Favourable serum calcification propensity with intraperitoneal as compared with subcutaneous insulin administration in type 1 diabetes

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Abstract

Background: Serum calcification propensity can be monitored using the maturation time of calciprotein particles in serum (T_{50} test). A shorter T_{50} indicates greater propensity to calcify; this is an independent determinant of cardiovascular disease. As the intraperitoneal (IP) route of insulin administration mimics the physiology more than the subcutaneous (SC) route in persons with type 1 diabetes (T1DM), we hypothesized that IP insulin influences determinants of calcium propensity and therefore result in a longer T_{50} than SC insulin administration.

Methods: Prospective, observational case-control study. Measurements were performed at baseline and at 26 weeks in age and gender matched persons with T1DM.

Results: A total of 181 persons, 39 (21.5%) of which used IP and 142 (78.5%) SC insulin were analysed. Baseline T_{50} was 356 (45) minutes. The geometric mean T_{50} significantly differed between both treatment groups: 367 [95% confidence interval (CI) 357, 376] for the IP group and 352 (95% CI 347, 357) for the SC group with a difference of –15 (95% CI –25, –4) minutes, in favour of IP treatment. In multivariable analyses, the IP route of insulin administration had a positive relation on T_{50} concentrations while higher age, triglycerides and phosphate concentrations had a positive relation.

Conclusion: Among persons with T1DM, IP insulin administration results in a more favourable calcification propensity time then SC insulin. It has yet to be shown if this observation translates into improved cardiovascular outcomes.

Keywords: cardiovascular, insulin, intraperitoneal, phosphate, serum calcification propensity, subcutaneous, T_{50}, type 1 diabetes mellitus

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Introduction

Among persons with type 1 diabetes mellitus (T1DM) there is an excess of cardiovascular morbidity and mortality as compared with persons without diabetes.\textsuperscript{1} Persons with T1DM are prone to vascular calcifications, which may aggravate the progression of vascular disease resulting in accelerated clinical manifestation of micro- and macrovascular complications and premature death.\textsuperscript{2,3} Consequently, there is a need for an improved understanding of the underlying mechanisms.\textsuperscript{1}

During the last few years, the blood mineral buffering system, which controls the precipitation of calcium and phosphate, has emerged as a novel cardiovascular risk factor.\textsuperscript{4} Here, precipitation of phosphate and calcium is seen in the perspective of a continuous interplay of inhibitors or promoters of calcium phosphate crystallization.
With the $T_{50}$ test a serum-based marker to assess the calcification in serum propensity has emerged.\textsuperscript{5} This kinetic test measures \textit{in vitro} the transformation time ($T_{50}$) of primary calciprotein particles (CPP1), consisting of complexes of calcium-phosphate and protein that are organized in amorphous nanoparticles, to secondary calciprotein particles (CPP2), which contain hydroxyapatite. A shorter $T_{50}$ indicates greater propensity to calcify, a consequence of disequilibrium between calcification stimulating and inhibiting factors.\textsuperscript{6} A shorter $T_{50}$ has been associated with ectopic calcification in the media of the vessel wall, atherosclerotic plaque progression and subsequent cardiovascular events in persons with chronic kidney disease (CKD) and end-stage renal disease (ESRD).\textsuperscript{1,5,7–15} Although vascular calcification is mainly observed in the coronary arteries and most pronounced among persons with T1DM persons with ESRD, it may already be present early in the course of T1DM.\textsuperscript{9}

Patients and methods

Study design, aims and outcomes
This was a multicentre, investigator-initiated study with a prospective, observational matched case-control design. Inclusion took place at Isala hospital (Zwolle, the Netherlands) and Diaconessenhuis hospital (Meppel, the Netherlands). The aim of the present analysis was to test the hypothesis that among persons with T1DM treated with IP insulin therapy there is a decreased calcification propensity (expressed as a higher $T_{50}$) as compared with treatment with SC insulin therapy. The primary outcome of this study was a comparison of IP insulin delivery to SC insulin delivery over the study period, with respect to $T_{50}$ levels. Secondary outcomes include (a) comparisons of IP and SC insulin delivery on determinants of serum calcification propensity including phosphate, calcium, magnesium, PTH and albumin concentrations, (b) sub-analyses for multiple daily SC injections (MDI) and continuous SC insulin infusion (CSI) treated persons and (c) a multivariable regression analysis with baseline $T_{50}$ as outcome variable.

