ± 1.3
HDL cholesterol (mmol/l)	1.11 ± 0.30	1.12 ± 0.26	1.02 ± 0.30[d]	1.06 ± 0.27[d]	1.11 ± 0.28	1.04 ± 0.29[d]
Triglycerides	2.2 ± 1.5	2.2 ± 1.6	2.2 ± 1.1	2.0 ± 1.1	2.2 ± 1.5	2.1 ± 1.1
Total cholesterol	6.3 ± 1.5	6.4 ± 1.1	5.9 ± 1.2[d]	6.0 ± 1.0[d]	6.3 ± 1.3	6.0 ± 1.1[d]
LDL cholesterol	4.1 ± 1.1	4.2 ± 0.9	3.9 ± 1.0[d]	4.0 ± 0.9[d]	4.2 ± 1.0	4.0 ± 1.0[d]
Systolic blood pressure (mmHg)	152 ± 25	149 ± 22	149 ± 23	145 ± 24[c]	150 ± 23	147 ± 23[c]
Diastolic blood pressure	84 ± 12	85 ± 12	83 ± 11	83 ± 12	84 ± 12	83 ± 12[c]
Insulin (pmol/l) (n=143)	71 (31-158)	67 (43-146)	79 (35-216)[c]	84 (43-200)[c]	69 (38-158)	81 (37-212)[d]
Pro-insulin (n=143)	3.9 (1.2-9.1)	4.8 (1.0-10.6)	4.1 (1.1-9.3)	4.5 (1.2-9.8)	4.3 (1.1-10.4)	4.4 (1.2-9.4)
Fibrinogen (g/l) (n=179)	3.5 ± 0.8	3.6 ± 0.8	3.9 ± 0.9[d]	3.9 ± 0.9[d]	3.5 ± 0.8	3.9 ± 0.9[d]
Von Willebrand factor (%)	128 ± 53	118 ± 48	123 ± 46	122 ± 52	123 ± 51	123 ± 49
Albumin-creatinine ratio (mg/mmol)						
Baseline <3.5 (n=174)	0.8 (0.4-1.8)	0.8 (0.4-2.0)	0.8 (0.4-3.0)	0.8 (0.3-2.6)	0.8 (0.4-1.9)	0.8 (0.4-2.6)[c]
Baseline ≥3.5 (n=40)	8.3 (3.7-137)	10.5 (4.4-73)	9.1 (0.6-89)	7.3 (1.6-74)	9.4 (3.8-73)	8.1 (0.9-74)
Serum creatinine (μmol/l)						
ACR at baseline <3.5	85 ± 17	82 ± 13	89 ± 17[c]	85 ± 13[c]	84 ± 15	87 ± 15[d]
ACR at baseline ≥3.5	87 ± 16	87 ± 16	95 ± 24[c]	94 ± 19[d]	87 ± 16	94 ± 22[d]

*Data are percentages, means ± standard deviations, or medians (10^{th}-90^{th} centile). HbA_{1c} = glycated haemoglobin, ACR = albumin creatinine ratio. * Mean duration of follow-up: 22 months, † cardiovascular history is defined as at least one of the following: myocardial infarction, angina pectoris, stroke, transient ischaemic attack, and intermittent claudication, ‡ hypertension is defined as a systolic blood pressure >165 mmHg, and/or a diastolic blood pressure >90 mmHg and/or blood pressure-lowering medication. [a] Group 6 versus group 8, p<0.05, [c] follow-up versus baseline, p <0.05, [d] follow-up versus baseline, p<0.01.*

We subsequently analysed changes in the outcome measures in patients who had completed two years of follow-up (n=166) by tertiles of change rates of HbA_{1c} (*table 2*). Results for tertiles of fasting glucose change rates were similar (data not shown). Triglycerides (*figure 1*), total cholesterol and blood pressure changed most favourably in the tertile with the HbA_{1c} decrease, as compared with the other two tertiles. These associations could not be ascribed to the prescription of more lipid-lowering agents or antihypertensives in that tertile (data not shown). Only in patients with an ACR <3.5 mg/mmol at baseline did changes in ACR show a significant favourable association with HbA_{1c} change rates. Changes in vWf were minor and not related to HbA_{1c} change rates. Overall, fibrinogen increased, but mainly in the lowest and middle tertiles of HbA_{1c} change (*table 2* and *figure 2*). We checked whether adjustment for weight change altered any of the relations under study. This was not the case (data not shown).
As shown in *tables 1* and *2*, there were relatively large mean changes in fibrinogen, which could not be explained by changes in glycaemic control (*table 2*), or by changes in the prevalence of smokers (data not shown). Upon further

