Přílohy:

ORIGINAL PAPER

Lipid peroxidation and impaired vascular function in patients with type 1 diabetes mellitus

Tomáš Pelcl[1] · Jan Škrha Jr.[1] · Jan Šoupal[1] · Milan Flekač[1] · Petr Kačer[2] · Jan Škrha[1] · Tomáš Navrátil[3] · Martin Prázný[1]

Abstract

Patients with type 1 diabetes mellitus (T1DM) develop microvascular complications during the course of the disease. Oxidative stress is the main mechanism of the microvascular damage. Currently no specific and reliable laboratory tests are available for screening of early microvascular changes. 56 patients with T1DM were included. We measured their serum levels of malonyl dialdehyde and reactive aldehydes with chain lengths C_6–C_{12}, which are known to be generated during lipid peroxidation, a process associated with oxidative stress. Serum levels of aldehydes were compared with the parameters of microvascular reactivity (MVR) examined by laser Doppler flowmetry and with the parameters of blood glucose control (glycated hemoglobin, glycemic variability using continuous glucose monitoring). In this cross-sectional observational study, higher levels of reactive aldehydes were associated with impaired skin MVR in T1DM. However, the parameters of glucose control were not associated with lipid peroxidation or MVR in our study. Therefore, we suggest that other than simple glycemic mechanism may be more important in the process of reactive aldehyde generation in T1DM.

Graphical abstract

Negative association between skin thermal hyperemia (blood flow after local heating expressed in % of basal perfusion) and serum octanal (ng cm^{-3}) $r = -0.55$, $p = 0.00001$
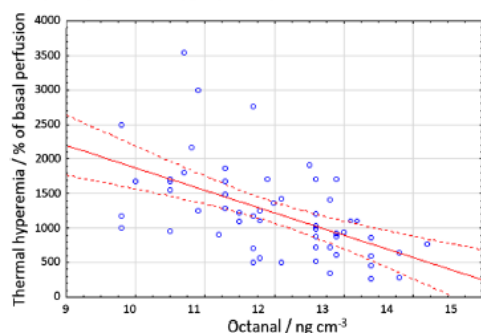
Keywords Oxidative stress · Diabetes mellitus · Aldehydes · Carbohydrates · Radicals

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Introduction

Oxidative stress plays an important role in the development of diabetes complications [1, 2]. It was hypothesized that high glucose variability (GV), the typical clinical feature of

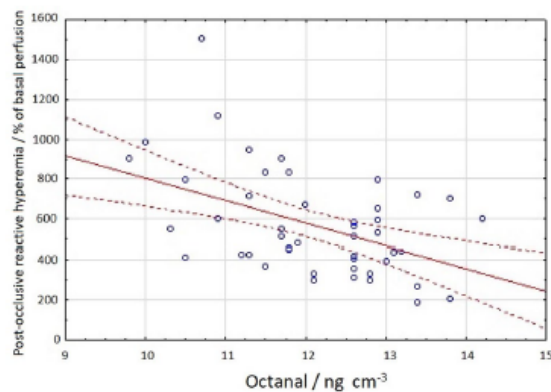Published online: 02 February 2019

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type 1 diabetes mellitus (T1DM), may, beyond hyperglycemia, additionally contribute to the generation of oxidative stress [3]. Although it may be useful to identify diabetic patients at high risk of vascular complications using biomarkers and/or functional tests of circulation, no simple and reliable tests exist so far. To contribute to the development of such test(s), we performed a novel combined approach including parameters of glucose control, functional tests of microvascular system, and serum biomarkers of oxidative stress.

Glucose control, a tool for evaluating the effectiveness of diabetes treatment, can be measured in several different ways. Glycated hemoglobin (HbA_{1c}) is a parameter of long-term glucose control. It reflects average glucose levels for past several months and is used as a gold standard for evaluating the patient's overall glucose control. HbA_{1c} is widely used and its correlation with long-term diabetic complications is well established [4]. However, HbA_{1c} does not reflect the short-term GV, which may be associated with chronic diabetes complications [2, 5, 6], by promoting oxidative stress [3]. High glucose variability is directly linked to the risk of hypoglycemia, contributing to the development of endothelial dysfunction [7]. Short-term glucose variability can be assessed in several ways. The most precise way is the analysis of the data from continuous glucose monitoring (CGM) using standard deviation (SD), coefficient of variation (CV), or various calculations, such as continuous overall net glycemic action (CONGA) or mean amplitude of glycemic excursions (MAGE) [8].
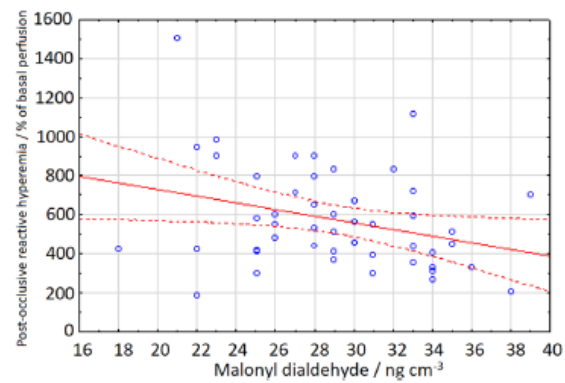
Functional tests of vascular system reflecting microvascular reactivity (MVR) can be assessed by evaluating the changes in the microvascular flow after ischemic (occlusive) or thermal stimulation. These non-invasive tests may detect impaired MVR in patients with endothelial dysfunction

caused by diseases such as diabetes mellitus [9] or chronic kidney disease [10].

Oxidative stress itself is a complex mechanism, which can be defined as an imbalance between oxidative and antioxidative processes in the body. Several biomarkers acting as by-products during reactions involving oxidative stress can be measured. Malonyl dialdehyde (MDA) and other reactive aldehydes formed by lipid peroxidation with C-chain length from 6 to 12 (i.e., hexanal to dodecanal) have been associated with oxidative stress [11]. Biomarkers of oxidative stress are elevated in patients with T1DM [12], however, their role is complex and not yet fully understood.

Fig. 2 Negative association between PORH (expressed in % of basal perfusion) and serum levels of MDA (ng cm^{-3}). $r = -0.30$, $p = 0.035$
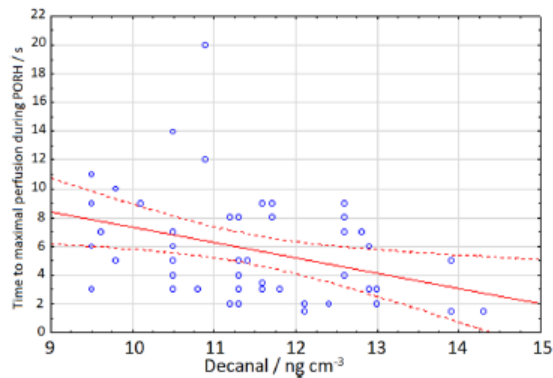
Fig. 1 Negative association between PORH (expressed in % of basal perfusion) and serum levels of octanal (ng cm^{-3}); $r = -0.48$, $p = 0.0006$
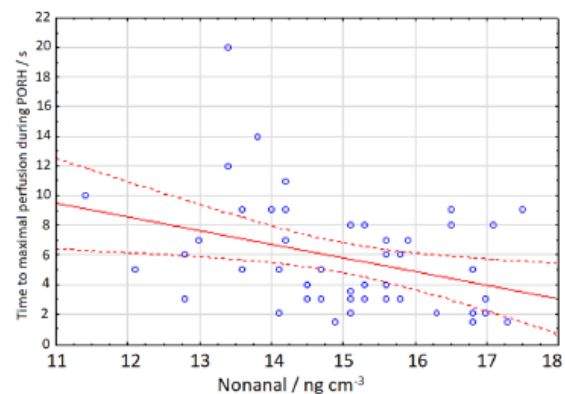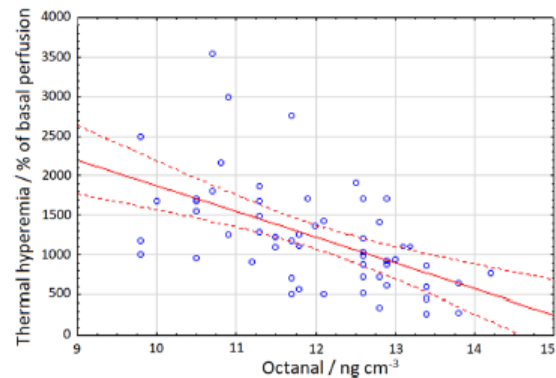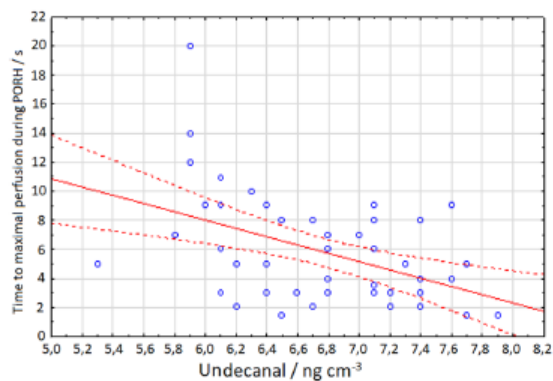
Fig. 3 Negative association between time to maximal perfusion during PORH (s) and serum levels of nonanal (ng cm^{-3}); $r = -0.35$, $p = 0.0143$
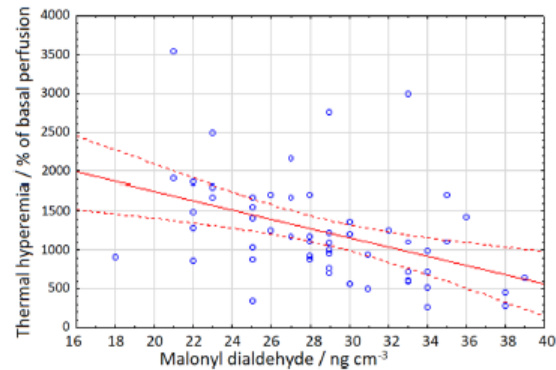
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Fig. 4 Negative association between time to maximal perfusion during PORH (s) and serum levels of decanal (ng cm^{-3}); $r = -0.39$, $p = 0.0123$

Fig. 6 Negative association between skin TH (expressed in % of basal perfusion) and serum levels of octanal (ng cm^{-3}); $r = -0.55$, $p = 0.00001$

Fig. 5 Negative association between time to maximal perfusion during PORH (s) and serum levels of undecanal (ng cm^{-3}); $r = -0.46$, $p = 0.001$

Fig. 7 Negative association between skin TH (expressed in % of basal perfusion) and serum levels of MDA (ng cm^{-3}); $r = -0.43$, $p = 0.0011$

Results and discussion

Skin MVR was measured by laser Doppler flowmetry. The changes in perfusion in post-occlusive reactive hyperemia (PORH) were negatively associated with serum levels of octanal and MDA ($r = -0.48$, $p = 0.0006$ and $r = -0.3$, $p = 0.035$, respectively), as shown in Figs. 1 and 2.

