

Clinical Research

## High cumulative insulin exposure: a risk factor of atherosclerosis in type 1 diabetes?

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### Abstract

**Background:** Since insulin therapy might have an atherogenic effect, we studied the relationship between cumulative insulin dose and atherosclerosis in type 1 diabetes. We have focused on patients with type 1 diabetes instead of type 2 diabetes to minimise the effect of insulin resistance as a potential confounder.

**Methods:** An observational study was performed in 215 subjects with type 1 diabetes treated with multiple insulin injection therapy. Atherosclerosis was assessed by measurement of carotid intima-media thickness (CIMT).

**Results:** The cumulative dose of regular insulin showed a positive and significant relation with CIMT: increase of 21  $\mu\text{m}$  in CIMT per S.D. of insulin use (95% CI: 8–35 adjusted for gender and age), which remained unchanged after adjustment for duration of diabetes, HbA1c, BMI, pulse pressure, physical activity and carotid lumen diameter. A similar relation was found for intermediate-acting insulin: 15.5  $\mu\text{m}$  per S.D. (2–29), which was no longer present after further adjustment.

**Conclusions:** These findings provide evidence that a high cumulative dose of regular insulin is a risk factor for atherosclerosis.

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**Keywords:** Type 1 diabetes; Carotid arterial wall thickness; Insulin; Insulin resistance; Insulin injections

### 1. Introduction

It is well established that patients with diabetes mellitus are at a two to four fold greater risk of cardiovascular disease (CVD) [1,2]. The increased risk has been attributed in part to hyperglycaemia. Indeed, the UK Prospective Diabetes Study (UKPDS) observed a graded positive association between glycaemic control and risk of cardiovascular disease

[3]. The benefits of aggressively glucose lowering by means of insulin therapy in type 2 diabetes remains, however, subject to debate. On the negative side, results from observational studies have indicated that high levels of endogenous insulin may increase CVD risk [4]. In addition, experimental studies in animals have shown that high exogenous insulin leads to increased atherosclerosis [5]. When high endogenous insulin levels are indeed a CVD risk factor one might expect a paradoxically increased risk of CVD in patients treated with exogenous insulin, as this leads to high (pharmacological) circulating insulin levels [6,7]. Such risk elevation may counteract the benefits of tight glucose control. As appears from the Diabetes Control and Complications Trial (DCCT) and the

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UKPDS, intensive blood-glucose control has been more effective in decreasing the risk of microvascular complications than in decreasing the risk of macrovascular complications [8,3]. The role of exogenous insulin in explaining this discrepancy is of major interest and, if proven correct may have clinical impact for the treatment of both type 1 and type 2 diabetes.

Though the symptoms of CVD do not appear in most patients until middle age or later, the development of atherosclerosis begins much earlier. A measure of the degree of atherosclerosis can be obtained using high-resolution B-mode ultrasonography to quantify arterial wall thickening. Intima-media thickness (IMT) of the carotid arteries is a marker of generalised atherosclerosis [9–11]. In non-diabetic subjects established cardiovascular risk factors, such as age, LDL-cholesterol, systolic blood pressure, body mass index, gender and low high-density-lipoprotein (HDL) cholesterol are associated with an increased CIMT and future CVD risk [12–14]. In type 1 diabetes CIMT is increased compared to matched healthy controls. Determinants of CIMT in type 1 diabetes are similar to subjects without diabetes [15–21].

The aim of the present study was to determine whether a high cumulative insulin dose is a risk factor for atherosclerosis, assessed by CIMT measurements, in patients with type 1 diabetes.

## 2. Methods

### 2.1. Study population

An observational study was performed in 215 patients with type 1 diabetes mellitus. Patients were recruited from the outpatient clinics of the Isala Clinics Zwolle and the Groene Hart Hospital Gouda. Type 1 diabetes mellitus was defined as age at onset <40 years in combination with insulin dependence from diagnosis. The main inclusion criteria for enrolment were age  $\geq 18$  years, multiple injection insulin therapy and duration of insulin treatment of at least 4 years. Two hundred and eighty-seven patients were invited to participate in the study, 215 patients responded and were seen from 2000 to 2001. The 72 non-responders did not significantly differ with the responders in major determinants of atherosclerosis risk (age, sex, duration of diabetes, family history). The Medical Ethics Committees of the participating institutions approved the study and all patients gave their written informed consent.