Table 2

Median baseline HbA_{1c} and adjusted change rates of serum lipids, blood pressure, serum pro-insulin and insulin, plasma fibrinogen and von Willebrand factor, and urinary albumin-creatinine ratio, according to tertiles of HbA_{1c} change rate during follow-up (n=166, two-year follow-up)

TERTILES OF HBA_{1C} CHANGE RATE (%/YEAR)		MEDIAN BASELINE HBA_{1C} (%)	MEAN CHANGE RATES (/YEAR) OF										ACR* (MG/MMOL)	
MEDIAN CHANGE RATE (10^{TH}-90^{TH} CENTILE)	N	(10^{TH}-90^{TH} CENTILE)	HDL (mM)	TG (mM)	TC (mM)	LDL (mM)	SBP (mm Hg)	DBP (mm Hg)	INSULIN† (pM)	PRO-I (pM)	FIBRINOGEN (g/l)	VWF (%)	BASELINE <3.5	BASELINE ≥3.5
-0.56 (-1.29- -0.19): decrease	55	8.3 (6.7-10.8)	-0.034	-0.17	-0.27	-0.14	-2.6	-0.9	6.3	0.13	0.22	-3.6	-0.12	-0.58
0.11 (-0.08-0.26): stable	56	6.4 (5.5-9.1)	-0.054	0.00	-0.11	-0.05	0.2	-0.8	4.8	-0.10	0.26	1.4	0.13	5.09
0.53 (0.29-0.99): increase	55	6.3 (5.5-8.3)	-0.044	0.08	-0.08	-0.05	1.1	1.5	0.3	0.73	0.15	0.3	0.51	0.96
		p_{trend}	NS	0.00	0.01	NS	NS	0.04	NS	NS	NS	NS	0.00	NS
		p_{ANOVA}	NS	0.01	0.02	NS	0.03	NS	NS	NS	NS	NS	0.01	NS

*All change rates of the outcome measures are adjusted for sex, diabetes duration, and randomisation group. Additional adjustment for change in body weight did not make substantial differences in any of the analyses. HbA_{1c} = glycated haemoglobin, HDL = HDL cholesterol, TG = triglycerides, TC = total cholesterol, LDL = LDL cholesterol, SBP/DBP = systolic/diastolic blood pressure, I = insulin, vWf = von Willebrand factor, ACR = albumin creatinine ratio, NS = not significant (p>0.05). * Subdivided into ACR <3.5 (n=133) and ≥3.5 mg/mmol (n=33) at baseline, † patients on insulin therapy at baseline and (or) at follow-up were excluded (n of those included: 32/46/38).*

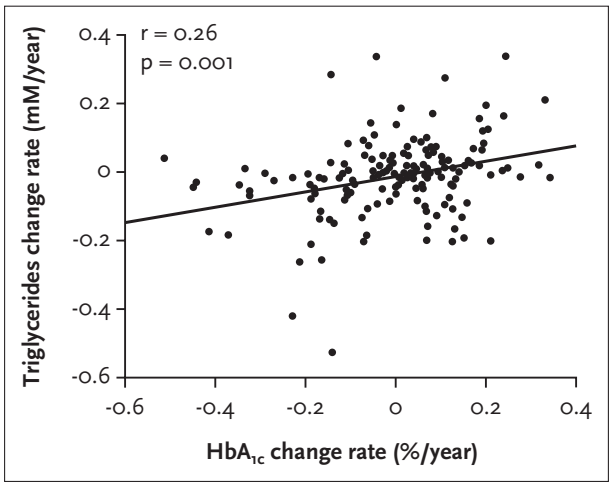

Figure 1

Scatterplot of the change rate of HbA_{1c} and the change rate of triglycerides

r = Pearson's correlation coefficient, p = p value corresponding to Pearson's correlation coefficient.