Time to maximal perfusion during PORH was negatively associated with serum levels of nonanal, decanal, and undecanal ($r = -0.35$, $p = 0.0143$; $r = -0.39$, $p = 0.0123$; and $r = -0.46$, $p = 0.001$, respectively), Figs. 3, 4 and 5.

MVR changes in perfusion measured during skin thermal hyperemia (TH) were negatively associated with serum levels of octanal and MDA ($r = -0.55$, $p < 0.0001$ and $r = -0.43$, $p = 0.001$, respectively), as presented in Figs. 6 and 7.

On the other hand, no associations between the serum levels of measured aldehydes and any parameters of glucose control, i.e., HbA$_{1c}$ ($r = -0.02$, $p = 0.88$), mean glucose calculated from CGM ($r = -0.03$, $p = 0.86$), total SD of CGM glucose ($r = -0.09$, $p = 0.53$), or CV of CGM glucose ($r = -0.04$, $p = 0.78$) were found. Similarly, parameters of MVR (TH) were not associated with parameters of glucose control i.e., HbA$_{1c}$ ($r = -0.09$, $p = 0.53$), or SD of CGM ($r = -0.04$, $p = 0.8$). This was already described in the previous study [13] with patients with type 2 diabetes mellitus (T2DM) though.

Markers of oxidative stress are nonspecific for diabetes, as higher levels are observed in several different conditions such as aging [14], arterial hypertension, arthritis, age-related cataract, bronchial asthma, or pneumoconiosis due

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to carcinogenic dust asbestos and silica [15, 16], and lung exposure to nanoparticles during iron oxide pigment production in industrial workers [17]. Several of these conditions are related to endothelial dysfunction caused by oxidative stress, often combined and interrelated, as in our previous study with chronic 2, 3, 7, 8-tetrachlordibenzo-*p*-dioxin (TCDD) intoxication of patients. In that study, 62.5% of patients develop T2DM in contrast with comparable Czech male population with only 17.6% of subjects. These patients also had elevated MDA and other reactive aldehydes. Due to its long half-life (8–10 years), both TCDD body deposit and high blood levels still persist and induce oxidative damage [18, 19]. Accordingly, the follow-up of this group of patients also showed impaired results of MVR using the same measurement techniques of laser Doppler flowmetry [20]. This illustrates the complex mechanism of oxidative damage and its influence on metabolic impairment, therefore, further research is needed.

Conclusion

In our cross-sectional observational study, a positive association of the levels of reactive aldehydes originating in lipid peroxidation with the impairment of skin MVR in T1DM was found. However, the parameters of glucose control and GV were not associated with lipid peroxidation markers or MVR in these patients. We, therefore, suggest that other than simple glycemic mechanisms may be probably more important in the process of reactive aldehydes generation in T1DM. As our study was not designed to prove the causality between the lipid peroxidation and the vascular dysfunction, further research should evaluate the role of reactive aldehydes in the development and/or prediction of diabetic vascular complications.

Materials and methods

We included 56 T1DM patients (22 males, 34 females), mean age 32 ± 8 years, HbA$_{1C}$ 62 ± 12 mmol mol^{-1} ($7.8 \pm 1.5\%$ DCCT), T1DM duration 14 ± 6 years, with body mass index 24.1 ± 2.8 kg m^{-2}. Serum levels of reactive aldehydes and MDA were measured by liquid chromatography–electrospray ionization–tandem mass spectrometry (LC–ESI–MS/MS). Masked CGM collected glucose data for 12 consecutive days to evaluate mean blood glucose and parameters of GV (standard deviation—SD, coefficient of variation—CV, and continuous overall net glycemic action—CONGA). CGM device iPro2 (Medtronic, USA) were used.

Skin MVR was measured by laser Doppler flowmetry (PeriFlux PF 4001 Master laser instrument and a PeriTemp 4005 Heater thermostatic unit, Perimed, Sweden) on the forearm of the non-dominant upper extremity during post-occlusive reactive hyperemia (PORH) and during thermal hyperemia (TH). Basal perfusion was measured for 2 min before the PORH test. The brachial artery was then occluded by a sphygmomanometer cuff inflated to a suprasystolic pressure for 3.5 min. We recorded maximal perfusion during hyperemia (PORH$_{max}$) measured in perfusion units (PU) and time needed to reach maximal perfusion (PORH$_t$) measured in seconds).

Perfusion during TH was measured as a % of basal perfusion, after a 5 min heating of the forearm to 44 °C by a thermostatic unit. Percent change in flow was calculated from baseline to the peak value during stimulations.

References

1. Giacco F, Brownlee M (2010) Circ Res 107:1058
2. Skrha J, Soupal J, Skrha J Jr, Prazny M (2016) Rev Endocr Metab Disord 17:103
3. Kuricova K, Pacal L, Soupal J, Prazny M, Kankova K (2016) Diabetes Res Clin Pract 114:1
4. The Diabetes Control and Complications Trial Research Group (1993) N Engl J Med 329:977
5. Hirsch IB, Brownlee M (2005) J Diabetes Complications 19:178
6. Soupal J, Skrha J Jr, Gajmon M, Horova E, Mraz M, Skrha J, Prazny M (2014) Diabetes Technol Ther 16:198
7. Pena AS, Couper JJ, Harrington J, Gent R, Fairchild J, Tham E, Baghurst P (2012) Diabetes Technol Ther 14:457
8. Service FJ (2013) Diabetes 65:1398
9. Fuchs D, Dupon PP, Schaap LA, Drailer R (2017) Cardiovasc Diabetol 16:11
10. Babos L, Jarai Z, Nemcsik J (2013) World J Nephrol 2:77
11. Antus B (2016) Oxid Med Cell Longev 2016:2930434
12. Fatima N, Faisal SM, Zubair S, Ajmal M, Siddiqui SS, Moin S, Owais M (2016) PLoS ONE 11:e0161548
13. Horova E, Pelcl T, Soupal J, Skrha J Jr, Flekac M, Vejrazka M, Skrha J, Prazny M (2018) Diabetes Res Clin Pract 139:300
14. Syslova K, Bohmova A, Mikoska M, Kuzma M, Pelclova D, Kacer P (2014) Oxid Med Cell Longev 2014:562860
15. Pelclova D, Fenclova Z, Kacer P, Kuzma M, Navratil T, Lebedova J (2008) Ind Health 46:484
16. Syslova K, Kacer P, Kuzma M, Najmonova V, Fenclova Z, Vlckova S, Lebedova J, Pelclova D (2009) J Chromatogr B Analyt Technol Biomed Life Sci 877:2477
17. Pelclova D, Zdimal V, Kacer P, Fenclova Z, Vlckova S, Syslova K, Navratil T, Schwarz J, Zikova N, Barosova H, Turci F, Komarc M, Pelcl T, Belacek J, Kukutschova J, Zakharov S (2016) J Breath Res 10:016004
18. Pelcl T, Skrha J Jr, Prazny M, Vlckova S, Fenclova Z, Navratil T, Malik J, Diblik P, Zikan V, Pelclova D (2018) Basic Clin Pharmacol Toxicol 123:356
19. Pelclova D, Navratil T, Vlckova S, Fenclova Z, Pelcl T, Kacerova T, Kacer P (2018) Monatsh Chem 149:1579
20. Pelclova D, Prazny M, Skrha J, Fenclova Z, Kalouskova M, Urban P, Navratil T, Senholdova Z, Smerhovsky Z (2007) Hum Exp Toxicol 26:705

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DTT

ORIGINAL ARTICLE

Comparison of Different Treatment Modalities for Type 1 Diabetes, Including Sensor-Augmented Insulin Regimens, in 52 Weeks of Follow-Up: A COMISAIR Study

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Abstract

Objective: To compare different treatment modalities for patients with type 1 diabetes (T1D) based on real-time continuous glucose monitoring (RT-CGM) or self-monitoring of blood glucose (SMBG) combined with multiple daily injections (MDIs) or continuous subcutaneous insulin infusion (CSII).

Research Design and Methods: Sixty-five T1D patients were followed up for a year. Of these, 27 started RT-CGM as part of a sensor-augmented insulin regimen (SAIR); within this SAIR group, 15 subjects started sensor-augmented pump (SAP) therapy and the remaining 12 continued with MDIs (MDIs + RT-CGM). A second group of 20 patients initiated CSII without RT-CGM, while a third group of 18 subjects continued on MDIs and SMBG. The main endpoints were reduction of HbA_{1c}, glycemic variability (GV), and incidence of hypoglycemia.

Results: After a year, the baseline mean HbA_{1c} in the SAIR group (8.3%) decreased to 7.1% ($P < 0.0001$); both SAIR subgroups, SAP and MDIs + RT-CGM, showed comparable improvement. The CSII group also had reduced HbA_{1c} (8.4% ± 0.9% vs. 7.9% ± 0.7%; $P < 0.05$). Both SAIRs were superior to MDIs ($P = 0.002$) and CSII ($P = 0.0032$). GV was also lowered, both in the SAIR ($P < 0.0001$) and CSII ($P < 0.05$) groups. Reduced incidence of hypoglycemia was observed only with SAIR (8% ± 4% vs. 6% ± 3%; $P < 0.01$).

Conclusion: Both SAIRs, SAP and MDIs + RT-CGM, provided significant and comparable decrease of HbA_{1c} with concurrent reduction of hypoglycemia. This improvement was greater than that seen with CSII. The combination of RT-CGM and MDIs can be a suitable alternative to SAP for some patients.