Patient selection
Cases were persons on IP insulin therapy using an implantable insulin pump (MIP 2007D, Medtronic/Minimed, Northridge, CA, USA) for the past 4 years without interruptions of >30 days. Inclusion criteria for cases have been described in detail previously.\textsuperscript{22} In brief, persons with T1DM, aged 18–70 years using CIPII and had an HbA1c $\geq$ 58 mmol/mol (7.5%) or at least five incidents of hypoglycaemia (defined as glucose < 4.0 mmol/l) per week were eligible. The SC control group were age and gender matched to the cases and consisted of persons with T1DM, using either MDI or CSI, for the past 4 years without interruptions of >30 days and an HbA1c at time of matching of $\geq$ 53 mmol/mol (7.0%). Exclusion criteria for the present study for both cases and controls included impaired renal function (plasma creatinine $\geq$ 150 µmol/l or Cockcroft–Gault $\leq$ 50 ml/min), cardiac disease (unstable angina or myocardial infarction within the previous 12 months or New York Heart Association class III or IV congestive heart failure), cognitive
impairment, current or past psychiatric treatment for schizophrenia, cognitive or bipolar disorder, current use of oral corticosteroids or suffering from a condition that necessitated corticosteroids use more than once in the previous 12 months, alcohol or drug abuse, current gravidity or plans to become pregnant during the study. The ratio of participants on the different therapies (CIPII:MDI:CSII) was 1:2:2.

Study protocol
There were four study visits. During the first visit, baseline characteristics were collected using a standardized case record form. During the second visit (5–7 days later) laboratory measurements were performed. During the third visit, 26 weeks after the first visit, clinical parameters were collected. During the fourth visit, 5–7 days after the third visit, laboratory measurements were performed. Throughout the study period, insulin (human insulin of E. Coli origin, 400 IU/ml, trade name: Insuman Implantable®, Sanofi-Aventis) was administered with an implantable pump for IP insulin users. Persons using CSII or MDI continued their own insulin regime consisting of fast-acting insulin analogues and for MDI also long-acting insulin analogues or Neutral Protamine Hagedorn-insulin (NPH). All persons received usual outpatient T1DM care. The implantable insulin pump used during this study and related procedures have been described in more detail previously.

The study protocol was registered prior to the start of the study (ClinicalTrials.gov identifier: NCT01621308 and NL41037.075.12) and approved by the local medical ethics committee. All participants gave written informed consent.

Measurements
Demographic and clinical parameters included: age, gender, weight, length, blood pressure, smoking and alcohol habits, co-morbidities, medication use, year of diagnosis of diabetes, presence of microvascular and macrovascular complications and previous insulin therapy (kind of insulin, dosage and, if applicable, the number of daily injections of the previous day). Blood pressure was measured using a blood pressure monitor (M6 comfort; OMRON Healthcare) using the highest mean of four measurements (two on each arm). Participants were instructed to visit the laboratory after 8 h of fasting. Calcification propensity was measured as previously described. In brief, serum was exposed to high and supersaturated concentrations of calcium and phosphate solutions in 96-well plates. Pipetting was performed using an automated high-precision pipetting system (Freedom EVO 100; Tecan, Männedorf, Switzerland). The transformation of CPP1 into CPP2 was then monitored at 37°C using time-resolved nephelometry (bmg labtech, Ortenberg, Germany). Nonlinear regression curves were calculated, allowing the determination of T50 time. Analytical coefficients of variation of various sera precipitating at T50 values at 130 and 450 min were CV_mean 3.4% and CV_max 5.4%, respectively. The Friedewald formula was used to quantitate levels of LDL cholesterol. A blinded continuous glucose measurement (CGM; iPro2, Medtronic, Northridge, CA, USA) device was inserted for a period of 6 days to measure 24-hour interstitial glucose profiles. The CGM device was inserted in the periumbilical area, and in pump users contralateral to the (implanted) insulin pump. Participants were instructed to perform a minimum of 4 blood glucose self-measurements daily during the CGM period, using a blood glucose meter (Contour XT; Bayer) to calibrate the sensor. All procedures related to the CGM were performed by one trained physician (PRvdD).

Statistical analysis
Results were expressed as mean [with standard deviation (SD)] or median [with interquartile range (IQR)] for normally distributed and non-normally distributed data, respectively. A significance level of 5% (two-sided) was used. Normality was examined with Q-Q plots. To evaluate the independent impact of several variables, including the route of insulin administration, on T50 concentrations a multivariate regression model was constructed. For this model, the baseline values were used since the most extensive characterization of the population (e.g. including c-peptide measurements) was performed at baseline. First, univariable linear regression analyses were applied to identify variables that are independently associated with T50. Subsequently, all variables that associated with T50 with a p value of < 0.1 were included in the multivariate linear regression using backward selection. The quality of the model was described using the accuracy of the prediction by the adjusted R² value. Differences between the IP and SC groups averaged over
the study period were estimated using the general linear model. A regression model based on covariate analysis was applied in order to adjust for possible baseline imbalances. In the model, the fixed factors CIPII and SC insulin therapy were used as determinants. The difference in scores was determined based on the β coefficient of the particular (CIPII or SC) group. Significance of the β coefficient was investigated with the Wald test based on a \( p < 0.05 \). The size of the β coefficient, with a 95% confidence interval (CI), gives the difference (Bonferroni corrected) between both treatment modalities over the study period adjusted for baseline differences. Statistical analyses were performed using SPSS (IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp.).