### 2.2. Assessment of carotid atherosclerosis

To measure intima-media thickness, ultrasonography of both carotid arteries was performed with a 7.5-MHz linear array transducer using a Philips ATL (Bothell, WA, USA) scanner. A careful search was performed for all interfaces at the near and far wall of the distal common carotid artery. The image was frozen on the R wave of the electrocardiogram and stored on videotape. This procedure was repeated at four

predefined angles for both sides (180, 150, 120, and 90° for the right carotid artery and 180, 210, 240, and 270° for the left carotid artery) using the Meijer's arc<sup>®</sup>. Images were obtained by five centre-specific sonographers, who completed a uniform certification program. This program included a detailed training and assessment session in which a high level of proficiency had to be demonstrated. Data on reproducibility on the intima-media thickness measurements come from several studies that we performed at the Julius Centre, using the same training, certification and reading procedures as the present study with the same readers. These studies showed an intraclass correlation coefficient ranging from 0.84 to 0.92 for repeated common CIMT measurement in single- and multi-centre studies [22–24].

The actual measurements of intima-media thickness were performed off-line. The frozen images on the videotape were digitised and displayed on a screen using additional dedicated software as described in detail by Liang et al. With a cursor, the interfaces of the distal common carotid artery were marked over a length of 10 mm using automated edge detection software [25]. The distance from the leading edge of the first bright line of the far wall (lumen–intima interface) to the leading edge of the second bright line (media–adventitia interface) indicates the intima-media thickness [26,27]. For the near wall the distance between the trailing edge of the first bright line to the trailing edge of the second bright line at the near wall provides the best estimate of the near wall intima-media thickness. The beginning of the dilatation of the distal common carotid artery served as a reference point for the start of the measurement. A common CIMT was determined for each individual as being the average of near and far wall measurements at all angles of both the left and right arteries, i.e. in similar fashion to previous studies [24,28,29]. We used a CIMT estimate based on near and far wall measurements rather than a far wall only, because several studies indicated that the average leads to stronger associations than the far wall only [9].

### 2.3. Cardiovascular risk factors

The patients were examined according to a standardised protocol. Patients were asked to complete a questionnaire at home on dietary habits and physical activity (estimated by the modified Baecke questionnaire) [30]. Alcohol consumption, smoking habits, use of medication including the actual insulin dose, medical history (including duration of diabetes and diabetic microvascular complications), family history of CVD, hypertension, lipid disorders or diabetes mellitus were recorded during the visit at the research centre.

Blood pressure was measured three times on the left arm with a semi-automated device (Dynamap) in supine position after the ultrasound procedure. Height, weight, and waist circumference were measured and body mass index ( $\text{kg}/\text{m}^2$ ) was calculated. The average of the last available levels of glycosylated haemoglobin A1c (HbA1c) total serum cholesterol, HDL-cholesterol, triglycerides, microalbumin and creatinin

from 2 years preceding inclusion were obtained from the patients' medical files. To overcome possible differences in laboratory methods, we created centre specific  $z$ -scores for the different biochemical parameters.

#### 2.4. Cumulative insulin use

The cumulative amount of insulin used was calculated from medical records since the diagnosis of diabetes, using the original correspondence from the diabetologist with the general practitioner as reliable source. In all patients, data were available since the first year of diabetes. Insulin dose (as noted by the attending physician) was multiplied by the time until a new dosage was prescribed. At least one record a year was taken into the assessment. This was done separately for short-acting analogues (like Lyspro and Aspart), regular insulin (like Actrapid and Humuline Regular) and intermediate-acting insulin (like Insulatard and NPH). To compare the different types of insulin, cumulative insulin doses were converted to  $z$ -scores [ $z = (x - \text{mean})/\text{S.D.}$ ]. We have used centre specific  $z$ -scores in order to adjust for potential differences, if present, in registration of insulin use between the two centres.

#### 2.5. Data analysis

Data are presented as mean and standard deviation. The relations between insulin and CIMT and the different parameters were evaluated using univariate and multivariate linear regression analyses. The results are presented as regression coefficients, with the 95% confidence interval (95% CI). CIMT was used as continuous variable in the analysis. Separate analyses were performed for regular insulin, intermediate-acting insulin, and the combination of regular insulin and short-acting analogues. Because patients use different combinations during the course of their disease, an individual patient can contribute to two analyses. Data were analysed using the SPSS 10.1 statistical software programme for Windows.