Introduction

THERE HAVE BEEN many advances in diabetes care technologies in the last few years, which have resulted in new opportunities for diabetes treatment. Despite some encouraging results, metabolic control remains suboptimal in most patients with type 1 diabetes.[1]

Successful treatment of type 1 diabetes requires both a precise insulin delivery system and reliable glucose monitoring systems. For delivery systems, the two most common are multiple daily injection (MDI) and continuous subcutaneous insulin infusion (CSII) therapies.[2,3] With both strategies, bolus insulin is dosed based on several factors, including carbohydrate content and glycemia. There are also two common monitoring systems: classical self-monitoring of blood glucose (SMBG) and real-time continuous glucose monitoring (RT-CGM). With SMBG, even if frequent monitoring is performed, some potentially important trends are always missed because

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they occurred between two measurements. In contrast, CGM gives the concentration of glucose in subcutaneous tissue approximately every 5 min and therefore provides much more data,[4] including glucose trends, to which patients can react to prevent hyper- and/or hypoglycemia.

Despite limited data, it is commonly believed that optimal diabetes management can best be achieved when an RT-CGM is used in combination with insulin pump therapy—sensor-augmented pump (SAP) therapy.[5] It has been reported that SAP improves glycemic control,[6,7] reduces time spent in hypoglycemia, increases time spent in the target zone,[7,8] and decreases glycemic variability (GV).[9,10]

In contrast, the efficacy of the combination of real-time CGM with MDIs is less described. Moreover, the accuracy and usability of CGM have gradually improved. Therefore, we need data from clinical studies with the newer generation of CGM devices. Finally, prospective studies simultaneously comparing head-to-head the different combinations of insulin delivery and monitoring systems—MDIs + SMBG, MDIs + RT-CGM, CSII + SMBG, and SAP—are lacking. Such a study would help to elucidate whether the observed benefit of SAP use is secondary to the RT-CGM technology, the type of insulin delivery, or both.

The aim of the study was to compare the efficacy of long-term use of sensor-augmented insulin regimens (SAIRs), that is, RT-CGM combined with either CSII or MDIs, on glycemic control compared with more common schemes based on classical SMBG.

Research Design and Methods

Study population

Sixty-five patients with type 1 diabetes were enrolled at the 3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University in Prague, Czech Republic. All subjects provided written informed consent before enrollment. Participants were included if they were aged >18 years, had a duration of diabetes of more than 2 years, and had an HbA$_{1c}$ level between 7.0% and 10% (53 and 86 mmol/mol). Only patients with insulin analogs were enrolled in this study. Subjects who had used CGM during the past 3 months were excluded from the study. Patients with ketoacidosis within the past 3 months and/or severe noncompliance and/or any concomitant therapy influencing glucose metabolism,

pregnant women, and women planning pregnancy were not allowed to participate either. Patients were divided into three groups with comparable baseline parameters (Table 1), taking into account their preferences and diabetologist's recommendation. At the baseline, 27 patients started to use RT-CGM as part of an SAIR, 20 patients initiated CSII therapy (without RT-CGM), and 18 patients continued on MDIs and SMBG only.

In the SAIR group, after a further consultation with the diabetologist, subjects could choose a combination of RT-CGM with either an insulin pump (SAP) or MDIs. Fifteen of them started to use SAP and the remaining 12 continued with MDIs (MDIs + RT-CGM).

A prerequisite for participation in the SAIR group was the willingness to use sensors >70% of the time. Similarly, patients in the groups without CGM had to be willing to monitor their glucose (SMBG) at least four times a day.

Study procedures

This was a nonrandomized, prospective, real-life clinical trial. Subjects were scheduled for a total of seven clinic visits (initial, at 2 weeks, 1 month, then 3, 6, 9, and 12 months). Initially, all patients were monitored by professional CGM (iPro2™; Medtronic, Northridge, CA) for 6 days. Throughout the study, subjects in the groups not using SAIR had professional CGM every 3 months. Participants in the CSII group wore one of two types of insulin pumps, MiniMed Paradigm Veo (Medtronic) and Animas Vibe (Animas Corporation, West Chester, PA). Participants in the SAP subgroup used either the MiniMed Paradigm Veo System with Enlite sensors (Medtronic) or Animas Vibe system with DexCom G4 sensors (Dexcom, San Diego, CA). The subgroup of patients with MDIs + RT-CGM used a DexCom G4 CGM system comprising a 7-day transcutaneous sensor, a transmitter, and a receiver. The patients were provided with a personal blood glucose meter (OneTouch [LifeScan, Milpitas, CA] or CONTOUR™ LINK [Bayer Diabetes Care, Basel, Switzerland]), which was used for diabetes self-management purposes and calibration of CGM. At the baseline, all subjects underwent a structured 4-day training program. In the first part of this program, specialists reviewed general principles of type 1 diabetes management. Patients were educated on how to prevent hypoglycemia and deal with it in a variety of situations. They

TABLE 1. BASELINE CHARACTERISTIC OF PATIENTS

Characteristic	SAIR group			CSII + SMBG group	MDIs + SMBG group
	All	SAP therapy	MDIs + CGM		
No.	27	15	12	20	18
Male [n (%)]	16 (59)	9 (60)	7 (58)	11 (55)	9 (50)
Age (years)	34 ± 10	33 ± 10	34 ± 10	35 ± 9	38 ± 17
Duration of diabetes (years)	15 ± 9	15 ± 9	16 ± 10	13 ± 10	14 ± 9
HbA$_{1c}$ (mmol/mol)	67.5 ± 10	66 ± 9	69.3 ± 8	67.9 ± 9	67.2 ± 9
HbA$_{1c}$ (%)	8.3 ± 9	8.2 ± 0.9	8.5 ± 1.1	8.4 ± 0.8	8.3 ± 0.8
Mean sensor glucose (mmol/L)	10.7 ± 1.5	10.4 ± 1.4	10.9 ± 1.6	10.7 ± 1.2	10.6 ± 1.4
BMI (kg/m^2)	25 ± 3	25 ± 3	25 ± 3	26 ± 4	24 ± 3

Values are presented as mean ± SD, P values <0.05 are statistically significant.
CGM, continuous glucose monitoring; CSII, continuous subcutaneous insulin infusion; MDIs, multiple daily injections; SAIR, sensor-augmented insulin regimen; SAP, sensor-augmented pump; SMBG, self-monitoring of blood glucose.

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were informed about the appropriate timing of preprandial insulin dosing. All patients underwent theoretical and practical education in carbohydrate counting and were encouraged to use flexible dosing of insulin throughout this study. Only patients in the SAIR and CSII groups completed theoretical training on the relevant devices, followed by treatment initiation and practical training with investigators.

Participants on SAIR were encouraged to make self-adjustments to their treatment using CGM values, hyper- and hypoglycemic alerts and trends, and also to incorporate results of SMBG into treatment changes. The target range for glucose was usually initially relatively wide, but we emphasized to patients that its successive narrowing is usually necessary for reduction of mean blood glucose and GV. An important part of the training was management of problems with CGM (troubleshooting) related to sweating, skin reactions, alarm settings, and appropriate calibration according to the type of CGM system. We highlighted to patients the importance of regular downloading and review of the data from CGM devices and insulin pumps. A bolus calculator was set for all patients with insulin pumps. Subjects in non-SAIR groups were encouraged to measure their blood glucose at least four times a day. All patients were instructed to use only the study blood glucose meter provided to them for all SMBG measurements taken

during this trial. Data from all CGM systems, insulin pumps, and blood glucose meters were downloaded for analysis.

Prespecified outcomes

The primary endpoint was the difference in HbA_{1c} between the groups after 52 weeks of follow-up. HbA_{1c} values were measured at the baseline, then every 3 months, and at the end of this trial. HbA_{1c} was analyzed by a high-performance liquid chromatography method on a Variant II analyzer (Bio-Rad, Hercules, CA). The normal reference range of HbA_{1c} in our laboratory is 4.0%–6.0% (20–42 mmol/mol).

Prespecified secondary endpoints were changes of GV expressed by the total standard deviation of blood glucose (SD_T), average daily glucose from CGM, % of time spent in range (4.0–10.0 mmol/L), and the incidence of hypoglycemia (% of time below 3.9 mmol/L).

At each clinic visit, patients were screened for adverse events and sensor insertion sites were inspected. Severe hypoglycemia was defined as an episode requiring assistance from another person or neurological recovery in response to restoration of plasma glucose to normal. Ketoacidosis was defined as an episode of hyperglycemia (>14 mmol/L) with low serum bicarbonate (<15 mmol/L), low pH (<7.3), or both

TABLE 2. INSULIN TREATMENT PATTERNS, SELF-MONITORING OF BLOOD GLUCOSE, AND BODY WEIGHT AT THE BASELINE AND AT THE END OF THE STUDY

	At the baseline	At the end	P	95% CI
No. of boluses/day (*n*)				
SAIR group	4.0±0.7	6.8±2.2	<0.0001*	1.8679 to 3.6860
SAP	4.0±0.8	7.2±2.3	<0.0001*	1.9379 to 4.6335
MDI + CGM	4.0±0.5	6.2±2	0.002	0.9308 to 3.4359
CSII	4.1±0.8	4.7±1.4	0.08	−0.08277 to 1.3628
MDIs	3.9±0.8	3.9±0.8	0.83	−0.6024 to 0.4847
Relative proportion of bolus insulin (%)				
SAIR group	49±7	53±5	0.004*	1.5371 to 7.8200
SAP	49±7	54±4	0.03*	0.3877 to 9.4790
MDI + CGM	48±6	53±5	0.07	−0.3165 to 9.08588
CSII	50±9	52±7	0.38	−2.8715 to 7.3715
MDIs	50±5	52±6	0.45	−2.3613 to 5.2502
The total daily dose of insulin (U)				
SAIR group	46±12	48±13	0.58	−4.9325 to 8.7843
SAP	45±12	47±13	0.65	−7.2315 to 11.3648
MDIs + CGM	48±12	50±13	0.75	−9.3076 to 12.8076
CSII	48±13	47±13	0.98	−8.2209 to 8.0209
MDIs	47±14	48±14	0.85	−8.5492 to 10.3270
Frequency of SMBG/day (*n*)				
SAIR group	3.8±1.2	3.2±1.0	0.049*	−1.1759 to −0.0019
SAP	3.7±0.8	3.6±1.0	0.84	−0.7408 to 0.6074
MDIs + CGM	3.9±1.6	2.7±0.6	0.02*	−2.2431 to −0.2402
CSII	3.6±0.7	3.6±0.7	0.95	−0.5001 to 0.4686
MDIs	3.6±1.3	3.7±1.4	0.88	−0.8935 to 1.0346
Body weight (kg)				
SAIR group	77.7±11	79.0±12	0.68	−4.9653 to 7.5208
SAP	76.1±10	77.3±9	0.74	−5.8359 to 8.1692
MDIs + CGM	79.6±13	81.0±14	0.80	−10.2345 to 13.0679
CSII	74.1±12	74.4±12	0.94	−7.3909 to 7.9909
MDIs	73±13	73.5±14	0.92	−8.7031 to 9.6698

Values are presented as mean±SD; *P values <0.05 are statistically significant.
CI, confidence interval.

together with either ketonemia or ketonuria that required treatment in a healthcare facility.