Results
Of the 183 participants eligible for analysis, 2 participants (using MDI) were excluded because of insufficient blood samples to perform measurements. Subsequently samples from 181 participants were analysed. Baseline characteristics of these participants are presented in Table 1. In brief, 63% were female, mean age was 50.0 (12.6) years, median diabetes duration 24.5 (17.0, 35.2) years, mean baseline HbA1c 63.7 (10.5) mmol/mol [8.0% (1.0%)] and median serum creatinine 68.0 (61.0, 75.5) μmol/l. In total, 39 (21.5%) of the participants used CIPII and 142 (78.5%) of the participants used the SC route of insulin administration. Within the SC group, 68 (47.9%) used MDI and 74 (52.1%) CSII.

At baseline, participants using CIPII had more frequent neuropathy, lower daily basal and higher bolus insulin dose, higher C-reactive protein concentrations, less time spent in the hypoglycaemic range, higher average glucose and less glucose variation during CGM measurements as compared with participants treated with SC insulin. The \( T_{50} \) time measured at baseline was normally distributed with a mean of 356 (45) minutes. Results of the uni- and multivariate model are presented in Table 2. According to the multivariate model, factors that had an independent, inverse relation with \( T_{50} \) were age, triglycerides and phosphate concentrations. IP administration of insulin showed a positive relation with \( T_{50} \). Among all subjects, \( T_{50} \) time was 362 (95% CI 354, 367) at baseline and 357 (95% CI 350, 365) at the end of the study period (difference −5 (95% CI −15, 6)). Within the IP and SC group, there were no differences observed in \( T_{50} \) over time (see Table 3). The geometric mean \( T_{50} \) over the study period among persons treated with IP insulin was 367 (95% CI 357, 376) minutes and 352 (95% CI 347, 357) minutes among persons treated with SC insulin. When comparing both groups, there was a difference over the study period of −15 (−25, −4) minutes. After further adjustment for differences in insulin dose, average and coefficient of variation of glucose levels measured during CGM and C-reactive protein (CRP) concentrations, the geometric mean \( T_{50} \) over the study period among persons treated with IP insulin remained significantly higher as compared with the SC group: 370 (95% CI 358, 381) versus 352 (95% CI 346, 357), difference −18 (95% CI −31, −5) minutes.

Discussion
The main finding of the present study is that persons with T1DM treated with CIPII have a decreased calcification propensity, that is, higher \( T_{50} \) as compared with persons treated with SC insulin. This difference remained significant after adjustment for potential risk factors. In multivariate analysis, the mode of insulin administration was also associated with \( T_{50} \) levels. Although the \( T_{50} \) difference between the IP and SC treatment groups seems to be modest (15 min), the results of this study may provide support for the hypothesis that the IP route of insulin administration per se may have a more favourable effect on vascular calcification than the SC route.

As IP insulin administration results in higher hepatic insulin concentrations than SC insulin administration, our findings could be explained by a more favourable portal to systemic insulin ratio.\textsuperscript{21,27,28} Effects of insulin on determinants of mineral stress could explain the findings of the current study.\textsuperscript{4} Several determinants were measured, yet only phosphate concentrations were (inversely) significantly associated with \( T_{50} \) levels in multivariable analysis. Although no baseline differences in phosphate concentrations were present between the IP and SC groups, this may indicate that IP insulin affects phosphate handling. On the other hand, it could be hypothesized that nonmeasured determinants of mineral stress were involved. In particular, the liver-derived plasma protein fetuin-A that self-assembles with calcium to form CCP1 and thus is a major regulator of
Table 1. Baseline characteristics.

