ORIGINAL ARTICLE

WILEY

Insulin induces a progressive increase in the resistance of subcutaneous tissue to fluid flow: Implications for insulin pump therapy

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Funding information
Medical University of Graz; Roche

Abstract

Aim: To determine the effect of insulin on the resistance of subcutaneous tissue to the flow of infusion fluids.

Materials and methods: Thirty subjects with type 1 diabetes wore two Accu-Chek Spirit Combo insulin pumps with Accu-Chek FlexLink infusion sets (Roche Diabetes Care, Mannheim, Germany) for 7 days. One pump was filled with insulin aspart (Novo Nordisk, Bagsvaerd, Denmark) and used for continuous subcutaneous insulin infusion (CSII). The other pump was filled with insulin diluting medium (IDM; Novo Nordisk) and used to deliver IDM subcutaneously at rates identical to those employed for CSII. Both infusion sites were assessed daily by measuring the pressure required to infuse various bolus amounts of IDM.

Results: On day 1, maximum pressure (P_{max}) and tissue flow resistance (TFR; calculated from measured pressure profiles) were similar for both infusion sites ($P > 0.20$). During the subsequent study days, the P_{max} and TFR values observed at the IDM infusion site remained at levels comparable to those seen on day 1 ($P > 0.13$). However, at the site of CSII, P_{max} and TFR progressively increased with CSII duration. By the end of day 7, P_{max} and TFR reached 25.8 */2.11 kPa (geometric mean */geometric standard deviation) and 8.64 */3.48 kPa*s/μL, respectively, representing a remarkable 3.5- and 20.6-fold increase relative to the respective P_{max} and TFR values observed on day 1 ($P < 0.001$).

Conclusion: Our results suggest that insulin induces a progressive increase in the resistance of subcutaneous tissue to the introduction of fluid; this has important implications for the future design of insulin pumps and infusion sets.

KEYWORDS
CSII, insulin analogues, insulin pump therapy, pharmacodynamics, type 1 diabetes

1 | INTRODUCTION

Insulin pump therapy has become a popular treatment option for people with type 1 diabetes.[1,2] This type of therapy, also known as continuous subcutaneous insulin infusion (CSII), uses a small, battery-operated, electromechanical pump to continuously deliver insulin through a fine-bore cannula inserted into the subcutaneous tissue.[1-3] In order to deliver accurate doses of basal and bolus insulin, insulin pumps must achieve a sufficiently high pumping pressure to overcome the flow resistance that the insulin solution encounters as it passes through the tubing and cannula (infusion set) and into the subcutaneous tissue. One important source of flow resistance is friction occurring in the infusion set among the moving fluid molecules themselves and between the fluid molecules and the inside walls of the infusion set. The magnitude of this infusion set-related flow resistance largely depends on the diameter and length of the infusion set's components, and the viscosity of the infusate.[4,5] Another important source of flow resistance is associated with the frictional forces that the extracellular matrix of the subcutaneous tissue exerts on the infusate molecules that pass through it.[6,7] The subcutaneous extracellular matrix occupies the space between the subcutaneous tissue cells (~10% of tissue volume) and is primarily composed of glycosaminoglycan chains that are enmeshed in a dense network of cross-linked collagen fibres.[6-9] This network structure behaves as though it were penetrated by pores with an estimated average pore radius of ~25 nm.[6] Since the cell membranes of the tissue cells (mostly fat cells) are virtually impermeable to the flow of fluids, infusate flow in the subcutaneous tissue is largely confined to the narrow pores of the extracellular matrix.[6,9] Thus, the subcutaneous tissue resistance to infusate flow mainly results from the frictional forces generated in the pores of the extracellular matrix. Therefore, the magnitude of this tissue-related flow resistance (TFR) largely depends on the structural properties of the extracellular matrix, such as porosity, pore size and shape, tortuosity and connectivity, as well as on the viscosity of the infusate.[6,7]

Although the flow resistance in the path of fluid flow is a key parameter in the design and sizing of pumps,[10] there have been only a very few attempts to assess this resistance for the insulin infusion set and the subcutaneous infusion site in humans.[11,12] In a study performed in 10 healthy subjects, Højbjerre et al[11] measured the infusion pressure required to deliver a 250-μL bolus of saline into subcutaneous tissue via a steel or plastic cannula. They found that, for both cannula types, the observed maximum infusion pressures tended to be higher on day 3 compared with day 1 of infusion site use, suggesting that prolonged use of a subcutaneous infusion site may result in an increase in the TFR at the infusion site. Likewise, in a study conducted in 12 healthy subjects and 12 people with diabetes, Patte et al[12] determined the pressure required to infuse a 300-μL bolus of saline through a steel cannula into subcutaneous tissue. However, in contrast to Højbjerre et al,[11] these authors observed that the tissue resistance pressure (calculated as the difference in pressure between infusing into tissue and infusing into air) did not change during a 3.5-day cannula wear period, indicating that prolonged use of a subcutaneous infusion site may not result in an increase in the TFR at the

infusion site. Unfortunately, in both studies only saline was used as the infusion fluid, and thus no reliable conclusions can be drawn from these studies concerning the hydraulic properties of the tissue at the site of CSII. Therefore, to clarify the conflicting results from the previous human studies and to ascertain whether there is a difference in the hydraulic properties between insulin-exposed and -unexposed tissue sites, the present study determined the TFR at the site of CSII in subjects with type 1 diabetes and compared it with that seen at an insulin-diluent infusion site in the same subjects.

2 | MATERIALS AND METHODS

2.1 | Study subjects

Thirty subjects were included in the study. They were of both sexes, in the age group of 18 to 64 years, and diagnosed with type 1 diabetes. Inclusion criteria were a glycated haemoglobin (HbA1c) level of <86 mmol/mol, and treatment with CSII. Subjects were excluded if they had evidence of clinically overt diabetic complications, had local lipodystrophy at insulin infusion sites, and/or had taken any vasoactive agents or anticoagulation medication. Each subject signed a written consent form after the purpose, nature, and potential risks of the study had been explained. The study was approved by the ethics committee of the Medical University of Graz and the Austrian Agency for Health and Food Safety (EU Clinical Trials Register no. EudraCT 2015-005311-32).