Statistical analysis

Statistical evaluation was performed by Statistics for Windows version 10 software (SPSS, Inc., Chicago, IL). Basic descriptive statistics were calculated for the relevant parameters. Analysis was performed by nonparametric tests (Kruskal–Wallis, Wilcoxon, and ANOVA repeated measurement). Data are expressed as mean–SD values. A value of $P < 0.05$ was considered statistically significant.

Results

### Baseline characteristics and adherence

Baseline characteristics were similar in the three groups (Table 1). Of the 65 patients enrolled, 62 completed all study visits. One subject from the CSII group and one from the SAIR group withdrew from the study after the third visit because of personal reasons. One patient from the MDI group was excluded from the analysis due to significant protocol violation. Mean sensor percentage use in the SAIR group was $85\% \pm 10\%$ of the time (median 85%) with no significant differences between the two subgroups—SAP or MDIs + RT-CGM ($85\% \pm 10\%$ [median 84%] vs. $85\% \pm 10\%$ [median 87%]; $P = 0.98$).

Compared with the baseline, at the end of this study in the SAIR group, there was a significantly higher number of boluses per day and the relative proportion of bolus insulin was higher, while no significant change in these parameters was seen in either SMBG group (Table 2). No change in the total daily dose of insulin between the baseline and the end of the study was observed for any study group (Table 2).

The average number of boluses per day at the end of the study was lower in both SMBG groups in comparison with the SAIR group (6.8 ± 2.2 vs. 4.3 ± 1.2; $P < 0.0001$). A higher frequency of boluses was seen in patients with CSII versus the self-reported boluses in the MDI only group (4.7 ± 1.4 vs. 3.9 ± 0.8; $P = 0.04$), while no significant difference between SAP and MDIs + RT-CGM was observed (7.2 ± 2.3 vs. 6.2 ± 2; $P = 0.25$). At the end of this trial, the total daily dose of insulin and the relative proportion of bolus insulin were not different between study groups (Table 2).

No significant change in body weight between the beginning and the end of the study was found for any study group (Table 2).

At the end of this study, the average number of blood glucose tests in non-SAIR groups was 3.7 ± 1.1 per day (median 3.6/day), with no significant differences between the groups with MDIs and CSII (3.7 ± 1.4 [median 3.3/day] vs. 3.6 ± 0.7 [median 3.5/day]; $P = 0.8$). In comparison with SMBG groups, the average frequency of finger-stick tests performed per day was numerically, but not statistically, lower in the SAIR group (3.2 ± 1.0 [median 3.1/day] vs. 3.7 ± 1.1 [median 3.6/day]; $P = 0.08$). However, regardless of the type of insulin delivery (SAP or MDIs + RT-CGM), there was lower frequency of SMBG in subjects who were using the DexCom G4 sensor ($n = 19$) in comparison with users of the MiniMed Paradigm Veo System with Enlite sensors ($n = 8$) (2.7 ± 0.6 vs. 4.3 ± 0.7, $P < 0.001$).
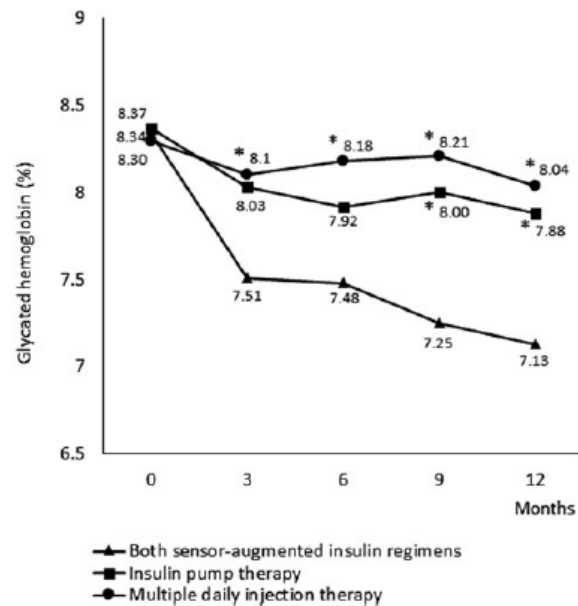
FIG. 1. Comparison of different treatment strategies for patient with type 1 diabetes during 1 year of follow-up: effect on HbA_{1c}. Mean glycated hemoglobin levels (%) at baseline and 3, 6, 9, and 12 months in all groups. Asterisks denote $P < 0.05$ for comparison between the sensor-augmented regimens group and the two SMBG groups (insulin pump and MDI therapy) at each time point. MDIs, multiple daily injections; SMBG, self-monitoring of blood glucose.

Primary and secondary endpoints

After a year, the SAIR group of patients had significantly lower HbA_{1c} ($8.3\% \pm 0.9\%$ vs. $7.1\% \pm 0.8\%$ [67.5 ± 10.4 mmol/mol vs. 54.5 ± 9.1 mmol/mol]; $P < 0.0001$) (Fig. 1). This improvement in HbA_{1c} was observed both in the subgroup with SAP ($8.2\% \pm 0.9\%$ vs. $7.1\% \pm 0.9\%$ [66 ± 9 mmol/mol vs. 53.9 ± 10 mmol/mol]; $P = 0.0025$) and with MDIs + RT-CGM ($8.5\% \pm 1.1\%$ vs. $7.2\% \pm 0.8\%$ [69.3 ± 12 mmol/mol vs. 55.3 ± 8.7 mmol/mol]; $P = 0.0034$) compared with the study baseline (Fig. 2).

CSII alone also led to significant reduction of HbA_{1c} ($8.4\% \pm 0.9\%$ vs. $7.9\% \pm 0.7\%$ [68.3 ± 9 mmol/mol vs. 62.7 ± 8 mmol/mol]; $P = 0.048$), while in the group just on MDIs, no significant decrease of HbA_{1c} was observed ($8.3\% \pm 0.8\%$ vs. $8.0\% \pm 0.9\%$ [67.2 ± 9 mmol/mol vs. 64.4 ± 10 mmol/mol]; $P = 0.40$) (Fig. 1).

At 1 year, the mean difference in HbA_{1c} between the SAIR group and the MDI group was -0.91% (-9.81 mmol/mol) (95% confidence interval [CI], -1.47% to -0.35% [-15.96 to -3.67 mmol/mol]; $P = 0.002$). Moreover, both SAIR strategies were superior to CSII; the mean difference was -0.75% (-8.11 mmol/mol) (95% CI, -1.23% to -0.26% [-13.41 to -2.81 mmol/mol]; $P = 0.0032$). The difference in HbA_{1c} between the SAIR group and the MDI group was significant from the third month and the difference between the SAIR group and the CSII group was significant from the ninth month (Fig. 1).

Importantly, superiority of both SAIRs in comparison with CSII only was not observed just for the SAP version of SAIR

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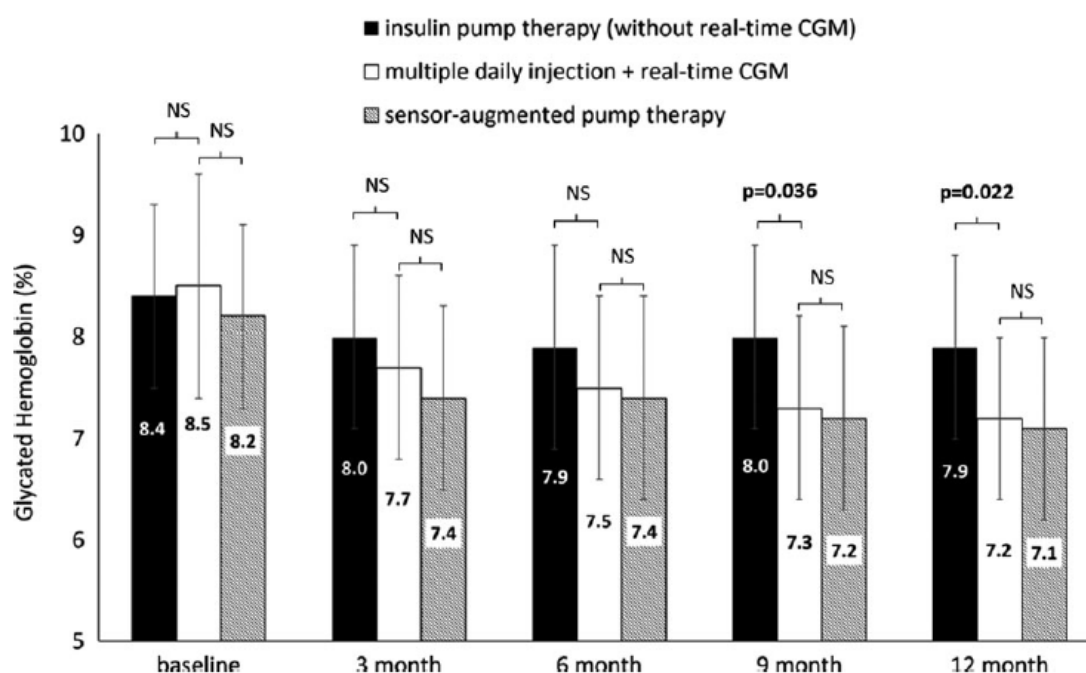
FIG. 2. Comparison of the effect on HbA$_{1c}$ in patients with MDIs + real-time CGM therapy with sensor-augmented pump therapy and insulin pump therapy during the 52-week follow-up study. Mean glycated hemoglobin levels (%) at baseline and 3, 6, 9, and 12 months. *P* values <0.05 are statistically significant. CGM, continuous glucose monitoring; NS, not significant.

but also for the MDI version of SAIR for a between-group difference favoring the MDI + RT-CGM subgroup of −0.66% (−7.4 mmol/mol) (95% CI, −1.23% to −0.10% [−13.64 to −1.6 mmol/mol]; *P* = 0.022). The difference in HbA$_{1c}$ between CSII and MDI + RT-CGM groups started to be significant from the ninth month of this study (Fig. 2).