|                      | IP (n=39)     | SC (n=141)    | MDI (n=67)    | CSII (n=74)   |
|----------------------|---------------|---------------|---------------|---------------|
| **Clinical**         |               |               |               |               |
| Female sex (%)       | 25 (64.1)     | 89 (62.7)     | 45 (66.2)     | 44 (59.5)     |
| Age (years)          | 49.6 (12.3)   | 50.1 (12.7)   | 52.5 (12.6)   | 47.9 (12.4)   |
| BMI (kg/m²)          | 25.0 [22.3, 29.4] | 26.2 [23.3, 28.5] | 25.8 [22.2, 28.5] | 26.2 [24.3, 28.7] |
| Systolic blood pressure (mmHg) | 136 [126.0, 151.5] | 133.5 [123.0, 147.4] | 134.0 [123.1, 151.1] | 132.5 [123.0, 144.6] |
| Diabetes duration (years) | 28.5 [22.1, 36.5] | 23.0 [16.1, 34.9] | 21.7 [12.8, 34.5] | 24.7 [16.7, 35.3] |
| Retinopathy present (%) | 17 (43.6) | 45 (31.7) | 17 (25.0) | 28 (37.8) |
| Neuropathy present (%) | 20 (51.3) | 31 (21.8) | 17 (25.0) | 14 (18.9) |
| Nephropathy present (%) | 2 (5.1) | 3 (2.1) | 1 (1.5) | 2 (2.7) |
| Macrovascular complication present (%) | 7 (17.9) | 19 (13.4) | 10 (14.7) | 9 (12.2) |
| Basal insulin dose (IU/day/kg) | 0.4 [0.3, 0.7] | 0.3 [0.2, 0.4] | 0.3 [0.2, 0.4] | 0.3 [0.2, 0.4] |
| Bolus insulin dose (IU/day/kg) | 0.2 [0.1, 0.3] | 0.3 [0.2, 0.4] | 0.4 [0.3, 0.5] | 0.2 [0.2, 0.3] |
| Total insulin dose (IU/day/kg) | 0.7 [0.5, 0.9] | 0.6 [0.5, 0.8] | 0.7 [0.5, 0.8] | 0.6 [0.4, 0.7] |
| **Biochemical**      |               |               |               |               |
| HbA1c (mmol/mol)     | 66.9 (14.4)   | 62.8 (8.9)    | 62.2 (9.2)    | 63.4 (8.8)    |
| HbA1c (%)            | 8.3 (1.3)     | 7.9 (0.8)     | 7.8 (0.8)     | 8.0 (0.8)     |
| Fasting glucose (mmol/l) | 8.4 [3.8] | 8.7 [3.7] | 8.6 [3.8] | 8.8 [3.7] |
| C-peptide            | 0.01 [0.01, 0.01] | 0.01 [0.01, 0.01] | 0.01 [0.01, 0.02] | 0.01 [0.01, 0.01] |
| C-reactive protein   | 2.0 [1.0, 5.8] | 1.0 [1.0, 3.0] | 1.0 [1.0, 3.2] | 1.0 [1.0, 2.0] |
| Creatinine (μmol/l)  | 70.0 [63.0, 76.0] | 67.0 [60.0, 75.3] | 66.0 [59.3, 74.0] | 68.0 [60.8, 76.3] |
| Alkaline phosphatase [U/l] | 74.0 [63.0, 94.0] | 68.0 [56.8, 85.0] | 71.5 [59.3, 89.5] | 66.5 [55.0, 84.3] |
| Triglycerids (mmol/l) | 1.0 [0.7, 1.6] | 0.8 [0.6, 1.0] | 0.8 [0.7, 1.2] | 0.8 [0.6, 1.0] |
| Calcium (mmol/l)     | 2.3 [2.1, 2.3] | 2.3 [2.1, 2.3] | 2.3 [2.2, 2.3] | 2.3 [2.1, 2.3] |
| Albumin (g/l)        | 44.3 [38.5, 46.4] | 42.4 [39.6, 44.4] | 41.8 [39.8, 44.3] | 42.5 [39.2, 44.6] |
| Phosphate (mmol/l)   | 1.0 [0.2]     | 1.0 [0.2]     | 1.1 [0.2]     | 1.0 [0.2]     |
| Magnesium (mmol/l)   | 0.8 [0.1]     | 0.8 [0.1]     | 0.8 [0.1]     | 0.7 [0.1]     |
| 25 (OH)D (nmol/l)    | 45.1 [30.6, 67.8] | 53.5 [41.4, 72.3] | 56.7 [40.3, 83.9] | 52.6 [42.3, 66.0] |
| PTH (pmol/l)         | 4.6 [3.8, 5.5] | 4.5 [3.5, 5.5] | 4.8 [3.7, 6.1] | 4.4 [3.3, 5.3] |
| Microalbuminuria:creatinine ratio | 1.2 [0.5, 1.8] | 0.9 [0.4, 1.7] | 1.0 [0.5, 2.1] | 0.8 [0.4, 1.4] |

(Continued)
mineralization may well be involved here.\(^9\) The lack of information on such unmeasured influences of \(T_{50}\) limits the generalizability of this study; future studies that focus on the role of IP and SC insulin administration on \(T_{50}\) should therefore include, for example, fetuin-A levels. Finally, given that there were no differences in markers of mineral metabolism or inflammation between the two groups, it is also possible that the difference between the two groups is due to residual confounding or confounding by indication. Since the diabetes of the patients treated with CIIPI is in general more complex as compared with patients treated with SC, it is possible that the observed difference in \(T_{50}\) between the two groups reflects unmeasured inflammation or other factors affecting \(T_{50}\) which are inherent to the patient population and not the CIIPII treatment itself. This may be supported by the observation that \(T_{50}\) was different between the groups at baseline, but no changes were observed in \(T_{50}\) after 26 weeks of treatment with CIIPII.

Previous short-term randomized studies demonstrated that IP insulin administration results in better short-term glycaemic control\(^{22,29}\) as compared with SC insulin therapy. During long-term follow-up, lower glucose variability\(^{30}\) and an increase in insulin-like growth factor-1 concentrations\(^{31,32}\) were observed as to SC insulin administration. After several years of IP insulin therapy, HbA1c concentration was at an equal or lower level than before initiation of CIIPI.\(^{33-36}\) There also seemed to be no differences in oxidative stress after long-term IP insulin as compared with SC insulin therapy.\(^{37}\) Taken together, we speculate that the favourable effects of IP insulin on serum calcification propensity observed in the present study are independent of glycaemia and oxidative stress - the lack of significance of glucose, HbA1c and CRP in our models may emphasize this.