2.2 | Study design

Eligible subjects wore two identical insulin pumps over a period of 7 days. One pump was used for CSII therapy and the other for the infusion of an insulin-free solution. At both infusion sites, the TFR was assessed shortly after establishing the infusion sites (day 0) and on each of the 7 subsequent days (days 1-7; Figure 1).

On day 0, subjects were admitted to the clinical research centre (CRC) at ~9:00 AM. On admission, blood glucose measurements were performed every 30 minutes using a glucose meter (FreeStyle Freedom Lite; Abbott Diabetes Care, Alameda, California). Employing the subjects' own insulin pumps, the insulin infusion rate was then adjusted on the basis of the glucose measurements to achieve glucose levels between 70 and 180 mg/dL. Concurrent with the glucose measurements and infusion rate adjustments, an insulin pump reservoir (Accu-Chek® Spirit Cartridge System; Roche Diabetes Care GmbH, Mannheim, Germany) was filled with insulin (NovoRapid; Novo Nordisk, Bagsvaerd, Denmark) and another with insulin-diluting medium (IDM) for NovoLog (Novo Nordisk). With the exception of the insulin molecule, the IDM contained all the ingredients of the insulin formulation including preservatives, such as phenol and m-cresol.[13] The filled reservoirs were then attached to infusion sets (Accu-Chek® FlexLink; Roche Diabetes Care GmbH) and inserted into insulin pumps (Accu-Chek® Spirit Combo, Roche Diabetes Care GmbH). The lengths
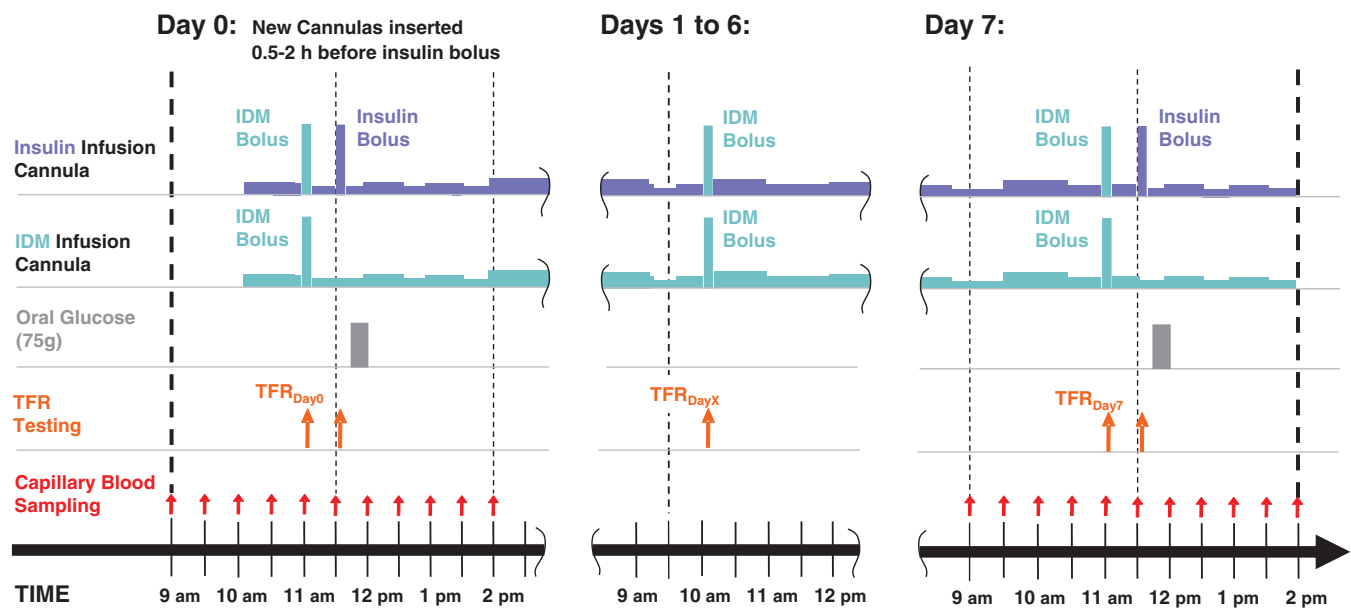
FIGURE 1 Schematic representation of the study protocol. In the morning of the first study day (day 0), two identical infusion set cannulas were inserted into subcutaneous tissue of 30 diabetes patients and used for the infusion of insulin (violet) and insulin-diluting medium (IDM; turquoise). On day 0 and on each of the following 7 days (days 1-7), the tissue flow resistance (TFR) was assessed at both infusion sites (orange arrows) by measuring the pressure required to infuse bolus amounts of IDM (turquoise). To perform these measurements, an insulin pump retrofitted with a pressure sensor and filled with IDM was attached to the two infusion cannulas (Supplemental Figure S1). On days 0 and 7, the determination of TFR was repeated at the insulin infusion site by measuring the pressure generated during the infusion of a bolus of insulin (violet). On the days when an insulin bolus was administered to determine the TFR (days 0 and 7), glucose concentrations in capillary blood were measured frequently (every 30 minutes, red arrows), and, to prevent hypoglycaemic events, glucose (75 g; grey) was ingested after insulin bolus delivery