At the baseline, no patient met the ADA/ESDA goal for HbA$_{1c}$ (<7.0% [53 mmol/mol]), while at the end of this trial, 48% of subjects in the SAIR group (eight patients in SAP and five patients in MDI subgroups), 16% (*n* = 3) of patients in the CSII group, and 18% (*n* = 3) of individuals on MDIs achieved the HbA$_{1c}$ target.

At 1 year, the average daily glucose level, as measured by RT-CGM or professional CGM, was significantly lower, both in the SAIR group (10.6 ± 1.5 mmol/L vs. 8.7 ± 1.4 mmol/L; *P* < 0.001) and in the CSII group (10.7 ± 1.2 mmol/L vs. 9.8 ± 1.1 mmol/L; *P* = 0.04). This improvement in average CGM glucose was accompanied by an increase in the time in range (4.0–10.0 mmol/L); 50% ± 11% versus 69% ± 11%; *P* < 0.0001, for SAIR and 51% ± 10% versus 59% ± 11%, *P* = 0.03, for CSII.

Compared with the baseline, GV was lower in the groups on SAIR (SD$_T$: 4.0 ± 0.7 mmol/L vs. 3.0 ± 0.5 mmol/L; *P* < 0.0001) and with CSII (SD$_T$ 3.9 ± 0.6 mmol/L vs. 3.4 ± 0.6 mmol/L; *P* < 0.05). Additionally, significant reduction of the time spent in hypoglycemia was observed only in patients with SAIR (8% ± 4% vs. 6% ± 3%; *P* < 0.01). For patients just on MDIs, no significant change in GV (SD$_T$ 3.8 ± 1.0 mmol/L vs. 3.8 ± 1.1 mmol/L; *P* = 0.93) and in hypoglycemia (6% ± 4% vs. 7% ± 5%; *P* = 0.68) was observed.

No difference in HbA$_{1c}$ (7.2% ± 0.8% vs. 7.3% ± 0.9% [54 ± 9 mmol/mol vs. 56 ± 10 mmol/mol]; *P* = 0.87), hypo-

glycemia (6% ± 4% vs. 6% ± 3%; *P* = 0.91), and GV (SD$_T$ 2.9 ± 0.5 mmol/L vs. 3.0 ± 0.4 mmol/L; *P* = 0.67) was observed in patients with the two types of CGM systems (DexCom G4 and Enlite sensor).

Adverse event

Throughout the study, two severe episodes of hypoglycemia were reported, one in the CSII group and one in the MDI group. No severe hypoglycemia in the SAIR group was reported. There was no ketoacidosis or sensor insertion site infection requiring assistance during a year of follow-up.

Discussion

To the best of our knowledge, this is the first prospective, 1-year real-life study simultaneously comparing four different treatment strategies based on different combinations of insulin delivery and monitoring systems. The sensor-augmented pump therapy for A1C Reduction (STAR3) study provided only comparison between SAP and MDIs where RT-CGM was not used.[6] Thus, it was not possible to determine the contribution of each component of the system on results. The SWITCH Study[7] showed that addition of RT-CGM to already established CSII therapy led to an improvement of glycemic control, while removal of RT-CGM resulted in a loss of this benefit. This implies that RT-CGM plays an important role in CSII patients. However, no patients with MDI therapy were investigated. The Juvenile Diabetes Research Foundation (JDRF) CGM studies investigated patients both on MDIs and CSII therapy. The JDRF, however, did not report the subgroup analyses comparing patients on MDI therapy with those on CSII therapy.[11,12]

Our study showed significant glycemic benefits in using RT-CGM, which were comparable for patients either on CSII or MDI therapy. Moreover, the usage of RT-CGM resulted in a sustained decrease of HbA_{1c} with a concurrent reduction of time spent in hypoglycemia, which has not always been described.[6]

Recent data from the T1D Exchange Clinic Registry[1] show that only $\sim 30\%$ of registered adults meet the ADA/ESDA goal for HbA_{1c} of <7.0% (53 mmol/mol). Given the inclusion criteria of our study, at the baseline, no patient met the ADA/ESDA goal either. However, after a year, our study showed that almost half of subjects in the SAIR group met the target for HbA_{1c}.

The HbA_{1c} decrease with SAIR in this study was accompanied by improvements in other secondary endpoints, including increased time in target range (4.0–10.0 mmol/L) and reduced GV. For describing GV in the present trial, we used total standard deviation (SD_T). It has been suggested that although SD_T has limitations,[13] more complex parameters of GV usually provide no additional information,[4] and thanks to its simplicity, it is easy to calculate SD_T as a component of routine diabetes management.[14]

In our study, treatment with CSII only also resulted in reduction of HbA_{1c} and GV, while in the group just on MDIs, significant decrease of HbA_{1c} and other endpoints was not achieved. One important result was that a combination of RT-CGM and MDIs was clearly superior to the improvement with CSII therapy, comparable with the superiority of SAP over CSII only. This is an important result because long-term studies comparing CSII without CGM and MDIs + RT-CGM are lacking.

In the 6-month follow-up study performed by Garg et al., RT-CGM provided similar benefits in glucose control for patients using either MDIs or SAP. However, in contrast with our trial, the significant decrease in HbA_1 was not seen, either in patients with MDIs + RT-CGM or SAP therapy. On the other hand, like numerous other trials,[6,11,12] the study was performed with the older generation of CGM. Since that time, experience with CGM and particularly the accuracy and usability of current systems have substantially improved,[15] which should translate into the results of newer studies. In a smaller study published by Tumminia et al., 14 patients (eight in the MDI + RT-CGM group and six in the SAP group) using RT-CGM >40% of the time significantly had decreased HbA_{1c} after 6 months. This effect was more evident in the MDI + RT-CGM group than the SAP group.[16]

In comparison with some other studies,[6,11,16] we observed higher adherence to the use of RT-CGM. This is important because sufficient sensor use is crucial to the success of CGM.[6,7,11,17] In the present study, 100% of participants in the SAIR group wore a sensor for more than 70% of the required time. This good adherence in using RT-CGM can be explained by the fact that patients were actively consulted about the treatment modality that best met their needs. In our study, we saw a greater frequency of boluses in the SAIR group compared with both SMBG groups. However, the higher number of boluses in the SAIR group does not correspond with the substantially lower frequency of finger sticks performed per day (6.8 boluses/day vs. 3.7 finger sticks/day). This is despite the fact that patients were encouraged to perform confirmatory finger sticks before each treatment decision. They often did not comply with this advice. This was apparent especially in patients with Dexcom G4 sensor. One possible explanation is that with the improved accuracy of the newer generation of CGM systems,[15] patients have more trust in CGM technology and sometimes provide the insulin dose adjustment without SMBG (although this procedure cannot yet be recommended for the systems used in this study).

Thus, more experience, improved accuracy, and usability of current CGM systems, high adherence to RT-CGM use, and patient's confidence in RT-CGM—all these aspects could be factors in our results.

There are also limitations. This was a nonrandomized study. Thus, although baseline HbA_{1c} was similar, the more motivated patients might have selected the insulin pumps and/or CGM. Another possible limitation is the different types of insulin pumps and CGM systems used in this study. However, this reflects real-life and day-to-day clinical practice. Moreover, the study is designed as a long-term follow-up and it is still ongoing after the first year. Thus, if we had not paid attention to patients' needs and randomized them, we would have expected a higher dropout and gradual loss of the ability to describe differences between study groups.

We believe that our findings could facilitate further discussion and possibly have an influence on diabetes care. Despite the potential benefits of using CSII therapy, with or without CGM,[7,18] many patients still report barriers to using it.[19,20] Some of these patients might be willing to use and benefit from another advanced technology—RT-CGM—where insulin is administered by MDIs.

In conclusion, in patients with type 1 diabetes with suboptimal glycemic control, both SAIRs, that is, SAP and MDIs + RT-CGM, were superior to MDIs or CSII therapy in reducing HbA_{1c}, hypoglycemia, and the other endpoints. Both SAIRs provided comparable glycemic benefits. Hence, a combination of real-time CGM and MDIs can be considered as an equivalent alternative to SAP therapy for patients who are not willing to or cannot use insulin pumps.

Author Disclosure Statement

No competing financial interests exist.

References

1. Miller KM, Foster NC, Beck RW, et al.: T1D Exchange Clinic Network. Current state of type 1 diabetes treatment in the U.S.: updated data from the T1D Exchange clinic registry. Diabetes Care 2015;38:971–978.
2. Nathan DM, Cleary PA, Backlund JY, et al.; Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group: Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes. N Engl J Med 2005;353:2643–2653.
3. Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and

progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–998.

4. Šoupal J, Škrha J Jr, Fajmon M, et al.: Glycemic variability is higher in type 1 diabetes patients with microvascular complications irrespective of glycemic control. Diabetes Technol Ther 2014;16:198–203.

5. Garg SK, Voelmle MK, Beatson CR, et al.: Use of continuous glucose monitoring in subjects with type 1 diabetes on multiple daily injections versus continuous subcutaneous insulin infusion therapy: a prospective 6-month study. Diabetes Care 2011;34:574–579.

6. Bergenstal RM, Tamborlane WV, Ahmann A, et al.; STAR 3 Study Group. Effectiveness of sensor-augmented insulin-pump therapy in type 1 diabetes. N Engl J Med 2010;363:311–320.

7. Battelino T, Conget I, Olsen B, et al.; SWITCH Study Group. The use and efficacy of continuous glucose monitoring in type 1 diabetes treated with insulin pump therapy: a randomised controlled trial. Diabetologia 2012;55:3155–3162.

8. Battelino T, Phillip M, Bratina N, et al.: Effect of continuous glucose monitoring on hypoglycemia in type 1 diabetes. Diabetes Care 34:795–800.

9. Garg SK, Schwartz S, Edelman SV: Improved glucose excursions using an implantable real-time continuous glucose sensor in adults with type 1 diabetes. Diabetes Care 2004;27:734–738.

10. Rodbard D, Jovanovic L, Garg S: Responses to continuous glucose monitoring in patients with type 1 diabetes using multiple daily injections and insulin pumps. Diabetes Technol Ther 2009;11:757–765.

11. Tamborlane WV, Beck RW, Bode BW, et al.; Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 2008;359:1464–1476.

12. Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group: Effectiveness of continuous glucose monitoring in a clinical care environment: evidence from the Juvenile Diabetes Research Foundation continuous glucose monitoring (JDRF-CGM) trial. Diabetes Care 2010;33:17–22.

13. Rodbard D: Interpretation of continuous glucose monitoring data: glycemic variability and quality of glycemic control. Diabetes Technol Ther 2009;11(Suppl 1):S-55–S-67.

