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Original Research

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 an inverse 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

Introduction

Among persons with type 1 diabetes mellitus (T1DM) there is an excess of cardiovascular morbidity and mortality as compared with persons without diabetes.[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.[2,3] Consequently, there is a need for an improved understanding of the underlying mechanisms.[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.[4] Here, precipitation of phosphate and calcium is seen in the perspective of a continuous interplay of inhibitors or promoters of calcium phosphate crystallization.

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With the T_{50} test a serum-based marker to assesses the calcification in serum propensity has emerged.[5] This kinetic test measures *in vitro* the transformation time (T_{50}) of primary calciprotein particles (CPP1), consisting of complexes of calcium-phosphate and protein that 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.[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).[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.[9]

Insulin appears to be involved in the blood mineral buffering system through several mechanisms. Insulin promotes, for example, cellular influx of phosphate from the extracellular fluid[16] resulting in a decrease of phosphate levels after acute insulin administration.[17] Hyperinsulinemia also increases levels of fibroblast growth factor (FGF)-23[17] and intraperitoneal (IP) insulin administration may increase concentrations of calcidiol.[18] In conjunction with the detrimental effects of hyperinsulinemia on the vascular endothelium, including increasing endothelium dysfunction and promoting oxidative stress,[19] this 'mineral stress' may contribute to the process of vascular calcification.[4]

With continuous IP insulin infusion (CIPII), insulin is infused directly in the IP cavity, resulting in higher insulin concentrations in the portal vein catchment area, higher hepatic extraction of insulin and lower peripheral plasma insulin concentrations as compared with SC insulin administration.[20,21] We hypothesized that IP insulin may positively influence major determinants of serum calcium propensity [including phosphate, calcium, magnesium, parathyroid hormone (PTH) and albumin concentrations] and therefore result in a decreased calcification propensity (expressed as a higher T_{50}) as compared with subcutaneous (SC) insulin therapy. Therefore, we investigated the effects of IP insulin administration, as compared with SC insulin therapy, on the T_{50} levels in persons with T1DM.

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) subanalyses for multiple daily SC injections (MDI) and continuous SC insulin infusion (CSII) 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.[22] In brief, persons with T1DM, aged 18–70 years using CIPII and had an HbA1c ≥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 CSII, for the past 4 years without interruptions of >30 days and a HbA1c at time of matching of ≥53 mmol/mol (7.0%). Exclusion criteria for the present study for both cases and controls included impaired renal function (plasma creatinine ≥150 µmol/l or Cockcroft–Gault ≤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.[23] 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.[24,25]

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.[5] 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 T_{50} 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.[26] 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 (PRvD).

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 T_{50} 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 T_{50}. Subsequently, all variables that associated with T_{50} 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 R^2 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 baseline 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.[21,27,28] Effects of insulin on determinants of mineral stress could explain the findings of the current study.[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/m^2)	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)[a]	17 (25.0)[a]	14 (18.9)[a]
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][a]	0.3 [0.2, 0.4][a]	0.3 [0.2, 0.4][a]
Bolus insulin dose (IU/day/kg)	0.2 [0.1, 0.3]	0.3 [0.2, 0.4][a]	0.4 [0.3, 0.5][a]	0.2 [0.2, 0.3][b]
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][a]
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)[c]	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][a]	1.0 [1.0, 3.2]	1.0 [1.0, 2.0][a]
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)[b]
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)

Table 1. (Continued)

	IP (*n* = 39)	SC (*n* = 141)	MDI (*n* = 67)	CSII (*n* = 74)
CGM measurements				
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]
Euglycemia (%)	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](OH)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.

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 CIPII 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 CIPII 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 CIPII.

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 CIPII.[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 *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 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)[c]	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.

Table 3. T_{50} outcomes within and between treatment groups.

	Baseline	End	Difference within group	Difference with IP
All	362 (354, 370)	357 (350, 365)	5 (−15, 6)	NA
IP	372 (358, 386)	362 (349, 375)	−10 (−29, 9)	NA
SC	352 (344, 359)	352 (346, 360)	1 (−9, 11)	−15 (−25, −4)
MDI	342 (332, 353)	346 (336, 355)	3 (−11, 18)	−23 (−37, −8)
CSII	360 (350, 370)	359 (350, 369)	−0.2 (−19, 9)	−8 (−22, 7)

CSII, continuous subcutaneous insulin infusion; IP, intraperitoneal; MDI, multiple daily injections; NA not applicable; SC, subcutaneous; T_{50}, maturation time of calciprotein particles in serum.
T_{50} levels are expressed in minutes.

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 T_{50},[1,5,7–15] this may have resulted in an overestimation of the actual T_{50} in our population. Still, based on currently available data, it seems that there is no increased calcification propensity (by means of the T_{50} 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 T_{50} with favourable cardiovascular prognosis, there is currently no data on the prognostic value of T_{50} in persons with T1DM.[13–15,38] Therefore the clinical relevance of the modest difference in T_{50} 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 T_{50} 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 T_{50} 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 persons[42,43] 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 T_{50} between the treatment groups. Finally, it should be noted that although the T_{50} 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 T_{50} 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,[45,46] our findings may become clinically relevant in due course.

Authors' note
PRVD: Design, inclusion of patients, measurements, statistical analysis, writing manuscript. FW: Design, interpretation of data, critically reviewing manuscript. AP: Design, measurements, critically reviewing manuscript. SJJL: Design, critically reviewing manuscript. TMV: Design, critically reviewing manuscript. KG.: Design, critically reviewing manuscript. JLH: Design, critically reviewing manuscript. NK: Design, critically reviewing manuscript. ROBG: design, critically reviewing manuscript. HVG: Design, measurements, critically reviewing manuscript. HJGB: Design, critically reviewing manuscript. All authors approved the final version of the manuscript.

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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