### 3. Results

General characteristics of the 215 patients participating in the study are presented in Table 1. The mean age of the patients was  $43.6 \pm 12.9$  years and the mean duration of diabetes was  $20.7 \pm 12.2$  years. Mean carotid IMT was  $664.6 \pm 47.7$   $\mu\text{m}$ . Ninety-eight patients (46%) ever used short-acting analogues, 196 patients (91%) ever used regular insulin and 204 patients (95%) ever used intermediate-acting insulin. The range of cumulative insulin dose was for short-acting analogues: 1326–232,308 U (median 27,393 U); for regular insulin: 3469–785,628 U (median 122,329 U); for intermediate-acting insulin 2448–1,063,038 U (median 110,144 U).

Table 1  
General characteristics of the study population

Age (years)	43.6 (12.9)
Women (%)	34
Duration of diabetes (Y)	20.7 (12.2)
Body mass index ( $\text{kg}/\text{m}^2$ )	25.8 (3.7)
Current smoking (%)	31
Previous smoking (%)	32
Systolic blood pressure (mmHg)	142.2 (21.0)
Diastolic blood pressure (mmHg)	82.0 (9.4)
Pulse pressure (mmHg)	60.2 (17.8)
HbA1c (%)	8.0 (1.1)
Total cholesterol (mmol/L)	4.9 (0.9)
Triglycerides (mmol/L)	1.0 (0.6)
HDL cholesterol (mmol/L)	1.5 (0.4)
Serum creatinin ( $\mu\text{mol}/\text{L}$ )	92.8 (36.8)
Proportion using ACE-inhibitors (%)	16
Proportion using lipid lowering drugs (%)	14
Daily insulin dose (IU/day)	56.1 (21.5)
Cumulative amount, short-acting analogues (IU) (median)	1326–232,308 (27,393)
Cumulative amount, regular insulin (IU) (median)	3469–785,628 (122,329)
Cumulative amount, intermediate-acting insulin (IU) (median)	2448–1,063,038 (110,144)
Physical activity score <sup>a</sup>	7.7 (1.1)
Common CIMT ( $\mu\text{m}$ )	664.6 (147.7)

Values are percentages or means with standard deviations in parentheses.

<sup>a</sup> Measured by the modified Baecke questionnaire.

The relation in univariate analysis of both clinical and biochemical parameters with CIMT is presented in Table 2. The cumulative use of the combination of all types of insulin showed a positive and significant relation with CIMT (increase of 21  $\mu\text{m}$  in CIMT per S.D. of insulin use; 95% CI: 8–35) adjusted for gender and age. The cumulative dose of regular insulin showed a positive and significant relation with thickening of the carotid intima-media (increase of 22  $\mu\text{m}$  in CIMT per S.D. of insulin use; 95% CI: 7–37). A similar relation was found for intermediate acting insulin (increase of 15.5  $\mu\text{m}$  in CIMT per S.D. of insulin use; 95% CI: 2–29). Fig. 1 shows the increase in CIMT per quartile of cumulative regular insulin exposure. Patients with the highest amount of insulin exposure (quartile 4) showed the greatest CIMT.

The results of the relation in univariate analysis of clinical and biochemical parameters with the different sorts of insulin are presented in Table 3. Only duration of diabetes and creatinin levels were significantly related to cumulative dose of regular insulin. Results of multivariate analysis of the combination of all types of insulin, regular insulin, intermediate-acting insulin and the combination of regular insulin and short acting analogues with CIMT as dependent factor are shown in Table 4. Co-variables in model I were selected because of their relation ( $p < 0.10$ ) with either CIMT or insulin in univariate analysis. The co-variables added in model II were chosen because of potential confounding.

For regular insulin the relation with CIMT remained unchanged when duration of diabetes, BMI, pulse pressure,