14. Suh S, Kim JH: Glycemic variability: how do we measure it and why is it important? Diabetes Metab J 2015;39:273–282.

15. Rodbard D: Continuous glucose monitoring: a review of successes, challenges, and opportunities. Diabetes Technol Ther 2016;18 Suppl 2:S2-3–S2-13.

16. Tumminia A, Crimi S, Sciacca L, et al.: Efficacy of real-time continuous glucose monitoring on glycaemic control and glucose variability in type 1 diabetic patients treated with either insulin pumps or multiple insulin injection therapy: a randomized controlled crossover trial. Diabetes Metab Res Rev 2015;31:61–68.

17. Raccah D, Sulmont V, Reznik Y, et al.: Incremental value of continuous glucose monitoring when starting pump therapy in patients with poorly controlled type 1 diabetes: the RealTrend study. Diabetes Care 2009;32:2245–2250.

18. Steineck I, Cederholm J, Eliasson B, et al.; Swedish National Diabetes Register. Insulin pump therapy, multiple daily injections, and cardiovascular mortality in 18,168 people with type 1 diabetes: observational study. BMJ 2015;350:h3234.

19. Liberman A, Buckingham B: Diabetes technology and the human factor. Diabetes Technol Ther 2016;18 Suppl 1:S101–S111.

20. Wong JC, Foster NC, Maahs DM, et al.; T1D Exchange Clinic Network. Real-time continuous glucose monitoring among participants in the T1D Exchange clinic registry. Diabetes Care 2014;37:2702–2709.

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Glycemic Outcomes in Adults With T1D Are Impacted More by Continuous Glucose Monitoring Than by Insulin Delivery Method: 3 Years of Follow-Up From the COMISAIR Study

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OBJECTIVE

This study assessed the clinical impact of four treatment strategies in adults with type 1 diabetes (T1D): real-time continuous glucose monitoring (rtCGM) with multiple daily insulin injections (rtCGM+MDI), rtCGM with continuous subcutaneous insulin infusion (rtCGM+CSII), self-monitoring of blood glucose with MDI (SMBG+MDI), and SMBG with CSII (SMBG+CSII).

RESEARCH DESIGN AND METHODS

This 3-year, nonrandomized, prospective, real-world, clinical trial followed 94 participants with T1D (rtCGM+MDI, $n = 22$; rtCGM+CSII, $n = 26$; SMBG+MDI, $n = 21$; SMBG+CSII, $n = 25$). The main end points were changes in A1C, time in range (70–180 mg/dL [3.9–10 mmol/L]), time below range (<70 mg/dL [<3.9 mmol/L]), glycemic variability, and incidence of hypoglycemia.

RESULTS

At 3 years, the rtCGM groups (rtCGM+MDI and rtCGM+CSII) had significantly lower A1C (7.0% [53 mmol/mol], $P = 0.0002$, and 6.9% [52 mmol/mol], $P < 0.0001$, respectively), compared with the SMBG+CSII and SMBG+MDI groups (7.7% [61 mmol/mol], $P = 0.3574$, and 8.0% [64 mmol/mol], $P = 1.000$, respectively), with no significant difference between the rtCGM groups. Significant improvements in percentage of time in range were observed in the rtCGM subgroups (rtCGM+MDI, 48.7–69.0%, $P < 0.0001$; and rtCGM+CSII, 50.9–72.3%, $P < 0.0001$) and in the SMBG+CSII group (50.6–57.8%, $P = 0.0114$). Significant reductions in time below range were found only in the rtCGM subgroups (rtCGM+MDI, 9.4–5.5%, $P = 0.0387$; and rtCGM+CSII, 9.0–5.3%, $P = 0.0235$, respectively). Seven severe hypoglycemia episodes occurred: SMBG groups, $n = 5$; sensor-augmented insulin regimens groups, $n = 2$.

CONCLUSIONS

rtCGM was superior to SMBG in reducing A1C, hypoglycemia, and other end points in individuals with T1D regardless of their insulin delivery method. rtCGM+MDI can be considered an equivalent but lower-cost alternative to sensor-augmented insulin pump therapy and superior to treatment with SMBG+MDI or SMBG+CSII therapy.

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Use of real-time continuous glucose monitoring (rtCGM) has emerged as a critical component of diabetes self-management for individuals treated with intensive insulin regimens, and it is now considered a standard of care for these patients (1–6).

Recent randomized clinical trials have demonstrated that use of rtCGM results in significant improvements in glycemic control and hypoglycemia and confers a higher quality of life to participants treated with multiple daily insulin injections (MDIs) compared with traditional self-monitoring of blood glucose (SMBG) (7–11). Similar improvements in A1C and hypoglycemia have also been observed in participants using rtCGM with insulin pump therapy (12,13). Significant reductions in severe hypoglycemia have also been observed in patients with type 1 diabetes (T1D) with problematic hypoglycemia who were treated with rtCGM in combination with either MDI (10) or insulin pump therapy (13). Importantly, a common observation in most rtCGM studies is that glycemic improvements and other benefits were dependent upon the persistence of sensor use (7–15).

Although randomized controlled trials (RCTs) are recognized as the highest level of evidence regarding the efficacy of rtCGM when used within tightly controlled settings, our understanding of the real-world use and benefits of rtCGM has been limited. Findings from RCTs often fail to reflect actual participant behaviors and resultant outcomes in real-world clinical practice (16–18). Moreover, there have been few long-term comparisons to evaluate the efficacy of rtCGM use in combination with the various insulin delivery methods (e.g., rtCGM + continuous subcutaneous insulin infusion [CSII] vs. rtCGM+MDI), and conclusive evidence of rtCGM benefits compared with SMBG has been sparse. Because diabetes management is primarily dependent on participant behavior, different research approaches are needed to more definitively assess these behavior-based interventions.

We recently reported findings from the Comparison of Sensor Augmented Insulin Regimens (COMISAIR) study, a 1-year, nonrandomized, real-world study that assessed the efficacy of long-term use of sensor-augmented insulin regimens (SAIR)–rtCGM combined with

either CSII (sensor-augmented pump [rtCGM+CSII]) or MDI (rtCGM+MDI)–on glycemic control compared with the addition of CSII (SMBG+CSII) or MDI (SMBG+MDI) (19) among 65 individuals with T1D. At study end, significant A1C reductions from baseline were observed in both the SAIR groups (rtCGM+CSII: −1.1% [−12.0 mmol/mol], $P = 0.0025$; rtCGM+MDI: −1.3% [−14.2 mmol/mol], $P = 0.0034$). Although SMBG+CSII use also led to a significant A1C reduction (0.5% [5.5 mmol/mol]), no significant reductions were seen in the SMBG+MDI group. The increase from baseline in average number of boluses per day was significantly greater in the rtCGM+CSII and rtCGM+MDI groups (3.2 and 2.2, respectively, both $P < 0.0001$) compared with SMBG+CSII (0.6, $P = 0.08$). No increase was seen in the SMBG+MDI group. Importantly, significant reductions in percentage of time in hypoglycemia (<70 mg/dL [<3.9 mmol/L]) were observed only in the SAIR groups, from 8 ± 4% to 6 ± 43%, $P < 0.01$.

In the current follow-up study, we investigated the effects of SAIR interventions on glycemic control and treatment persistence among a larger participant cohort after 3 years, providing further supportive evidence for the use of rtCGM in the management of T1D.

RESEARCH DESIGN AND METHODS

The COMISAIR-2 study was the 3-year follow-up of the COMISAIR trial (19), which compared the efficacy of the long-term use of SAIR regimens among individuals. Participants were recruited from the participant population treated at the 3rd Department of Internal Medicine, 1st Faculty of Medicine, Charles University. This report includes results from an additional 29 participants whose complete 1-year data were not available at the conclusion of the initial COMISAIR trial. The study was approved by an independent ethics review board and conducted in accordance with the Declaration of Helsinki (20). All subjects provided written informed consent before enrollment.

Inclusion criteria were as follows: age >18 years, >2 years T1D duration, A1C 7.0–10% (53–86 mmol/mol), treated with analog insulins, willingness to use sensors >70% of the time or perform SMBG four or more times per day, and

willingness to participate in a 4-day training program at baseline. Exclusion criteria were as follows: use of rtCGM within the previous 3 months, ketoacidosis within the previous 3 months, concomitant therapy influencing glucose metabolism, pregnant or planning pregnancy, and demonstrated nonadherence to current treatment regimen.

Procedures
Enrolled participants were scheduled for a total of 15 clinic visits (baseline, at week 2, and then at months 1, 3, 6, 9, 12, 15, 18, 21, 24, 27, 30, 33, and 36). A detailed description of the study procedures was previously published (19).

At the initial visit, investigators confirmed eligibility and initiated professional CGM (iPro2; Medtronic, Northridge, CA) in all participants for 6 days. Throughout the study, participants in the groups not using SAIR had professional CGM every 3 months. Participants then attended a structured 4-day training program that addressed basic insulin administration skills, including timing and dosing of preprandial insulin, prevention of hypoglycemia, and theoretical and practical carbohydrate counting. Participants were encouraged to use flexible insulin dosing.

During training, all treatment modalities (rtCGM+MDI, rtCGM+CSII, SMBG+MDI, and SMBG+CSII) were introduced to participants. In collaboration with study clinicians, participants selected their treatment modality according to their individual needs and preferences. Investigator influence on participant decisions was minimal (6% of cases), and no participant was discouraged from using one of the SAIR regimens. Participants in the SAIR and CSII groups completed theoretical training on the relevant devices, followed by treatment initiation and practical training (including insulin adjustment) with investigators.