of the infusion set cannula (6, 8 or 10 mm) and tubing (30, 60, 80 or 110 cm) were selected according to the subject's preferences. Once the subjects' glucose levels were maintained in the target range, the infusion sets were primed and the two cannulas inserted on opposite sides of the abdomen or hips (Figure 1). Afterwards, both pumps were programmed with the same basal insulin infusion profile that had been used by the subject prior to the study period. The subject's own infusion set and insulin pump were then removed and the basal infusion of insulin and IDM started. Subsequently, all subjects were instructed on the use of the insulin pump from Roche. After 0.5 to 2 hours of insulin and IDM infusion (Figure 1, day 0), the subject's pumps were detached and an additional pump (Accu-Chek® Spirit Combo), retrofitted with a disposable pressure sensor (DPT-100; Utah Medical Products, Midvale, Utah) between the pump reservoir and infusion set tube, was filled with IDM and connected to the subject's infusion set cannulas (Supplemental Figure S1). After attaching the pump, 10-, 50-, 100- and 250-μL boluses of IDM were delivered and the infusion pressures measured. Subsequently, in order to assess whether there was a difference between the infusion pressure required for infusing IDM and that for infusing insulin, another pump (Accu-Chek® Spirit Combo) with a disposable pressure sensor, was filled with insulin (NovoRapid) and connected to the subject's insulin infusion cannula. Then, an insulin bolus was administered and the infusion pressure measured (Figure 1, day 0). The size of the administered insulin bolus equalled the subject's usual insulin dose to cover 75 g of glucose. To facilitate the comparison with IDM bolus administrations, the insulin

bolus was divided into two portions, one equal to 5 U, and one equal to the total dose minus 5 U. After these measurements, the previously used pumps were re-attached to the corresponding infusion cannulas and the insulin and IDM infusion continued. Fifteen minutes after bolus administration, the subjects were asked to ingest 75 g glucose dissolved in 300 mL water (Figure 1, day 0). If, after glucose ingestion, the capillary blood glucose levels slowly decreased to approximately 200 mg/dL, the subjects were allowed to leave the CRC. However, if the glucose levels decreased below 60 mg/dL, the subjects were asked to ingest additional glucose and to wait until euglycaemic glucose levels were re-attained. On leaving the CRC, subjects were reminded to continue infusing the IDM and insulin at the same rates and bolus sizes at home. Furthermore, subjects were asked to perform at least seven blood glucose measurements per day and to immediately contact the study team when correction boluses failed to decrease their glucose levels. In addition, they were asked to keep a written diary containing the estimated carbohydrate intake, the insulin bolus amounts, results of the blood glucose measurements as well as the time of meals, bolus administrations, and glucose measurements.

The next day (day 1), the subjects were re-admitted to the CRC (Figure 1, days 1-6). On arrival, the subject's pumps were disconnected and the pump with the pressure sensor was attached to the infusion cannulas instead. Afterwards, 10-, 50-, 100- and 250-μL boluses of IDM were administered and the infusion pressures measured. Subsequently, the detached pumps were re-connected, and the subjects were allowed to leave the CRC. The IDM bolus

administrations and the simultaneous infusion pressure measurements were then repeated on days 2, 3, 4, 5 and 6 at approximately the same time of day (Figure 1, days 1-6).
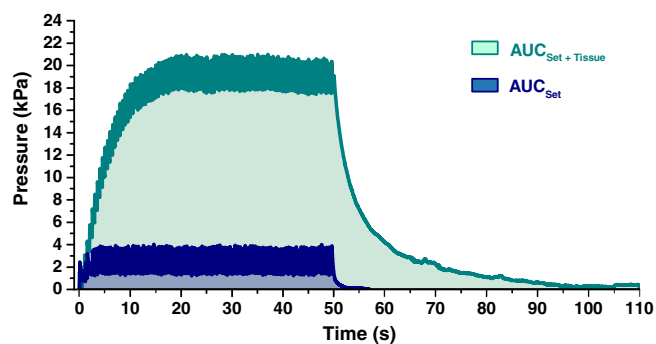
On day 7, the subjects were admitted to the CRC at ~09:00 AM. Afterwards, the experimental procedures applied were the same as those used on day 0, except that no new infusion cannulas were inserted on the morning of the study day (Figure 1, day 7).

2.3 | Study endpoints

The study endpoints were computed from the infusion pressure time courses recorded for the boluses infused through the infusion set into air (P_S) and through the infusion set into tissue (P) (Figure 2). TFR, which was considered the primary endpoint, was calculated as TFR = $(AUC_{set+tissue} - AUC_{set})/V_b$, where $AUC_{set+tissue}$ is the area under the P curve, AUC_{set} is the area under the P_S curve, and V_b is the delivered bolus volume. Detailed derivation of this equation is given in the Supplemental Methods and Supplemental Figure S2. Secondary endpoints included the maximum pressure values observed during bolus delivery into tissue (P_{max}) and into air (P_{Smax}), the means of the pressure values recorded during bolus delivery into tissue (P_{mean}) and into air (P_{Smean}), as well as the flow resistance associated with the infusion set only (R_S), which was calculated as $R_S = AUC_{set}/V_b$.

2.4 | Statistical methods

Normal probability plots and Kolmogorov–Smirnov (KS) tests were used to test for normality of data distribution. As all datasets followed a log-normal distribution (P values from the KS tests were all > 0.10; Supplemental Figure S3), statistical comparisons were performed on log-transformed data using the two-tailed paired t-test and one-factor