The present study is the first, to the best of our knowledge, to investigate the effects of the route of insulin (IP \textit{versus} SC) on the calcium propensity, measured using the \(T_{50}\) score. When comparing the \(T_{50}\) outcomes in the current study with outcomes in the general population living in the northern part of the Netherlands (using the PREVEND cohort consisting of 981 persons, mean age 58 (11) years, 74\% male and mean \(T_{50}\) of 334 (58) minutes, unpublished data), levels seem to be comparable. Besides differences between populations with respect to age and

### Table 1. (Continued)

| CGM measurements | \(\text{IP (n=39)}\) | \(\text{SC (n=141)}\) | \(\text{MDI (n=67)}\) | \(\text{CSII (n=74)}\) |
|------------------|------------------|------------------|------------------|------------------|
| Hypoglycaemia (%) | 2.0 [0.0, 6.5] | 6.0 [1.2, 12.0]\(^a\) | 10.0 [4.0, 15.0]\(^a\) | 3.0 [1.0, 7.0]\(^b\) |
| Euglycaemia (%)   | 29.0 [19.0, 45.5] | 37.0 [25.0, 51.0] | 40.0 [29.0, 58.0] | 34.0 [25.0, 43.0] |
| Hyperglycaemia (%)| 64.0 [47.0, 78.5] | 56.0 [38.0, 68.0] | 49.0 [31.0, 61.0]\(^a\) | 61.0 [51.0, 71.0]\(^b\) |
| Mean              | 10.6 [2.4] | 9.4 [1.8]\(^a\) | 9.0 [1.8]\(^a\) | 9.8 [1.7]\(^b\) |
| SD                | 3.9 [1.0] | 3.9 [0.9] | 4.0 [0.9] | 3.8 [0.8] |
| CV                | 37.2 [8.4] | 41.9 [8.9]\(^a\) | 44.8 [9.6]\(^a\) | 39.3 [7.4]\(^b\) |
| MAGE              | 7.7 [2.6] | 7.9 [2.5] | 7.9 [2.7] | 7.8 [2.3] |
| MODD              | 3.9 [1.1] | 4.1 [1.4] | 4.2 [1.7] | 4.1 [1.1] |

Data are presented as n (%), mean (SD) or median [IQR].

\(^a\)\(p<0.05\) as compared with CIPII.

\(^b\)\(p<0.05\) for MDI versus CSII.

\(p\) values are based on ANOVA (Bonferroni corrected) analysis. Retinopathy, neuropathy and nephropathy categories do not add up.

\(25(\text{OH})\text{D}\), 25-hydroxyvitamin D; ANOVA, analysis of variance; BMI, body mass index; CIPII, continuous IP insulin infusion; CSII, continuous intraperitoneal insulin infusion; CV, coefficient of variation; Gamma-GT, Gamma-glutamyl transpeptidase; IP, intraperitoneal; IQR, interquartile range; MAGE, mean average glucose excursions; MDI, multiple daily injections; MODD, mean of daily differences; PTH, parathyroid hormone; SC, subcutaneous; SD, standard deviation.

Missing values: mean of CGM measurements \(n=14\); CV \(n=14\); MAGE \(n=14\); MODD \(n=15\); fasting glucose \(n=23\).
Table 2. Univariable and multivariable analysis with baseline $T_{50}$ as outcome variable.

|                                | Univariable St. Beta | $p$ value | Multivariable St. Beta | $p$ value | Part correlation |
|--------------------------------|----------------------|-----------|------------------------|-----------|------------------|
| Gender (male = 1)              | $-0.261$             | 0.001     | $-0.102$               | 0.189     | $-0.108$         |
| Age (years)                    | $-0.153$             | 0.044     | $-0.215$               | 0.004     | $-0.233$         |
| BMI (kg/m²)                    | $-0.094$             | 0.214     |                        |           |                  |
| Systolic blood pressure (mmHg) | $0.117$              | 0.122     |                        |           |                  |
| Diabetes duration (years)      | $0.078$              | 0.303     |                        |           |                  |
| Retinopathy present (yes = 1) | $0.075$              | 0.323     |                        |           |                  |
| Neuropathy present (yes = 1)  | $0.152$              | 0.045     | $0.138$                | 0.057     | 0.156            |
| Nephropathy present (yes = 1) | $0.082$              | 0.282     |                        |           |                  |
| Macrovascular complication present (yes = 1) | $0.003$ | 0.972 |                   |           |                  |
| Total insulin dose [IU/day/kg] | $0.049$              | 0.523     |                        |           |                  |
| HbA1c [mmol/mol]               | $0.004$              | 0.954     |                        |           |                  |
| Fasting glucose [mmol/l]       | $0.130$              | 0.105     |                        |           |                  |
| C-peptide                      | $-0.018$             | 0.818     |                        |           |                  |
| C-reactive protein             | $-0.121$             | 0.116     |                        |           |                  |
| Creatinine [µmol/l]            | $0.175$              | 0.020     | $0.140$                | 0.071     | 0.148            |
| Alkaline phosphatase [U/l]     | $0.157$              | 0.038     | $0.128$                | 0.075     | 0.146            |
| Triglycerides                  | $-0.134$             | 0.077     | $-0.264$               | $<0.001$  | $-0.301$         |
| Calcium [mmol/l]               | $-0.066$             | 0.393     |                        |           |                  |
| Albumin [g/l]                  | $-0.019$             | 0.801     |                        |           |                  |
| Phosphate [mmol/l]             | $-0.434$             | $<0.001$  | $-0.329$               | $<0.001$  | $-0.365$         |
| Magnesium [mmol/l]             | $0.005$              | 0.952     |                        |           |                  |
| 25 [OH]D [nmol/l]              | $-0.046$             | 0.198     |                        |           |                  |
| PTH [pmol/l]                   | $-0.081$             | 0.291     |                        |           |                  |
| Urine microalbumin:creatinine ratio | $-0.038$   | 0.619     |                        |           |                  |
| CGM - Hypoglycaemia (%)        | $-0.122$             | 0.123     |                        |           |                  |
| CGM - Euglycemia (%)           | $-0.072$             | 0.361     |                        |           |                  |
| CGM - Hyperglycaemia (%)       | $0.116$              | 0.145     |                        |           |                  |
| CGM - Mean                     | $0.151$              | 0.056     | $0.093$                | 0.215     | 0.102            |
| CGM - SD                       | $0.111$              | 0.160     |                        |           |                  |
| CGM - CV                       | $-0.018$             | 0.818     |                        |           |                  |
| CGM - MAGE                     | $0.122$              | 0.122     |                        |           |                  |
| CGM - MODD                     | $0.144$              | 0.068     | $0.071$                | 0.320     | 0.082            |
| Route of insulin administration [IP = 1] | $0.188$ | 0.013 | $0.168$               | 0.021     | 0.189            |