Table 2  
Association of clinical and biochemical parameters with CIMT

	CIMT ( $\mu\text{m}$ )
Age (years)	8.21 [7.15; 9.28]*
Female gender	-42.03 [-84.03; -0.03]*
Duration of diabetes (years)	2.28 [0.96; 3.61]*
Body mass index ( $\text{kg}/\text{m}^2$ )	3.15 [-0.73; 7.02]
Waist (cm)	1.43 [0.09; 2.76]*
Systolic blood pressure (mmHg)	0.74 [-0.01; 1.48]
Diastolic blood pressure (mmHg)	0.47 [-1.03; 1.96]
Pulse pressure (mmHg)	0.97 [0.04; 1.91]*
HbA1c (%)	2.61 [-10.57; 15.80]
Total Cholesterol (mmol/L)	5.43 [-11.38; 22.25]
Triglycerides (mmol/L)	-10.26 [-33.96; 13.44]
HDL Cholesterol (mmol/L)	-19.19 [-57.33; 18.95]
Creatinine ( $\mu\text{mol}/\text{L}$ )	-0.12 [-0.51; 0.26]
Physical activity score	13.34 [0.12; 26.56]*
Carotid lumen diameter ( $\mu\text{m}$ )	49.13 [26.71; 71.55]*
Current smoking	0.89 [-29.83; 31.61]
Previous smoking	22.97 [-9.78; 55.73]
Alcohol use (g)	-0.29 [-1.25; 0.67]
Family history of CVD	-2.93 [-16.82; 10.96]
Short-acting analogues (a) $n = 98$ , S.D.	-13.95 [-39.11; 11.22]
Regular insulin (b) $n = 196$ , S.D.	21.93 [6.59; 37.27]*
Intermediate-acting insulin (c) $n = 204$ , S.D.	15.50 [1.68; 29.31]*
Combination of regular insulin and short-acting analogues, S.D.	21.53 [6.22; 36.84]*
All types of insulin, S.D.	21.38 [7.74; 35.03]*
Insulin daily dose (IU)	0.47 [-0.25; 1.18]

Values are expressed as linear regression coefficients, adjusted for gender and age (if appropriate) with 95% confidence interval in parentheses, and reflect changes in CIMT (in  $\mu\text{m}$ ) per unit increase of the clinical parameter, or the age/gender-adjusted difference between the categories of dichotomous variables.

\*  $p < 0.05$ .

physical activity and carotid lumen diameter were taken into account. For the combination of all types of insulin and for intermediate acting insulin the relation was no longer present (Table 4). Multivariate analysis with waist circumference

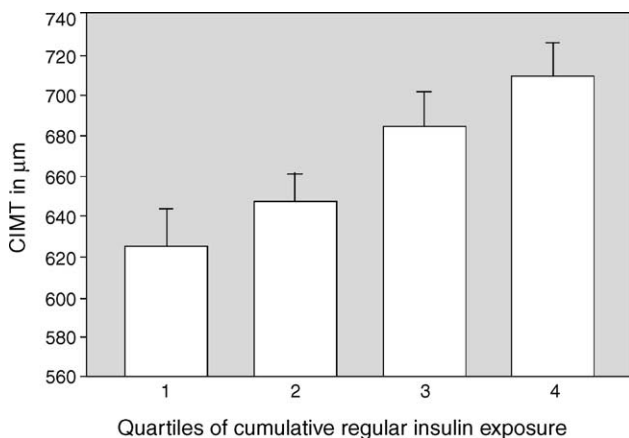


Fig. 1. Carotid intima-media thickness by quartiles of cumulative regular insulin exposure (adjusted for age, gender, duration of diabetes, HbA1c, pulse pressure, carotid lumen diameter, physical activity and body mass index).

instead of BMI did not change the association. Moreover, the multivariate analysis was repeated with other covariates (systolic blood pressure, diastolic blood pressure, total cholesterol and previous smoking), which did not change the relation we found. Additional analyses were performed in two groups after stratification by the median value of BMI (lower or higher than a BMI of  $25.2 \text{ kg}/\text{m}^2$ ). The results were identical and therefore no stratified results are presented.

#### 4. Discussion

In this study in patients with type 1 diabetes cumulative dose of regular insulin was related to generalised atherosclerosis. Cumulative dose of regular insulin showed a positive and significant relation with thickening of the carotid artery intima and media (increase of  $22 \mu\text{m}$  in baseline CIMT per standard deviation increase of insulin use). This constitutes a 3.3% difference in CIMT. Adjustment for potential confounders did not change the relation. Cumulative dose of intermediate-acting insulin showed no relation with CIMT. As a result of lack of power no conclusion could be made on the relation between short-acting analogues and CIMT. To the best of our knowledge this is the first empirical evidence to show that cumulative insulin treatment is related to atherosclerosis.