repeated-measures analysis of variance. A P value below 0.05 was considered to indicate statistical significance. All data are presented as the geometric mean*/one geometric standard deviation (geoMean*/geoSD), unless otherwise indicated. From these measures of location and variability, the lower and upper tolerance limit (TL) values, which include with confidence γ at least proportion β of the infusion pressures, can be calculated as $TL_{lower} = geoMean/(geoSD)^K$ and $TL_{upper} = geoMean*(geoSD)^K$, where K is the tolerance factor, which depends on β, γ and sample size n.[14,15] Sample size calculations were based on the desired precision of estimates of the population standard deviation of the maximum infusion pressure ([16,17]; Supplemental Methods).
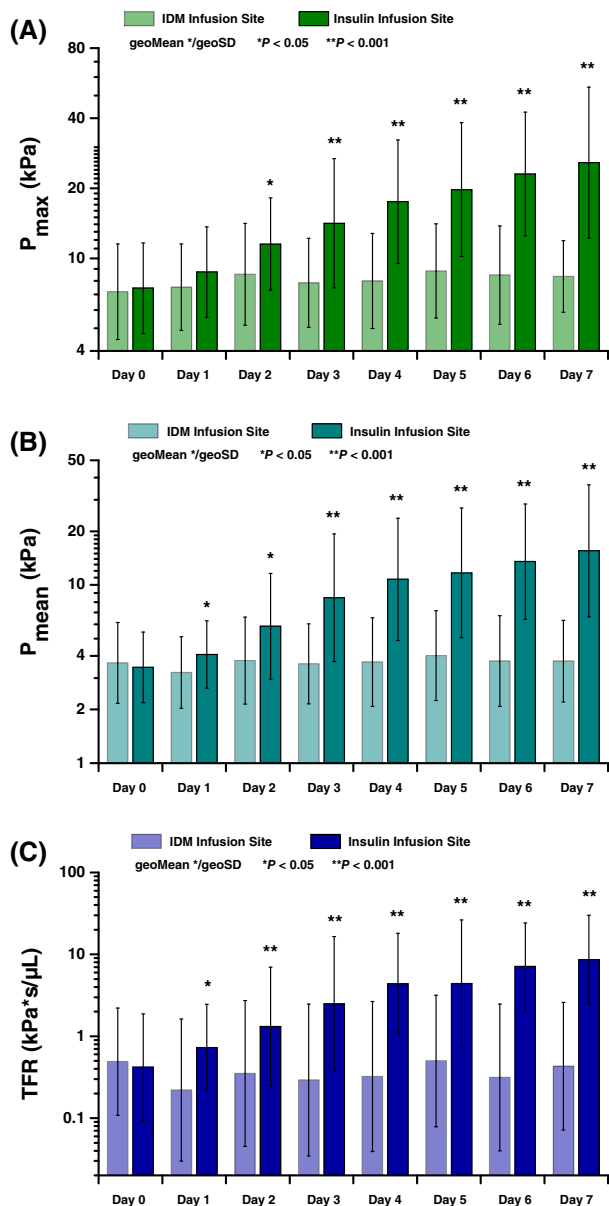
3 | RESULTS

3.1 | Subject characteristics

Thirty-five subjects with type 1 diabetes were invited to take part in the study. Of these, five were excluded due to screening errors. The 30 subjects who completed the study (eight women and 22 men) had a mean ± SD age of 43.5 ± 12.5 years (range 21-64 years) and a mean ± SD body mass index of 26.9 ± 4.0 kg/m² (range 20.9-39.5 kg/m²). Their mean ± SD diabetes duration was 24.5 ± 10.5 years (range 9-46 years), and their mean ± SD HbA1c level was 63 ± 9 mmol/mol (7.9 ± 0.8%; range 46-80 mmol/mol [6.4-9.5%]; normal range 23-41 mmol/mol [4.3-5.9%]).

3.2 | Infusion set function

All subjects wore both infusion sets for the full 7 days. Twenty-three of the 30 subjects wore infusion cannulas with a cannula length of 8 mm, another four wore cannulas with a length of 6 mm, and three subjects wore cannulas with a length of 10 mm. The infusion cannulas were connected to the pumps' reservoirs by infusion set tubes with a length of either 60 cm (15 subjects), 80 cm (12 subjects), 110 cm (2 subjects), or 30 cm (1 subject). No infection at the cannula insertion sites or uncorrectable hyperglycaemia occurred in any of the subjects. However, in seven instances, the adhesive tape of the cannula housing loosened, causing the fluid to leak around the infusion set cannula. In each of these cases, leakage occurred only at the site of insulin infusion after more than 4 days of infusion site use. To prevent further leakage from these infusion sites, the cannula housing adhesive tapes were resecured to the subjects' skin using additional adhesive strips (Fixomull; BSM Medical, Hamburg, Germany) and/or liquid tissue adhesive (Indermil; Henkel, Dublin, Ireland). Furthermore, on day 0, there were four instances in which grossly elevated infusion pressures were measured during bolus delivery via the 60 newly inserted cannulas (two cases occurred at the insulin infusion site and two at the IDM infusion site). Cannulas at these infusion sites were then removed and replaced by shorter infusion set cannulas (two had a length of 8 mm and two had a length of 6 mm). No malfunctions of the insulin pumps or pressure sensing system were noted during the study.

FIGURE 2 Representative infusion pressure time courses observed for an 100-μL bolus of insulin-diluting medium, infused either through the infusion set into air (P_S; blue curve) or through the infusion set into insulin infusion site tissue (cyan curve). The flow resistance associated with the infusion set is calculated as the area under the observed P_S curve (AUC_{set}) divided by the delivered bolus volume (V_b). The tissue flow resistance is obtained by subtracting the AUC_{set} from the area under the observed P curve ($AUC_{set+tissue}$) and dividing the result by V_b

FIGURE 3 Average infusion pressures (P_{max}, P_{mean}) and tissue flow resistances (TFR) obtained for 100-μL boluses of insulin-diluting medium (IDM) administered at the insulin and IDM infusion sites during the 7-day study period. **A**, P_{max} values observed at the IDM (light green) and insulin (green) infusion sites. **B**, P_{mean} values observed at the IDM (light cyan) and insulin (cyan) infusion sites. **C**, TFR values observed at the IDM (light blue) and insulin (blue) infusion sites. Data are geoMean */geoSD (n = 30). Note that the y-axes are in logarithmic scale. Comparisons between infusion sites were performed using the two-tailed paired t-test on log-transformed data

3.3 | Pressures and flow resistances observed during bolus infusion through infusion sets into air

The average P_{Smax}, P_{Smean}, and R_S values observed during the infusion of IDM boluses through the infusion set into air are given in Supplemental Table S1. As can be seen, average P_{Smax}, P_{Smean} and R_S values obtained for the various bolus amounts of IDM remained unchanged over the 7-day study period ($P > 0.10$). During the bolus infusions, P_{Smax} values ranged from 1.8 to 15.6 kPa, with a geoMean of 6.0 kPa, while R_S values ranged from 0.53 to 2.43 kPa*s/μL, with a geoMean of 1.11 kPa*s/μL. Furthermore, when 50-μL boluses of insulin were infused through the infusion set into air, the P_{Smax}, P_{Smean}, and R_S values obtained (Supplemental Table S2 and Figure 3) were comparable to those measured during the infusion of 50-μL boluses of IDM ($P > 0.24$; Supplemental Table S1).