$R^2$ for the multivariable model: 0.325. Calcium concentrations are corrected for albumin.

$25$(OH)D, 25-hydroxyvitamin D; BMI, body mass index; CGM, continuous glucose monitoring; CSII, continuous intraperitoneal insulin infusion; CV, coefficient of variation; Gamma-GT, Gamma-glutamyl transpeptidase; IP, intraperitoneal; MAGE, mean average glucose excursions; MDI, multiple daily injections; MODD, mean of daily differences; PTH, parathyroid hormone; SC, subcutaneous; SD, standard deviation; St., standardized; $T_{50}$, maturation time of calciprotein particles in serum.
gender distribution, it should be taken in mind that patients with current cardiac problems were excluded from the present study. As cardiovascular disease is associated with low T50,1,5,7–15 this may have resulted in an overestimation of the actual T50 in our population. Still, based on currently available data, it seems that there is no increased calcification propensity (by means of the T50 test) among persons with T1DM as compared with the general population.

Currently, IP insulin administration using an implantable pump is limited to a selected group of persons (worldwide approximately <300 persons with, for example, ‘brittle’ diabetes, frequent hypoglycaemic episodes, SC insulin resistance) due to the high costs of this treatment option. As such, this study is a unique contribution to the literature. Although previous studies (mostly in CKD and ESRD populations) associated increased T50 with favourable cardiovascular prognosis, there is currently no data on the prognostic value of T50 in persons with T1DM.13–15,38 Therefore the clinical relevance of the modest difference in T50 between the IP and SC treatment group found in this study remains to be determined.

Strengths of the present study include the inclusion of subjects who have been using their current route of therapy for at least 4 years, thus creating a stable situation, and measurements made on two time points. Limitations of this study should be mentioned. First and foremost, a major limitation is the nonrandomized design therefore no conclusions can be made regarding causality. Second, although the current analysis was pre-specified as a secondary outcome in the original study protocol, no separate power calculation was performed to detect potentially relevant differences in T50 between treatment groups. Therefore the results in this study are presented with 95% CIs.39–41 Third, as most variables were available at baseline, the multivariable analysis was only performed at baseline. Fourth, despite the multivariate analysis demonstrating a significant relation between the route of insulin delivery on the T50 score irrespective of total insulin dose, it should be noted that in the present study, the insulin dose at baseline was higher among persons treated with IP insulin as compared with SC treated persons. This finding may be explained by the increased formation of insulin antibodies among CIPII treated persons42,43 or a pronounced hepatic first-pass effect of insulin after IP administration (estimated to range between 50% and 100%21,44). Fifth, due to limited (financial) resources available, we were unable to measure FGF-23 and fetuin-A levels in the present study. As mentioned before, this could certainly be of interest from a mechanistic point of view and it hampers further analysis of the differences found in T50 between the treatment groups. Finally, it should be noted that although the T50 score (as a proxy of mineral stress) was a strong and independent risk factor for cardiovascular events in previous studies among for example, persons with advanced CKD, renal failure or renal transplant recipients,13–15,38 this has not been observed in persons with T1DM without overt renal failure yet, including the current study. We encourage future studies that explore the use of the T50 score in predicting cardiovascular events in T1DM.

Concerning the external validity of our findings, the limited number of persons treated with IP insulin make it unlikely that, on the short-term,
our findings translate into significantly less cardiovascular events. Nevertheless, as IP insulin is a last-resort treatment option for T1DM, the group of CIPII treated persons is considered selected, more complex and more prone to development of complications as compared with SC treated persons. As the IP route of insulin administration seems promising for use in fully automated closed-loop systems, our findings may become clinically relevant in due course.