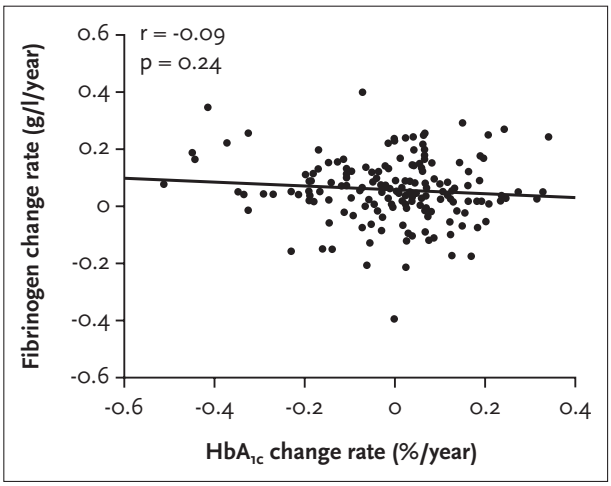

Figure 2

Scatterplot of the change rate of HbA_{1c} and the change rate of fibrinogen

r = Pearson's correlation coefficient, p = p value corresponding to the Pearson's correlation coefficient.

analysis, there were significant baseline differences in fibrinogen levels among patients on diet treatment, those on tablets, and those using insulin, which remained when adjusted for age, sex and diabetes duration. These differences were still present at follow-up. Patients using insulin had significantly higher fibrinogen levels than those on diet, while those on tablets had intermediate fibrinogen levels. From baseline to follow-up, fibrinogen increased from 3.7 ± 0.9 to 4.3 ± 0.9 g/l (mean ± SD, p=0.03) in patients who started insulin treatment (n=23), and from 3.3 ± 0.6 to 3.8 ± 0.9 g/l (p<0.001) in those who changed from diet alone to tablet treatment (n=35). In the subgroup who retained baseline treatment throughout the study (n=93), fibrinogen increased from 3.5 ± 0.8 to 3.8 ± 0.9 g/l (p=0.003). In contrast, in the subgroup who stayed on diet treatment alone throughout the study (n=37), fibrinogen remained unchanged (3.4 ± 0.7 g/l), despite significant worsening of glycaemic control. Among individuals who started insulin treatment at some point during the observation period, the change in fibrinogen levels (measured at 0, 6, 12, 18 and 24 months) was about twice as large in the six-month interval in which the

insulin was started (0.28 g/l) as in the six-month intervals preceding and following the interval in which insulin was started (0.14 g/l). For comparison, plasma glucose continued to decrease after starting insulin, body mass index continued to increase, triglycerides decreased and ACR decreased.
The vWf levels during the one-year interval with and without the initiation of insulin treatment showed a slight increase (7.2%) and a slight decrease (-5.2%), respectively. Changes in HDL cholesterol were not clearly associated with any specific treatment step.
The duration of storage of serum and plasma samples (range: 18 to 48 months) did not significantly correlate with the measured levels of insulin, proinsulin and vWf (correlation coefficients: .01, .09, and .07, respectively). This suggests that the quality of the blood samples did not deteriorate significantly during storage at -20°C.