3.4 | Infusion pressures and flow resistances observed at insulin and IDM infusion sites

The average P_{max}, P_{mean} and TFR values obtained for the subcutaneous sites of insulin and IDM infusion are shown in Table 1, Figure 3 and Supplemental Table S3. As can be seen, P_{max}, P_{mean} and TFR values obtained on the first study day (day 0) were similar for both infusion sites ($P > 0.20$). During the subsequent study days, the P_{max}, P_{mean} and TFR values observed at the IDM infusion site remained at levels comparable to those seen on day 0 ($P > 0.13$). However, at the insulin infusion site, the P_{max}, P_{mean} and TFR values progressively increased with increasing duration of infusion site use (Figure 3, Table 1, Supplemental Table S3). By the end of the study period, P_{max}, P_{mean} and TFR reached levels of 25.8*/2.11 kPa, 15.5*/2.35 kPa, and 8.64*/3.48 kPa*s/μL, respectively, representing a 3.5-, 4.6- and 20.6-fold increase relative to the respective P_{max}, P_{mean} and TFR values observed on day 0 ($P < 0.001$). At the IDM infusion sites, P_{max} values ranged from 2.3 to 39.4 kPa, and TFR values ranged from 0.01 to 27.46 kPa*s/μL, while at the insulin infusion sites, P_{max} values ranged from 2.7 to 128.8 kPa, and TFR values ranged from 0.01 to 157.03 kPa*s/μL. From the geoMean and geoSD values observed for P_{max} on day 7, the upper TLs below which 75%, 90% and 95% of the population of P_{max} values after 7 days of CSII will lie, were estimated to be 78.9, 127.5, and 173.1 kPa, respectively. Furthermore, when 50-μL boluses of insulin were administered on days 0 and 7 at the insulin infusion sites, the observed P_{max}, P_{mean} and TFR values (Figure 4 and Supplemental Table S2) were comparable to those obtained on days 0 and 7 when 50-μL boluses of IDM were administered at these sites ($P > 0.22$, Table 1 and Supplemental Table S3).

4 | DISCUSSION

The present investigation demonstrates that insulin induces a progressive increase in the resistance of subcutaneous tissue to the flow of infusion fluids. When the subcutaneous infusion sites were evaluated on the first day of their use, the TFR at the site of rapid-acting insulin infusion was found to be similar to that observed at the site of the insulin-free IDM infusion, but less than half the resistance caused by the infusion set itself (~0.4 vs. 1.1 kPa*s/μL; Figures 3 and 4). Thus, during the first day of infusion site use, the majority of the pressure provided by the pump to administer insulin was to overcome the resistance of the infusion set (~70%). However, during the subsequent

TABLE 1 Average P_{max} and tissue flow resistance values obtained for insulin and insulin-diluting medium infusion sites

P_{max} observed at IDM infusion sites, kPa[a,b]

Day	10 μL	50 μL	100 μL	250 μL
0	6.8*/1.61	7.2*/1.64	7.2*/1.61	7.9*/1.69
1	7.0*/1.53	7.6*/1.53	7.5*/1.53	7.9*/1.52
2	7.4*/1.59	8.9*/1.70	8.5*/1.65	8.6*/1.60
3	6.8*/1.57	7.7*/1.54	7.9*/1.55	7.7*/1.55
4	6.7*/1.41	7.4*/1.53	8.0*/1.60	8.1*/1.58
5	7.6*/1.49	8.7*/1.56	8.8*/1.59	9.3*/1.61
6	7.4*/1.50	8.4*/1.60	8.5*/1.63	8.6*/1.59
7	7.5*/1.36	8.4*/1.44	8.4*/1.42	8.6*/1.42
p^g	0.707	0.151	0.318	0.497

P_{max} observed at insulin infusion sites, kPa[a,b]

Day	10 μL	p^c	50 μL	p^d	100 μL	p^e	250 μL	p^f
0	6.4*/1.48	0.401	6.7*/1.52	.342	7.4*/1.56	0.696	7.9*/1.68	0.998
1	7.8*/1.57	0.072	8.6*/1.56	.129	8.7*/1.56	0.061	8.8*/1.45	0.080
2	9.3*/1.46	0.001	11.8*/1.60	.013	11.5*/1.58	0.006	11.8*/1.54	0.001
3	9.7*/1.63	<0.001	14.6*/1.88	<.001	14.1*/1.90	<0.001	14.0*/1.82	<0.001
4	10.6*/1.66	<0.001	17.8*/1.86	<.001	17.5*/1.84	<0.001	14.0*/1.82	<0.001
5	11.5*/1.79	<0.001	20.4*/1.94	<.001	19.7*/1.94	<0.001	19.1*/1.89	<0.001
6	11.5*/1.83	<0.001	23.6*/1.78	<.001	23.0*/1.84	<0.001	21.9*/1.83	<0.001
7	11.0*/1.79	<0.001	23.7*/1.94	<.001	25.8*/2.11	<0.001	21.8*/2.06	<0.001
p^g	<0.001		<0.001		<0.001		<0.001	

TFR observed at IDM infusion sites, kPa*s/μL[a,b]