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Conflict of interest statement
AP is an employee and stockholder of Calciscon. All other authors declare they have no conflict of interest.

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References
1. Ferranti SD, de Boer IH, de Fonseca V, et al. Type 1 diabetes mellitus and cardiovascular disease: a scientific statement from the American heart association and American diabetes association. Diabetes Care 2014; 37: 2843–2863.
2. Olson JC, Edmundowicz D, Becker DJ, et al. Coronary calcium in adults with type 1 diabetes: a stronger correlate of clinical coronary artery disease in men than in women. Diabetes 2000; 49: 1571–1578.
3. Stabley John N and Towler Dwight A. Arterial calcification in diabetes mellitus. Arterioscler Thromb Vasc Biol 2017; 37: 205–217.
4. Pasch A, Jahn-Dechent W and Smith ER. Phosphate, calcification in blood, and mineral stress: the physiologic blood mineral buffering system and its association with cardiovascular risk. Int J Nephrol. Epub ahead of print 2 September 2018. DOI: 10.1155/2018/9182078.
5. Pasch A, Farese S, Gräber S, et al. Nanoparticle-based test measures overall propensity for calcification in serum. J Am Soc Nephrol 2012; 23: 1744–1752.
6. Johnson RC, Leopold JA and Loscalzo J. Vascular calcification: pathobiological mechanisms and clinical implications. Circ Res 2006; 99: 1044–1059.
7. Al-Aly Z. Medial vascular calcification in diabetes mellitus and chronic kidney disease: the role of inflammation. Cardiovasc Hematol Disord Drug Targets 2007; 7: 1–6.
8. Niskanen L, Siitonen O, Suhonen M, et al. Medial artery calcification predicts cardiovascular mortality in patients with NIDDM. Diabetes Care 1994; 17: 1252–1256.
9. Snell-Bergeon JK, Budoff MJ and Hokanson JE. Vascular calcification in diabetes: mechanisms and implications. Curr Diab Rep 2013; 13: 391–402.
10. Vattikuti R and Towler DA. Osteogenic regulation of vascular calcification: an early perspective. Am J Physiol Endocrinol Metab 2004; 286: E686–E696.
11. Bostom A, Pasch A, Madsen T, et al. Serum calcification propensity and fetuin-a: biomarkers of cardiovascular disease in kidney transplant recipients. Am J Nephrol 2018; 48: 21–31.
12. Dahdal S, Dervetzis V, Chalikias G, et al. Serum calcification propensity is independently associated with disease activity in systemic lupus erythematosus. PLoS One 2018; 13: e0188695.
13. Smith ER, Ford ML, Tomlinson LA, et al. Serum calcification propensity predicts all-cause mortality in predialysis CKD. *J Am Soc Nephrol* 2014; 25: 339–348.

14. Lorenz G, Steubl D, Kemmner S, et al. Worsening calcification propensity precedes all-cause and cardiovascular mortality in haemodialyzed patients. *Sci Rep* 2017; 7: 13368.

15. Pasch A, Block GA, Bachtler M, et al. Blood calcification propensity, cardiovascular events, and survival in patients receiving hemodialysis in the EVOLVE trial. *Clin J Am Soc Nephrol* 2017; 12: 315–322.

16. Raskin P and Pak CY. The effect of chronic insulin therapy on phosphate metabolism in diabetes mellitus. *Diabetologia* 1981; 21: 50–53.

17. Winther K, Nybo M, Vind B, et al. Acute hyperinsulinemia is followed by increased serum concentrations of fibroblast growth factor 23 in type 2 diabetes patients. *Scand J Clin Lab Invest* 2012; 72: 108–113.

18. Colette C, Pares-Herbute N, Monnier L, et al. Effect of different insulin administration modalities on vitamin D metabolism of insulin-dependent diabetic patients. *Horm Metab Res* 1989; 21: 37–41.

19. Kashiwagi A, Shinozaki K, Nishio Y, et al. Endothelium-specific activation of NAD(P)H oxidase in aortas of exogenously hyperinsulinemic rats. *Am J Physiol* 1999; 277: E976–E983.

20. Nathan DM, Dunn FL, Bruch J, et al. Postprandial insulin profiles with implantable pump therapy may explain decreased frequency of severe hypoglycemia, compared with intensive subcutaneous regimens, in insulin-dependent diabetes mellitus patients. *Am J Med* 1996; 100: 412–417.

21. Selam JL, Bergman RN, Raccah D, et al. Determination of portal insulin absorption from peritoneum via novel nonisotopic method. *Diabetes* 1990; 39: 1361–1365.

22. Logtenberg SJ, Kleefstra N, Houweling ST, et al. Improved glycemic control with intraperitoneal versus subcutaneous insulin in type 1 diabetes: a randomized controlled trial. *Diabetes Care* 2009; 32: 1372–1377.