To appreciate these results some issues need to be addressed. We have focussed on patients with type 1 diabetes instead of type 2 diabetes to minimise the effect of insulin resistance as a potential confounder of the relation between insulin dose and atherosclerosis. As insulin resistance is strongly related to both atherosclerosis and high insulin need, it would be difficult to draw conclusions about the atherogenic effects of insulin from a study in type 2 diabetes. To examine the possibility of a detrimental effect of insulin on the arterial wall, we studied the relation of cumulative insulin dose with atherosclerosis in individuals with type 1 diabetes. However, if high cumulative insulin use proves to be a risk factor of atherosclerosis, it may have implications in particular for the treatment of type 2 diabetes, this is because these patients often use considerable amounts of insulin.

Our study is based on 215 patients with type 1 diabetes who have been under medical supervision in a district hospital in the Netherlands, at least 4 years. It is unlikely that non-response is related to either insulin-use or atherosclerotic status. In the Dutch hospital system letters to the GP are traditionally used as summary for the clinician and therefore very detailed. This provides a unique reliable long-term data source for prescribed insulin dose. An important drawback of the present study is the lack of long-term data on glycaemic control. Although the data on the previous 2 years are strongly related to cumulative glycaemic control [31], data over a similar period as insulin use would be better.

The outcome measurement, CIMT is considered to be an appropriate measure for generalised atherosclerosis [32–34]. It is strongly related to several cardiovascular risk factors

Table 3

Association of clinical and biochemical parameters with intermediate acting insulin, regular insulin and combination of regular insulin and short-acting analogues

	Regular insulin	Intermediate-acting insulin	Combination of regular insulin and short-acting analogues
Age (years)	0.005 [−0.006; 0.01]	0.03 [0.02; 0.04]*	0.003 [−0.007; 0.01]
Sex	−0.05 [−0.3; 0.2]	−0.4 [−0.7; −0.08]*	−0.06 [−0.3; 0.2]
Duration of DM (years)	0.04 [0.03; 0.05]*	0.06 [0.05; 0.07]*	0.04 [0.03; 0.05]*
Body mass index (kg/m <sup>2</sup> )	0.02 [−0.02; 0.06]	0.04 [0.001; 0.08]*	0.02 [−0.01; 0.06]
Waist (cm)	0.01 [−0.003; 0.02]	0.009 [−0.005; 0.02]	0.01 [−0.001; 0.02]
SBP (mmHg)	−0.003 [−0.01; 0.005]	0.01 [0.03; 0.02]*	−0.003 [−0.01; 0.004]
DBP (mmHg)	−0.007 [−0.02; 0.008]	−0.005 [−0.02; 0.01]	−0.008 [−0.02; 0.006]
Pulse pressure (mmHg)	−0.001 [−0.01; 0.008]	0.02 [0.01; 0.03]*	−0.002 [−0.01; 0.007]
HbA1c (%)	−0.006 [−0.1; 0.1]	−0.05 [−0.2; 0.08]	0.0004 [−0.1; 0.1]
Total Cholesterol (mmol/L)	−0.001 [−0.2; 0.2]	−0.08 [−0.3; 0.09]	−0.03 [−0.2; 0.1]
Triglycerides (mmol/L)	0.05 [−0.2; 0.3]	−0.09 [−0.3; 0.2]	0.03 [−0.2; 0.3]
HDL Cholesterol (mmol/L)	0.008 [−0.4; 0.4]	−0.07 [−0.5; 0.3]	−0.006 [−0.4; 0.4]
Creatinin (μmol/L)	0.006 [0.002; 0.01]*	−0.002 [−0.006; 0.002]	−0.0007 [−0.004; 0.002]
Physical activity	−0.04 [−0.2; 0.08]	−0.04 [−0.2; 0.1]	−0.05 [−0.1; 0.04]
Carotid lumen diameter (μm)	−0.2 [−0.4; 0.04]	0.2 [−0.03; 0.5]	0.1 [−0.05; 0.3]
Current smoking	−0.007 [−0.3; 0.3]	−0.3 [−0.6; 0.06]	−0.2 [−0.4; 0.04]
Previous smoking	0.2 [−0.2; 0.5]	0.1 [−0.2; 0.4]	0.09 [−0.1; 0.3]
Alcohol use (g)	−0.0009 [−0.009; 0.008]	−0.004 [−0.01; 0.05]	−0.001 [−0.007; 0.004]
CIMT (μm)	0.002 [0.001; 0.003]*	0.002 [0.0; 0.003]*	0.002 [0.0; 0.003]*
Family history of CVD	0.01 [−0.0; 0.1]	0.03 [−0.1; 0.2]	0.003 [−0.09; 0.09]

Values are expressed as linear regression coefficients, adjusted for gender and age, with 95% confidence interval in parentheses, and reflect changes in S.D. of insulin (z-score). SBP, systolic blood pressure; DBP, diastolic blood pressure.