Day no.	10 μL	50 μL	100 μL	250 μL
0	0.65*/6.34	0.49*/4.52	0.49*/5.19	0.37*/4.88
1	0.32*/7.51	0.28*/7.38	0.32*/7.46	0.22*/6.88
2	0.40*/8.60	0.49*/7.76	0.49*/7.28	0.32*/7.07
3	0.56*/7.23	0.42*/8.45	0.42*/7.41	0.27*/8.07
4	0.42*/8.00	0.37*/8.26	0.37*/7.02	0.31*/6.53
5	0.66*/6.27	0.67*/4.97	0.50*/6.37	0.39*/9.31
6	0.59*/7.82	0.41*/8.46	0.31*/7.89	0.33*/6.89
7	1.00*/4.02	0.55*/5.51	0.43*/6.01	0.36*/6.33
p^g	0.129	0.346	0.263	0.781

TFR observed at insulin infusion sites, kPa*s/μL[a,b]

Day no.	10 μL	p^c	50 μL	p^d	100 μL	p^e	250 μL	p^f
0	0.42*/6.47	0.332	0.35*/3.99	0.359	0.42*/4.46	.680	0.36*/4.89	0.976
1	0.91*/4.19	0.012	0.89*/3.07	0.002	0.73*/3.38	.002	0.70*/2.98	0.001
2	2.51*/4.68	<0.001	1.84*/3.70	<0.001	1.32*/5.30	<.001	1.38*/3.96	<0.001
3	5.72*/5.61	<0.001	3.49*/5.57	<0.001	2.48*/6.66	<.001	2.17*/6.44	<0.001
4	6.86*/6.14	<0.001	5.20*/4.79	<0.001	4.38*/4.12	<.001	3.78*/3.33	<0.001
5	8.15*/4.98	<0.001	6.74*/4.51	<0.001	4.40*/6.01	<.001	3.61*/5.56	<0.001
6	10.40*/6.03	<0.001	11.1*/3.48	<0.001	7.14*/3.38	<.001	4.51*/5.03	<0.001
7	9.84*/7.56	<0.001	11.7*/3.83	<0.001	8.64*/3.48	<.001	5.79*/3.37	<0.001
p^g	<0.001		<0.001		<0.001		<0.001	

aData are geometric mean */geometric standard deviation, n = 30.
bObtained using 10-, 50-, 100-, and 250-μL boluses of IDM.
cComparison for 10-μL boluses between infusion sites with two-tailed paired t-test on log-transformed data.
dComparison for 50-μL boluses between infusion sites with two-tailed paired t-test on log-transformed data.
eComparison for 100-μL boluses between infusion sites with two-tailed paired t-test on log-transformed data.
fComparison for 250-μL boluses between infusion sites with two-tailed paired t-test on log-transformed data.
gComparison between days with repeated-measures ANOVA on log-transformed data.
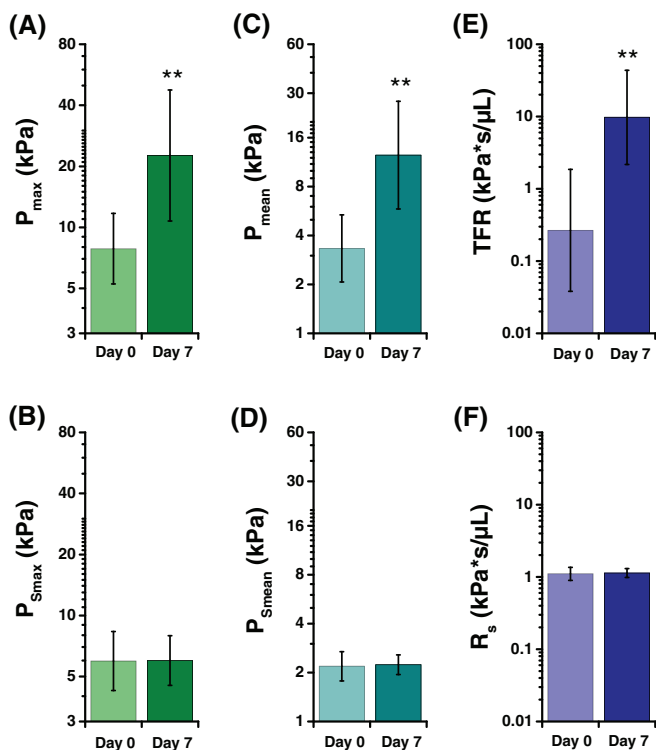
FIGURE 4 Average infusion pressures (maximum pressure [P_{max}], mean pressure [P_{mean}], maximum pressure generated when infusing through the infusion set into air [P_{Smax}], mean pressure generated when infusing through the infusion set into air [P_{Smean}]) and flow resistances (tissue flow resistance [TFR], infusion set-related flow resistance [R_S]) obtained for 5-U boluses of insulin infused on study days 0 and 7. **A**, P_{max} values observed at the insulin infusion sites on day 0 (light green) and day 7 (green). **B**, P_{Smax} values observed on day 0 (light green) and day 7 (green) during bolus delivery through the infusion set into air. **C**, P_{mean} values observed at the insulin infusion sites on day 0 (light cyan) and day 7 (cyan). **D**, P_{Smean} values observed on day 0 (light cyan) and day 7 (cyan) during bolus delivery through the infusion set into air. **E**, TFR values observed at the insulin infusion sites on day 0 (light blue) and day 7 (blue). **F**, R_S values observed on day 0 (light blue) and day 7 (blue) during bolus delivery through the infusion set into air. All data are geometric mean */geometric standard deviation (n = 30). Note that the y-axes are in logarithmic scale. Comparisons between study days were performed using the two-tailed paired t-test on log-transformed data. **$P < 0.001$

days, we found that the TFR at the insulin infusion site progressively increased with usage duration, whereas the TFR at the IDM infusion site remained at levels comparable to those seen on the first day of infusion site use (Figure 3). By the end of the 7-day study period, the TFR at the insulin infusion site reached a level approximately eight times higher than that of the infusion set resistance. Thus, after several days of continuous use of the insulin infusion site, the pressure provided by the pump to administer insulin highly increased and the vast majority of this pressure was to overcome the TFR at the infusion site (eg, ~90% on day 7). Furthermore, it is important to note that the infusion sets for delivering insulin and IDM were of identical design and the delivered IDM contained all ingredients present in the insulin formulation, except the insulin protein.[13] Thus, the concomitant

observation of a lack of an increase in TFR at the IDM infusion site reveals that the insulin protein itself caused the increase in TFR and not some other insulin formulation ingredient (eg, phenol preservative) or some design feature of the infusion set (eg, size or material property of the cannula).