23. van Dijk PR, Logtenberg SJ, Hendriks SH, et al. Intraperitoneal versus subcutaneous insulin therapy in the treatment of type 1 diabetes mellitus. *Neth J Med* 2015; 73: 399–409.

24. Haveman JW, Logtenberg SJ, Kleefstra N, et al. Surgical aspects and complications of continuous intraperitoneal insulin infusion with an implantable pump. *Langenbecks Arch Surg* 2010; 395: 65–71.

25. van Dijk PR, Logtenberg SJ, Groenier KH, et al. Effect of i.p. insulin administration on IGF1 and IGFBP1 in type 1 diabetes. *Endocr Connect* 2014; 3: 17–23.

26. Friedewald WT, Levy RI and Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* 1972; 18: 499–502.

27. Bratusch-Marrain PR, Waldhäusl WK, Gasić S, et al. Hepatic disposal of biosynthetic human insulin and porcine C-peptide in humans. *Metabolism* 1984; 33: 151–157.

28. Giacca A, Caumo A, Galimberti G, et al. Peritoneal and subcutaneous absorption of insulin in type I diabetic subjects. *J Clin Endocrinol Metab* 1993; 77: 738–742.

29. Haardt MJ, Selam JL, Slama G, et al. A cost-benefit comparison of intensive diabetes management with implantable pumps versus multiple subcutaneous injections in patients with type I diabetes. *Diabetes Care* 1994; 17: 847–851.

30. van Dijk PR, Groenier KH, DeVries JH, et al. Continuous intraperitoneal insulin infusion versus subcutaneous insulin therapy in the treatment of type 1 diabetes: effects on glycemic variability. *Diabetes Technol Ther* 2015; 17: 379–384.

31. van Dijk PR, Logtenberg SJ, Chisalita SI, et al. Different effects of intraperitoneal and subcutaneous insulin administration on the GH-IGF-1 axis in type 1 diabetes. *J Clin Endocrinol Metab* 2016; 101: 2493–2501.

32. van Dijk PR, Logtenberg SJ, Chisalita SI, et al. After 6 years of intraperitoneal insulin administration IGF-1 concentrations in T1DM patients are at low-normal level. *Growth Horm IGF Res* 2015; 25: 316–319.

33. Logtenberg SJ, van Ballegooie E, Israël-Bultman H, et al. Glycaemic control, health status and treatment satisfaction with continuous intraperitoneal insulin infusion. *Neth J Med* 2007; 65: 65–70.

34. Gin H, Renard E, Melki V, et al. Combined improvements in implantable pump technology and insulin stability allow safe and effective long term intraperitoneal insulin delivery in type 1 diabetic patients: the EVADIAC experience. *Diabetes Metab* 2003; 29: 602–607.

35. Schaepelynck P, Renard E, Jeandidier N, et al. A recent survey confirms the efficacy and the safety of implanted insulin pumps during long-term use in poorly controlled type 1 diabetes.
36. van Dijk PR, Logtenberg SJ, Groenier KH, et al. Continuous intraperitoneal insulin infusion in type 1 diabetes: a 6-year post-trial follow-up. *BMC Endocr Disord* 2014; 14: 30.

37. van Dijk PR, Waanders F, Logtenberg SJJ, et al. Different routes of insulin administration do not influence serum free thiols in type 1 diabetes mellitus. *Endocrinol Diabetes Metab* 2019; 2: e00088.

38. Keyzer CA, de Borst MH, van den Berg E, et al. Calcification propensity and survival among renal transplant recipients. *J Am Soc Nephrol* 2016; 27: 239–248.

39. Senn SJ. Power is indeed irrelevant in interpreting completed studies. *BMJ* 2002; 325: 1304.

40. Altman DG, Moher D and Schulz KF. Peer review of statistics in medical research. Reporting power calculations is important. *BMJ* 2002; 325: 491; author reply 491.

41. Bacchetti P. Peer review of statistics in medical research: the other problem. *BMJ* 2002; 324: 1271–1273.

42. Olsen CL, Chan E, Turner DS, et al. Insulin antibody responses after long-term intraperitoneal insulin administration via implantable programmable insulin delivery systems. *Diabetes Care* 1994; 17: 169–176.

43. Jeandidier N, Boivin S, Sapin R, et al. Immunogenicity of intraperitoneal insulin infusion using programmable implantable devices. *Diabetologia* 1995; 38: 577–584.

44. Radziuk J, Pye S, Seigler DE, et al. Splanchnic and systemic absorption of intraperitoneal insulin using a new double-tracer method. *Am J Physiol* 1994; 266: E750–E759.

45. Dassau E, Renard E, Place J, et al. Intraperitoneal insulin delivery provides superior glycaemic regulation to subcutaneous insulin delivery in model predictive control-based fully-automated artificial pancreas in patients with type 1 diabetes: a pilot study. *Diabetes Obes Metab* 2017; 19: 1698–1705.

46. Chakrabarty A, Gregory JM, Moore LM, et al. A new animal model of insulin-glucose dynamics in the intraperitoneal space enhances closed-loop control performance. *J Process Control* 2019; 76: 62–73.