\*  $p < 0.05$ .

[35,36] and has been shown to be a good predictor of CVD [37]. Since most conventional risk factors are positively associated with subclinical atherosclerosis, individuals with the highest CIMT are expected to be more at risk of developing CVD in later life than subjects with the lowest CIMT. In type 1 diabetes CIMT was increased compared to matched healthy controls [38]. In various studies among patients with type 1 diabetes, CIMT has been shown to correlate with different cardiovascular risk factors. None of the studies observed that all possible risk factors were significantly related to increased CIMT, but mostly a sum of risk factors. Our findings are in agreement with these studies.

It is important to realise that insulin use and glycaemic control are tightly related. It may be questioned to what extent a relation between cumulative insulin use and risk of atherosclerosis is not simply a reflection of the relation of poor glycaemic control and CIMT? Two comments can be made. To be a confounder a variable needs to be related to both

exposure (cumulative insulin use) and the outcome (CIMT). HbA1c, as the average value over a 2-year period, was related to daily insulin use [39] and has been shown to be an appropriate reflection of cumulative glycaemic control [31]. Yet, in our study, no relation was found between HbA1c and cumulative insulin use (Table 3). While perhaps surprising, this is partly explained by the practicalities of diabetes treatment. In clinical practice, when a patient's HbA1c rises, the doctor increases the insulin use. This results in a higher cumulative insulin estimate. Yet due to the increase in insulin, the HbA1c falls, leading to no association between the two (Table 3). Following this argument, glycaemic control cannot confound the relation of cumulative insulin to CIMT since it does not relate to cumulative insulin use. Another potential explanation might be that higher glucose levels at the time of diagnosis initiate atherosclerotic changes and an increased insulin dose, the latter resulting in increased cumulative insulin use.

Table 4

Relation of cumulative insulin use with CIMT

	Combination of all types of insulin	Regular insulin	Intermediate acting insulin	Combination of regular insulin and short-acting analogues
Crude	51.01 [33.39; 68.63]*	28.74 [6.91; 50.56]*	49.28 [32.00; 66.56]*	26.25 [4.19; 48.30]*
+Age and gender	21.38 [7.74; 35.03]*	21.93 [6.59; 37.27]*	15.50 [1.68; 29.31]*	21.53 [6.22; 36.84]*
Model I	3.76 [−17.76; 25.28]	23.18 [5.53; 40.83]*	−12.65 [−30.73; 5.44]	21.20 [3.47; 38.94]*
Model II	−1.39 [−28.54; 25.75]	23.63 [0.40; 46.86]*	−16.53 [−38.63; 5.58]	20.57 [−2.88; 44.01]#

Values are expressed as linear regression coefficients with 95% confidence interval in parentheses, and reflect changes in CIMT (in μm) per S.D. increase of insulin dose. Model I: adjusted for: age, gender, duration of diabetes, pulse pressure, carotid lumen diameter, physical activity and body mass index. Model II: Model I plus adjustment for HbA1c, log triglycerides, HDL-cholesterol, actual smoking, blood pressure lowering medication and lipid lowering medication, family history of type 2 diabetes and albumin excretion rate.

\*  $p < 0.05$ .

#  $p < 0.10$ .

The second comment is, to what extent is the required insulin dose a reflection of the underlying hyperglycaemia? From clinical practice it is well known that patients differ considerably in the amount of daily insulin needed to control glucose levels within certain ranges [8]. In addition to glucose levels, body constitution, eating habits and physical activity may also play a role. To evaluate the extent to which daily insulin use is explained by these factors, we estimated the explained variance ( $R^2$ ) of daily insulin use by age, gender, HbA1c, weight, triglycerides, physical activity and current smoking [39]. The variance explained by these factors was only 40% and thus variability in insulin use (and most likely also cumulative insulin use) is only modestly explained by usual determinants, including glycaemic control. This finding supports the notion that cumulative insulin is only very modestly a reflection of poor glycaemic control. Finally, in the current analyses of our data we adjusted the relation of cumulative insulin to CIMT for all these factors in an attempt to control as much as possible for 'glycaemic control' and the relation persisted significant. Based on the above reasoning, we believe that the reported relation is indeed a reflection of insulin use and not of glycaemic control.