Participants using SAIR were encouraged to make self-adjustments to their treatment using rtCGM values (hyperglycemia and hypoglycemic alerts and trends) and to incorporate results of SMBG into treatment changes. Participants in non-SAIR groups were encouraged to measure their blood glucose at least four times per day. All participants were instructed to use only the study

Table 1—Baseline characteristics

Characteristic	rtCGM + MDI (n = 22)	rtCGM + CSII (n = 26)	SMBG + CSII (n = 25)	SMBG + MDI (n = 21)	P value
Male (%)	59	50	48	52	0.89
Age (years)	32.6 ± 11.5	32.3 ± 9.9	33 ± 9.3	35 ± 15	0.95
Duration of diabetes (years)	13.7 ± 9.8	14.6 ± 7.8	13.4 ± 8.4	13.5 ± 8.8	0.86
A1C (mmol/mol)	66.6 ± 10.0	66.5 ± 10.2	67.3 ± 9	67 ± 8.6	0.95
A1C (%)	8.2 ± 0.9	8.2 ± 0.9	8.3 ± 0.8	8.3 ± 0.8	0.92
Mean sensor glucose (mmol/L)	10.5 ± 1.4	10.3 ± 1.5	10.4 ± 1.6	10.4 ± 1.3	0.89
BMI (kg/m^2)	26 ± 4	25 ± 4	25 ± 3	25 ± 3	0.91
Body weight (kg)	76.6 ± 14	72.5 ± 15	74 ± 11	73.7 ± 13	0.96
The total daily dose of insulin (units)	48.1 ± 15	46.2 ± 11.5	46.7 ± 11.4	48.8 ± 13.5	0.93
Relative proportion of bolus insulin (%)	48.7 ± 3.9	48.7 ± 4	50.1 ± 4.4	50 ± 4.4	0.61
No. of boluses/day (n)	3.9 ± 0.9	3.8 ± 0.8	3.8 ± 0.9	3.8 ± 0.7	0.99
Frequency of SMBG/day (n)	3.7 ± 1	3.7 ± 1.2	3.8 ± 1.1	3.6 ± 1	0.95

Values are presented as mean ± SD.

blood glucose meter provided to them for all SMBG measurements taken during this trial.

At each clinic visit, participants were screened for adverse events, sensor insertion sites were inspected (SAIR participants), and data from all rtCGM systems, insulin pumps, and blood glucose meters were downloaded for analysis.

Glucose Monitoring Devices

Participants in the CSII group wore one of two types of insulin pumps: MiniMed Paradigm Veo (Medtronic) and Animas Vibe (Animas Corporation, West Chester, PA). Participants in the rtCGM + CSII subgroup used either the MiniMed Paradigm Veo System with Enlite sensors (Medtronic) or Animas Vibe system with DexComG4 sensors (Dexcom, San Diego, CA). The subgroup of participants who selected rtCGM + MDI therapy used a DexCom G4 rtCGM system. The iPro2 was used for glucose monitoring in all participants at baseline and every 3 months in SMBG participants. All participants were provided with a personal blood glucose meter (OneTouch [Life-Scan, Milpitas, CA] or CONTOUR LINK [Bayer Diabetes Care, Basel, Switzerland]), which was used for diabetes self-management purposes and calibration of rtCGM. We highlighted to participants the importance of regular downloading and review of the data from rtCGM devices and insulin pumps. A bolus calculator was set for all participants with insulin pumps.

Outcomes

The primary end point was the difference in A1C between the groups after 3 years of follow-up. Secondary end points were as follows: change in glycemic variability (expressed as the total SD of blood glucose, average daily glucose from CGM, and percentage of time spent in range 70–180 mg/dL [3.9–10.0 mmol/L]), percentage of time <70 mg/dL (<3.9 mmol/L), rtCGM usage (SAIR groups), change in average number of boluses per day, and incidence of hypoglycemia.

Measures

A1C values were measured at the baseline and then every 3 months until study end. A1C was analyzed by a high-performance liquid chromatography method on a Variant II analyzer (Bio-Rad, Hercules, CA). The normal reference range of A1C in our laboratory is 4.0–6.0% (20–42 mmol/mol). Initially, all patients were monitored by professional CGM for 6 days. Then, throughout the study, subjects in the groups not using rtCGM were assessed by professional CGM for 6 days every 3 months.

Severe hypoglycemia was defined as an episode requiring assistance from another person or neurological recovery in response to restoration of plasma glucose to normal. Ketoacidosis was defined as an episode of hyperglycemia (>252 mg/dL [>14 mmol/L]) with low serum bicarbonate (<15 mmol/L), low pH (<7.3), or both together with either ketonemia or ketonuria that required treatment in a health care facility.

Statistical Analysis

The basic characteristics of each group were analyzed using nonparametric tests (Kruskal-Wallis and ANOVA). The data of repeated measurements (obtained every 3 months) such as the mean glucose levels, time in/below target range, and glycemic variability were compared using a linear mixed-effects model. P values <0.05 were considered statistically
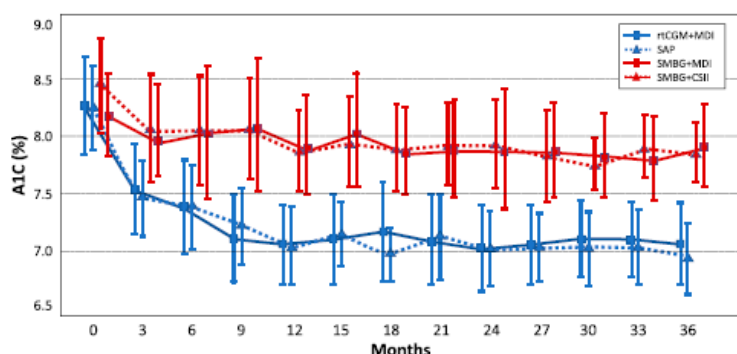

Figure 1—Change in A1C from baseline by study group. SAP, sensor-augmented pump.

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Table 2—Significance of A1C change over 36 months

	Month 3	Month 6	Month 9	Month 12	Month 15	Month 18	Month 21	Month 24	Month 27	Month 30	Month 33	Month 36
rtCGM+MDI	0.0017	0.0006	<0.0001	<0.0001	<0.0001	0.0003	<0.0001	<0.0001	0.0001	0.0004	0.0006	0.0002
rtCGM+CSII	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0001	<0.0001
SMBG+MDI	1.0000	1.0000	1.0000	0.3914	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
SMBG+CSII	1.0000	1.0000	1.0000	0.0183	0.1778	1.0000	0.9125	0.9740	0.7677	0.2954	1.0000	0.3574

significant. Analyses were conducted using the R statistical package, version 3.1.1. Data are expressed as mean ± SD values.

RESULTS

Baseline Characteristics and Adherence

A total of 94 participants were enrolled in the study; 88 completed all study visits. Among the six participants who discontinued the study, two SMBG+CSII participants and one rtCGM+CSII participant withdrew for personal reasons, one SMBG+CSII participant decided to initiate rtCGM after 1 year, one rtCGM+MDI initiated rtCGM+CSII, and one SMBG+MDI participant died due to breast cancer. Baseline characteristics were similar in the four study groups (Table 1).

All SAIR participants wore their sensors >70% of the time. No significant changes in total insulin dose or body weight were observed in any of the study groups.

Primary and Secondary End Points

Change in A1C

At 3 years, the rtCGM+MDI and rtCGM+CSII groups had significantly lower A1C (7.0% [53 mmol/mol], $P =$ 0.0002, and 6.9% [52 mmol/mol], $P <$ 0.0001, respectively), compared with the SMBG+MDI and SMBG+CSII groups (8.0% [64 mmol/mol], $P = 1.000$, and 7.7% [61 mmol/mol], $P = 0.3574$, respectively). No significant differences in A1C between the rtCGM+MDI and rtCGM+CSII groups ($P = 0.61$) or SMBG+MDI and SMBG+CSII ($P = 0.69$) were observed.

Significant reductions in A1C were seen in the rtCGM+MDI and rtCGM+CSII groups at all follow-up visits throughout the 3-year study period (Fig. 1 and Table 2). Significant A1C reductions were seen in the SMBG+CSII group only at month 12 ($P = 0.0183$); no significant reductions were seen in the SMBG+MDI group. Supplementary Table 1 presents A1C changes in each study group at all study visits.

Forty-eight percent ($n = 23$) of SAIR participants achieved <7.0% A1C at 3 years (rtCGM+MDI, 43% [$n = 9$]; rtCGM+CSII, 56% [$n = 14$]) compared with 9% ($n = 2$) of SMBG+CSII and 16% ($n = 3$) of SMBG+MDI participants.

Between-group comparisons of A1C changes showed significant differences between the SAIR and SMBG groups at 3 years, favoring use of rtCGM (Table 3). No significant differences between the SAIR subgroups or SMBG subgroups were observed.

Significant differences between the rtCGM+MDI group and SMBG groups were observed beginning at month 6, whereas the differences between the rtCGM+CSII group and SMBG groups were observed beginning at month 3.

Average Sensor Glucose

Significant differences in improvements in average sensor glucose were seen in the rtCGM+MDI and rtCGM+CSII groups but not in the SMBG+CSII or SMBG+MDI groups (Table 3). No significant between-group differences within the SAIR or SMBG subgroups were observed.

Glycemic Variability

Significant differences in glycemic variability were observed between rtCGM+MDI versus SMBG+MDI, rtCGM+CSII versus SMBG+MDI, and SMBG+CSII versus SMBG+MDI (Table 3). No significant differences were seen between rtCGM+MDI and rtCGM+CSII. Significant improvements in time in range and time spent in hypoglycemia were observed at 3 years in the rtCGM+MDI, rtCGM+CSII, and SMBG+CSII groups but not the SMBG+MDI group (Fig. 2).

Time in Range

Improvements in time in range (70–180 mg/dL [3.9–10.0 mmol/L]) among SAIR subgroups were significantly greater than observed in the SMBG subgroups:

rtCGM+MDI versus SMBG+MDI, 14.21% (95% CI 6.45 to −22, $P = 0.0007$); rtCGM+MDI versus SMBG+CSII, 11.13% (95% CI 4.46–17.81, $P = 0.0016$); rtCGM+CSII versus SMBG+MDI, 17.58% (95% CI, 10.9–24.27, $P < 0.0001$); and rtCGM+CSII versus SMBG+CSII, 14.5% (95% CI 8.82–20.19, $P < 0.0001$). No differences were seen between the rtCGM+MDI and rtCGM+CSII groups. Significant reductions in percentage of time below range (<70 mg/dL [<3.9 mmol/L]) were seen in the rtCGM+MDI ($P = 0.0387$) and rtCGM+CSII ($P = 0.0235$) groups but not the SMBG+CSII (0.4847) or SMBG+MDI ($P = 1.000$) groups (Fig. 2).