There are several possible explanations for the observed effect of insulin on the resistance of the subcutaneous tissue to fluid infusion. One explanation may be related to the tendency of insulin to form fibrils and/or isoelectric precipitates when exposed to stressful environmental conditions, such as elevated temperatures, pH changes, mechanical agitation, or contact with hydrophobic surfaces.[18] Since such conditions may arise during CSII,[18] it seems possible that fibrils and/or precipitates were formed in the delivery system and that they were entrained by the fluid flow and deposited in the pores of the subcutaneous extracellular matrix. Hence, deposits of fibrils and/or precipitates may have gradually diminished the available pore flow area, which, in turn, may have caused the progressive increase in the TFR seen at the insulin infusion site. Another possible explanation for our observation may be inferred from insulin's role in wound healing. Insulin has long been recognized as a promoter of wound healing.[19] Wounds treated with insulin show improved neovascularization, earlier re-epithelialization, increased migration of various cells including macrophages, fibroblasts, endothelial cells and keratinocytes into the wound bed, and a higher collagen deposition by fibroblasts.[19,20] Furthermore, a study comparing subcutaneous tissue responses to infusion cannulas delivering various infusion fluids to diabetic swine found that, 7 to 10 days after cannula insertion, the cell density in the tissue surrounding the cannulas for insulin infusion was substantially higher than that in the tissue surrounding the cannulas for saline infusion.[21] In view of these findings, it seems very likely that collagen fibrils and cells, such as macrophages and fibroblasts, progressively accumulated in the tissue adjacent to the insulin infusion site and thus may have diminished the available area for fluid flow and increased the path length for fluid flow by imposing a tortuous pathway. This, in turn, may have increased the resistance to fluid flow.[6] The histological correlate of the increased TFR found at insulin infusion sites may, therefore, be the accumulation of cells and collagen fibrils at these infusion sites, and the very high TFR observed on days 4 to 7 of the study (Figure 3) may indicate that the cell and collagen fibril accumulation at the insulin infusion sites has progressed substantially over the course of the study.

After continuous use of the insulin infusion sites for more than 4 days, seven out of 30 infusion set tapes (23.3%) became loose and infusate fluid leaked out from the insertion sites. The occurrence of fluid leakage at the insulin infusion sites was invariably accompanied by infusion pressure values that were substantially decreased when compared with the pressure values observed on previous study days or with those observed after re-securing the adhesive tapes on the subject's skin. The frequency of insulin leakage and adhesive failure found in the present study (23.3%) was similar to that previously observed for other infusion set brands (23%).[22] Interestingly, the subjects' mean blood glucose concentrations observed on the day on which infusate leakage was detected (mean ± SE: 10.63 ± 0.89 mmol/

L) were similar to those observed on the two days preceding the detection (10.97 ± 1.0 and 10.73 ± 0.96 mmol/L; $P > 0.83$). However, the bolus insulin-to-carbohydrate ratios observed on the day of leakage detection (3.37 ± 0.41 IU/CU) were significantly higher than those observed on the two days preceding detection (2.66 ± 0.40 and 2.37 ± 0.22 IU/CU; $P = 0.049$). These results suggest that the subjects in whom insulin leakage had been detected may have compensated for the partial loss of insulin from their leaking infusion sites by increasing the amount of bolus insulin, thereby maintaining blood glucose control. Since current insulin pumps do not have leakage detection alarms, leakage from the insulin infusion sites most often goes unnoticed for several hours until hyperglycaemia and/or the distinctive insulin odour is detected. However, in the present study, due to the pressure information obtained by the pressure sensor placed between the pump reservoir and the infusion set connector (Supplemental Figure S1), we were able to detect all leaks from the infusion sites in a timely manner. Furthermore, of the 60 newly inserted infusion cannulas, four offered a very high resistance to the bolus infusion of IDM (6.7%). Since the generated infusion pressure was several times higher than that usually observed on the first day of cannula use, the four cannulas were removed and replaced by shorter infusion cannulas. The most likely reason for the observed high resistance to the infusate flow may have been that the cannula tips passed right through the subcutaneous fat layer and reached a more compact tissue layer, such as the muscle fascia.[23]

The rate of malpositioning of infusion cannulas found in the present study (6.7%) is somewhat higher than that obtained for the same infusion set brand (3.1%) but is similar to that observed for other infusion set brands (8.9%).[24] Unfortunately, with current insulin pumps, most malplacements of infusion cannulas may not be recognized until hyperglycaemia and/or high pressure alarms occur hours later.[25,26] However, again by using the pressure information from the pressure sensor attached to the pump, we were able to detect malpositioning of infusion cannulas right after their occurrence. Therefore, our results further suggest that building a precise pressure-monitoring capability into the insulin pump would permit a rapid detection of both insulin leakage from the infusion site and malplacement of infusion cannulas.