Our findings may be partly explained by the presence of the insulin resistance syndrome (the metabolic syndrome, even in type 1 diabetes). Although insulin sensitivity was not directly assessed, adjustment for potential confounders related to insulin resistance, like HDL, TG, waist circumference or BMI and SBP, did not materially change the relations (Table 4). These results support the view that insulin is a determinant of CIMT and not insulin resistance.

The insulin hormone may have several direct actions on the vasculature, both protective and harmful. A putative favourable effect of insulin is the production of the vasodilator nitric oxide (NO) by endothelial cells through enhancement of nitric oxide synthase (NOS). Studies performed *in vitro* on endothelial cells have confirmed that insulin enhances the expression of NOS inducing the release of NO [40,41]. Another favourable effect of insulin is its anti-inflammatory potential, as observed in human aortic endothelial cells *in vitro* [42,43]. A possible harmful effect of insulin is its potential to increase the production of endothelin, a potent vasoconstrictor and mitogen [44]. Insulin promotes adverse vascular effects by stimulating various growth factors acting through the mitogen-activated protein kinase (MAPK) signalling pathway. MAPK may also mediate the effect of insulin on vascular smooth muscle cell production of plasminogen activator inhibitor-1, which attenuates fibrinolysis [45].

There is only circumstantial evidence that exposure to high levels of exogenous insulin adversely affects the risk of CVD [46–52]. The only observational study on this subject in patients with type 1 diabetes is the EDIC study, which is a follow-up study of the DCCT patients [15]. In the EDIC no effect was found after 8 years intensive insulin treatment on carotid artery wall thickness. Recently however, the findings of the EDIC study showed that the effect of intensive insulin

treatment on CIMT may only be found after a lag of time period (>12 years) [53]. It should be noted that the difference in lifetime insulin use is probably much greater than the difference during the randomised treatment period of the DCCT.

Evidence on the relation of cumulative insulin and cardiovascular risk may come from a few randomised controlled trials that compared intensive insulin treatment with conventional insulin treatment in relation to macrovascular disease. It should be realised that these trials were designed to determine the benefits of improved glucose control, rather than the effects of increased risk with cumulative insulin dosage, and focussed on microvascular complications rather than on macrovascular events. The results of these trials are not consistent. Both direct (beneficial), neutral and inverse relations have been found between insulin treatment and macrovascular disease, all not statistically significant [3,54–57]. However, no data on the relation between the amount of insulin used and cardiovascular events were presented.

We found that cumulative dose of regular insulin is positively and significantly related with thickening of the intima-media. No independent relation was observed for intermediate acting insulin. This might be partly explained by the difference in pharmacokinetics and pharmacodynamics between regular insulin and intermediate-acting insulin. Regular insulin is usually administered two to four times a day, resulting in insulin peaks just after injection, followed by a quick clearance within 5–7 h. Intermediate-acting insulin is applied once or twice a day and has a different pharmacokinetic profile. After injection the insulin level of intermediate acting insulin declines much slower and the insulin peak is lower compared to regular insulin. The maximum insulin concentration after an injection of regular insulin is more than twice as high as the insulin concentration is after an injection with NPH [58]. It might be that the insulin profile of intermediate acting insulin is less harmful than the profile of regular insulin.

Confirmation of our findings is warranted. As such it is of interest to evaluate further the data of DCCT and UKPDS focussing on cumulative insulin use and CIMT or macrovascular events. Trials in type 2 diabetes on the comparison of insulin versus oral treatment on progression of CIMT are of particular relevance in this respect. The ongoing Outcome Reduction with an Initial Glargine Intervention (ORIGIN) study might provide more evidence, although in this study the glucose levels will probably be higher in the usual care group that prevents direct conclusions on the potential atherogenic effect of insulin (<http://www.clinicaltrials.gov/ct/show/NCT00069784>). If exogenous insulin has atherogenic effects, as found in our study, we are inclined to be prudent in prescribing high insulin doses, especially in type 2 diabetes. Furthermore, insight in environmental and genetic determinants of insulin use (in both type 1 and type 2 diabetes) is of importance to decrease the amount of insulin needed.

In conclusion, the results of this study in type 1 diabetes provide evidence that a high cumulative use of regular insulin may increase the risk of atherosclerosis.

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