DISCUSSION

No differences between the two intervention arms were found, except for a slightly lower fasting plasma glucose level. The lack of a greater contrast in fasting glucose levels, and in HbA_{1c}, may have been due to two obstacles. Firstly, the eligibility criteria employed may not have been sufficiently restrictive. Secondly, compliance with the study protocol may not have been sufficient to achieve the desired divergence between the treatment arms. Therefore, we reanalysed our data and focused on the question whether different levels of success in lowering the main parameter of antihyperglycaemic treatment, HbA_{1c}, would be accompanied by different changes in various cardiovascular risk factors and indicators. As expected, we found that triglycerides, total cholesterol, blood pressure and ACR (only when <3.5 mg/mmol at baseline) exhibited significantly more favourable changes in the tertile with decreasing HbA_{1c}, compared with the other tertiles. In contrast, these favourable changes were not found for proinsulin, insulin, fibrinogen and vWf (*table 2*). For fibrinogen and vWf, this is in accordance with earlier studies.[35-37]
Moreover, in the whole group, fibrinogen even showed a significant increase. In conclusion, improvement of glycaemic control – with the strategy employed, i.e. a conventional step-up regime consisting of metformin or sulphonylurea in increasing dosages and insulin if the assigned target values were not reached with metformin or sulphonylurea – was associated with concurrent favourable changes in some, but not all, the cardiovascular risk factors and indicators.
Our results concerning the favourable association of HbA_{1c} with ACR in those subjects without microalbuminuria and the absence of such an association in those with microalbuminuria are in accordance with findings in type 1 diabetes that intensified glycaemic control may not have favourable effects on the progression of the urinary albumin excretion rate once microalbuminuria exists.[38] In addition, Levin *et al.* reported that creatinine clearance among subjects with microalbuminuria deteriorated more rapidly than among subjects without microalbuminuria, regardless of intensity of glycaemic treatment.[39]
Glycaemic changes were not significantly associated with changes in fibrinogen. However, fibrinogen increased after the initiation of insulin treatment (or after an increase of pharmacological treatment in general). Adjustment for diabetes duration, age and sex did not substantially affect differences between those who started insulin and the rest of the population (data not shown). Furthermore, fibrinogen showed a gradual overall increase during the study. However, the yearly change rate was far greater than the relationship of fibrinogen with age in nondiabetics would suggest (except in the subgroup who remained on diet treatment alone throughout the study). In a cross-sectional study, De Boever *et al.* found a 0.14 g/l increase per ten years in a nondiabetic population aged between 35 and 59 years.[40] These findings may be of particular interest. Firstly, considerable variations in cardiovascular event rates could be attributable to relatively small variations in fibrinogen levels. Meade *et al.* have shown that a fibrinogen level elevation of 0.6 g/l was associated with an 84% increased risk of stroke within the next five years.[41] In addition, Danesh *et al.* showed, in a meta-analysis of prospective studies, an 80% increased risk for coronary heart disease for each increment in fibrinogen of 1.0 g/l.[42] Secondly, the VA CSDM feasibility trial reported an unexpectedly high incidence of cardiovascular events in a group of male type 2 diabetic patients who started insulin treatment.[14] Fibrinogen level rose significantly in the first year and returned to baseline at two years.[43]
Insulin and proinsulin have been recognised as potentially atherogenic substances, although it is not clear whether insulin by injection has effects comparable with those seen in hyperinsulinaemia related to insulin resistance.[44,45] A study in the offspring of hypertensive men demonstrated that fibrinogen is closely related to plasma insulin and most components of the insulin resistance syndrome.[46] In contrast De Feo *et al.* demonstrated that induced insulin deficiency was associated with an increase in fibrinogen, suggesting an acute phase protein response.[47] *In vitro* experiments lend support to possible adverse effects of proinsulin and insulin on fibrinolysis, acting via both liver and endothelium.[48] The present findings could be in accordance with an effect of treatment intensification on the production of fibrinogen by hepatocytes *in vivo*. *In vitro* studies, however, have shown inconsistent results in this respect. Incubation of hepatocytes with insulin for less than 24 hours had no influence on fibrinogen synthesis,[49,50] while studies with longer incubation showed no effect,[51] decreased[52] or increased fibrinogen synthesis.[53] Moreover,

in our study fibrinogen change rate was not related to insulin and proinsulin change rates (data not shown). In sum, our data do not provide an explanation for the fibrinogen changes that were observed.

In conclusion, our study suggests that further improvement of glycaemic control in moderately well-controlled type 2 diabetes is not associated with concurrent favourable changes in some cardiovascular risk indicators that are typically associated with type 2 diabetes. We stress that our findings on glycaemic control cannot be distinguished from the therapeutic strategy employed, i.e. a conventional step-up regime consisting of metformin or sulphonylurea in increasing dosages and insulin if the assigned target values were not reached. Therefore, we cannot exclude that improvement of glycaemic control through other strategies may yield better results in terms of cardiovascular risk factor amelioration. Nevertheless, our findings may provide a framework for understanding the disappointing effects of intensified glycaemic control on risk of macrovascular disease. Our finding that fibrinogen levels may increase after starting insulin treatment of particular concern and warrants further study.

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