Our observation that insulin progressively increases the resistance of subcutaneous tissue to fluid flow has several important implications for the design of future insulin pumps and infusion sets. Since the power required for pumps to drive the fluid flow is proportional to the flow resistance,[10] an increase in TFR after prolonged use of an infusion site will also be accompanied by an increase in the power consumption of the pump. Thus, pumps used for administering insulin into the same tissue site over extended durations of time (ie, beyond the recommended duration of 2-3 days[3,21]) will draw more power from their batteries, resulting in decreased battery life. Consequently, to maintain a sufficient battery life, the batteries of such pumps may have to be enlarged or their energy density increased. Furthermore, most current insulin pumps feature an occlusion detection alarm that is triggered if the current of the pump motor and/or the force sensed at the pump piston exceeds a certain threshold.[27] The alarm thresholds employed by insulin pumps vary from manufacturer to

manufacturer, with some using thresholds below 100 kPa (Insulet: 68.9 kPa; Medtronic: 86.1 kPa) and some above 100 kPa (Animas: 103.4 kPa; Tandem: 206.8 kPa; Roche: <400 kPa).[28-32] The estimation of the TLs for P_{max} in the present study suggests that a significant proportion of infusion pressures encountered after 7 days of CSII use (ie, 25% of P_{max} values >79 kPa; 10% of P_{max} values >128 kPa; 5% of P_{max} values >173 kPa) may exceed the occlusion alarm thresholds employed by some of the current insulin pumps. Therefore, to reduce the probability of false occlusion alarms during extended periods of infusion site use (ie, more than 2-3 days), future pumps may need to adjust the occlusion alarm thresholds to account for the component of the infusion pressure that is required to overcome the TFR. In addition, in the present study, fluid leakage was detected on study days 5 to 7 at seven out of 30 insulin infusion sites (23.3%), while no fluid leakage was noticed at the 30 sites of IDM infusion. Further leakage from the sites of insulin infusion was prevented by re-securing the cannula housing adhesive tapes to the subjects' skin with additional adhesive strips and/or liquid tissue adhesive. This observation strongly suggests that the relatively high incidence of fluid leakage from the insulin infusion sites found in the present and previous studies[22,33] may have resulted from the high infusion pressures needed to overcome the gradually increasing TFR at these infusion sites (Figures 3 and 4). Therefore, to reduce the probability of leakage failures during extended periods of infusion site use (ie, periods exceeding 2-3 days), new infusion sets exhibiting increased mechanical and adhesive strength to withstand the high infusion pressures, may need to be developed.

An alternative to the above-proposed changes in the design of insulin pumps and infusion sets may be the use of techniques that inhibit the increase in TFR at the insulin infusion site. The choice of these techniques, however, may largely depend on the mechanism by which insulin induces the increase in TFR. For instance, if the increase in TFR is due to fibrils and/or precipitates occluding the pores of the extracellular matrix, techniques that stabilize the insulin solution may need to be applied. Such techniques may include the addition of fibril-inhibiting agents to the insulin solution[18] or the use of infusion set materials that minimize the loss of the insulin-stabilizing preservatives from the insulin solution.[34,35] However, if the increase in TFR is attributable to the accumulation of cells and collagen fibrils at the infusion site, techniques that inhibit wound healing, such as the coinfusion of glucocorticoids,[36] may be used. Thus, to select the most effective technique to inhibit the increase in TFR, further studies are needed to identify the mechanism by which insulin induces the increase in TFR.

Limitations of the present study include the use of a conventional infusion set that is designed to be worn for 3 days. Thus, the observed relationship between infusion pressure and rate of leakage failures may not be generalizable to the novel infusion sets designed to be worn for up to 7 or 10 days.[34,37] A future study applying extended-wear infusion sets will be needed to determine whether the pressure-leakage rate relationship observed for conventional infusion sets is different from that for extended-wear infusion sets. Because the 7-day survival rates recently observed for extended-wear infusion sets (73-80%)[34,37] are not substantially higher than those found for some

conventional infusion sets (eg, 66% in Waldenmaier et al[38]; 70% in the present study), it will be interesting to see whether there are clinically relevant differences in the pressure-leakage rate relationship for conventional and extended-wear infusion sets. Furthermore, although the number of subjects was adequate to characterize the frequency distribution of infusion pressures and TFRs for the subcutaneous insulin and IDM infusion sites, a larger number would have equated to a higher precision in the determination of the TLs for infusion pressure and TFR.[39] Thus, studies with larger sample size would be needed to more precisely determine the TLs for these parameters. Finally, the present study used a rapid-acting insulin with the standard concentration of 100 units/mL (U-100). Because during infusion of a higher concentration insulin (eg, U-500)[40] the tissue volume exposed to the infused insulin may be substantially smaller than that during infusion of the U-100 insulin, it is likely that the time course of action of concentrated insulin upon TFR is significantly different from the time course of action found with the U-100 insulin. Thus, a future study applying concentrated and standard U-100 insulins will be needed to determine whether there is a difference in the time-action profiles of these insulin formulations.

CONFLICT OF INTEREST

A.H.L. is an employee of Roche Diabetes Care. T.R.P. has received research support from AstraZeneca and Novo Nordisk, and has served on advisory panels for Adocia, Arecor, AstraZeneca, Bristol-Myers Squibb, Eli Lilly, Novo Nordisk, and Roche Diabetes Care. W.R. and M.J. have filed patent applications relating to the work described in this article. There are no other potential conflicts of interest relevant to this article.

AUTHOR CONTRIBUTIONS

M.T., M.J., A.C.T. and A.S. helped to design the experiments, performed the experiments, conducted data analysis, interpreted the data and helped to draft the manuscript. J.F. and R.S.A. contributed to the setting up and performing of the experiments, and interpreted the data. A.H.L. and T.R.P. helped to design the experiments, provided advice and assistance in performing experiments and statistical analysis, and interpreted the data. W.R. designed experiments, planned the statistical analysis, supervised the experiments, conducted data analysis, interpreted the data and wrote the manuscript. All authors reviewed and edited the manuscript.

PEER REVIEW

The peer review history for this article is available at https://publons.com/publon/10.1111/dom.14594.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study but are not included in the article or in the on-line supplementary files are available from the corresponding author upon reasonable request.

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