Insulin Boluses

At study end, the average number of boluses per day was lower in both SMBG groups in comparison with the rtCGM groups (6.9 ± 1.9 vs. 4.5 ± 1.1, $P < 0.0001$). A higher frequency of boluses was seen in participants with SMBG+CSII versus the self-reported boluses in the SMBG+MDI group (4.9 ± 1.2 vs. 4.1 ± 0.8, $P = 0.02$). No significant difference between rtCGM+CSII and rtCGM+MDI was observed (7.1 ± 1.9 vs. 6.6 ± 1.9, $P = 0.4$) (Supplementary Table 2).

rtCGM Use

Mean percentage use of rtCGM in the SAIR groups was high throughout the study period, with slight but notable increases from year 1 (rtCGM+MDI, 85.7 ± 9%; rtCGM+CSII, 86.7 ± 10%) and year 3 (rtCGM+MDI, 88.0 ± 8%; rtCGM+CSII, 87.0 ± 8%). No significant differences between the subgroups were observed (Supplementary Table 2).

SMBG Use

The average frequency of fingerstick tests performed per day was lower in the SAIR group compared with the SMBG group (3.0 ± 0.9 vs. 3.8 ± 1.2, $P = 0.001$). It is important to note that the rtCGM devices required twice daily calibration with fingerstick testing. Within the SAIR group, daily SMBG frequency

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Table 3—Between-group differences in A1C, average sensor glucose, and glycemic variability (SD) at 3 years

Study groups	A1C, % (mmol/mol)			Average sensor glucose, mg/dL (mmol/L)			Glycemic variability, mg/dL (mmol/L)		
	Difference	95% CI	P value	Difference	95% CI	P value	Difference	95% CI	P value
rtCGM+MDI vs. SMBG+MDI	−0.87 (−9.5)	−1.38 to −0.35 (−15.2 to −3.8)	0.0016	−27.0	−42.3 to −12.24 (−2.35 to −0.68)	0.0007	−12.24 (−0.68)	−18.36 to 5.94 (−1.02 to −0.33)	0.0003
rtCGM+MDI vs. SMBG+CSII	−0.78 (−8.5)	−1.23 to −0.33 (−13.4 to −3.6)	0.0011	−21.1	−32.76 to 9.18 (−1.82 to −0.51)	0.0009	−4.5 (−0.25)	−10.62 to 1.44 (−0.59 to −0.08)	0.14
rtCGM+MDI vs. rtCGM+CSII	0.12 (−1.3)	−0.35 to 0.59 (−3.8 to 6.4)	0.61	1.98	−10.44 to 14.4 (−0.58 to 0.8)	0.75	−0.18 (−0.01)	−6.3 to 5.76 (−0.35 to 0.32)	0.95
rtCGM+CSII vs. SMBG+MDI	−0.99 (−10.8)	−1.45 to −0.52 (−15.8 to −5.7)	<0.0001	−29.2	−42.66 to −15.84 (−2.37 to −0.88)	<0.0001	−12.06 (−0.67)	−18.54 to 5.58 (−1.03 to 0.31)	0.0006
rtCGM+CSII vs. SMBG+CSII	−0.9 (−9.8)	−1.3 to −0.5 (−14.2 to −5.5)	<0.0001	−23.0	−33.48 to −12.6 (−1.86 to −0.7)	<0.0001	−4.32 (−0.24)	−10.62 to 1.98 (−0.59 to −0.11)	0.17
SMBG+CSII vs. SMBG+MDI	−0.09 (−1.0)	−0.52 to 0.35 (−5.7 to 3.8)	0.69	−6.2	−19.08 to 6.66 (−1.06 to 0.37)	0.33	−7.56 (−0.42)	−14.04 to 1.26 (−0.78 to −0.07)	0.02

was significantly lower among Dexcom G4 sensor users ($n = 32$) compared with Medtronic Enlite sensor users ($n = 14$): 2.7 ± 0.6 vs. 3.9 ± 0.8, $P < 0.001$ (Supplementary Table 2).

Adverse Events

Seven severe episodes of hypoglycemia were reported during the 3-year study period: two within the SMBG+CSII group, three in the SMBG+MDI group, one within the rtCGM+CSII group (which occurred when the participant was not wearing the sensor), and one within the rtCGM+MDI group. Three episodes of ketoacidosis occurred: one in the SMBG+CSII group, one in the SMBG+MDI group, and one in the rtCGM+CSII group; all cases were adjudicated. Four allergic reactions to sensor wear occurred but did not result in study discontinuation. No infections requiring assistance were reported during the 3-year study period.

CONCLUSIONS

To our knowledge, this is the first prospective, real-world, 3-year study to simultaneously compare four different treatment strategies based on different combinations of glucose monitoring systems and insulin delivery methods. As reported here, use of rtCGM among adults with T1D treated with MDI or CSII therapy was associated with 3 years of sustained improvements from baseline in A1C, glycemic variability, and time in range, with significantly greater reductions in time spent below range (<70 mg/dL [<3.9 mmol/L]); both time in range and time below range are now emerging as important metrics of glycemic control. Importantly, we observed comparable improvements in both the rtCGM+CSII and rtCGM+MDI groups, suggesting equivalent efficacy regardless of the insulin delivery method used.

Although similar improvements in glycemic control have been shown in previous RCTs (7–13), our findings demonstrate the long-term sustainability of rtCGM use, its clinical benefits, and its implications regarding medication adherence within the context of real-world diabetes self-management. The consistently high percentage of time that participants wore their sensors during the 3-year study period suggests that rtCGM was perceived to be a valuable tool in
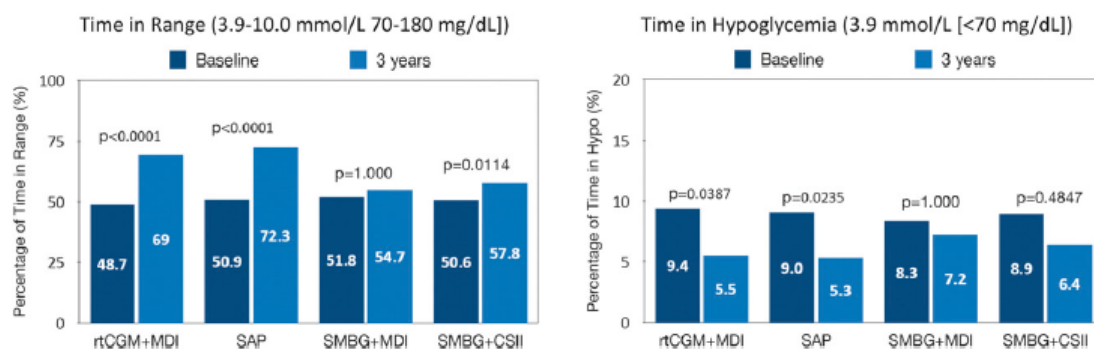
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Figure 2—Changes in percentage of time in range and time in hypoglycemia. SAP, sensor-augmented pump.

their self-management regimens, and it may also explain the significant increase in the number of daily boluses observed in the SAIR groups; no changes in daily bolusing were seen in the SMBG groups. Additionally this persistence in CGM use correlates with the increased number of participants getting to the goal, suggesting the perceived value translated into improved clinical outcomes.

From a clinical perspective, the glycemic improvements observed among rtCGM users will likely lead to significant reductions in long-term complications (21). However, our findings also have important implications for payers. As reported by Gilmer et al. (22), a 1.0% reduction in A1C from 8.0 to 7.0% is associated with ~$820 in savings over 3 years in adults with diabetes but without heart disease and hypertension; the savings are even greater when one or both of these comorbidities are present.

In addition to the long duration of assessment, another strength is the use of a real-world study design. Although the efficacy and clinical utility of rtCGM have been demonstrated in numerous RCTs (7–13), they do not necessarily reflect the behaviors and clinical responses of participants in real life because RCTs strictly control the setting and delivery of interventions to minimize the effect of external factors on outcomes (16–18). Nor do they inform us about the long-term sustainability and clinical impact of rtCGM use beyond the defined study durations. In our study, we allowed participants to choose the insulin/ monitoring option that met their individual needs, which reflects real-life decision-making in most clinical practices.

Additionally, an increasing number of payers and regulatory agencies are recognizing the inherent limitations of RCTs in providing real-world evidence (RWE) about the efficacy of medications and use of medical devices in clinical practice. As such, they are now focusing on RWE to inform their decisions. For example, both the U.S. Food and Drug Administration and European Medicines Agency are asking manufacturers to provide RWE in combination with RCT findings when evaluating both the short- and long-term safety and effectiveness of new drug and medical device submissions, particularly in the assessment of medical devices in real-world clinical practice (23–26).

The study has notable limitations. Because this was a nonrandomized study, it is possible that there were some unmeasured factors that could impact our findings. For example, it is possible that the more motivated study participants may have selected to use rtCGM. Although one would expect motivated participants to achieve greater improvements than participants who are less motivated, we observed no significant between-group differences in motivation. Because all subjects were willing to participate in a "Dose Adjustment for Normal Eating (DAFNE)-like" 4-day training program, motivation likely only had a minor impact on results, if any. Moreover, if we had not allowed participants to choose the regimens that met their individual needs and preferences, we would have likely seen a much higher discontinuation rate, which would have resulted in a gradual loss in our ability to describe differences between study groups. Another potential limitation is that different types of insulin pumps and rtCGM systems were used in this study. However, as reported, changes in A1C between the study subgroups were comparable, which suggests that device differences did not impact our findings. Additionally, with the exception of patients with insulin pumps (CGM+CSII and SMBG+ CSII groups), all bolusing data gathered from the other study groups were self-reported. Although it is possible that participants may have overreported their bolusing frequency, given the higher number of boluses within the rtCGM groups, which appear to correlate with better glycemic outcomes versus SMBG groups, we believe the impact of overreporting was minimal.

Importantly, our findings demonstrate that the use of rtCGM with MDI can be considered an equivalent but more cost-effective treatment alternative to sensor-augmented insulin pumps for many individuals with T1D. For example, in a recent analysis of the Multiple Daily Injections and Continuous Glucose Monitoring in Diabetes (DIAMOND) trial (8), Skandari and colleagues (27) found that among rtCGM+CSII participants, the total per-person 28-week costs were $8,272 vs. $5,623 among rtCGM+MDI users; the difference was primarily attributed to CSII use. The increasing focus on reducing costs while improving outcomes may impact reimbursement decisions regarding current and future sensor-augmented insulin pump systems.

In conclusion, in individuals with T1D with suboptimal glycemic control, use of rtCGM was superior to SMBG in reducing A1C, hypoglycemia, and the other end points regardless of the insulin delivery method used; both methods provided comparable glycemic benefits. Our findings may provide guidance to clinicians

when discussing treatment/monitoring options with their participants.

References

1. National Institute for Health and Care Excellence. Type 1 diabetes in adults: diagnosis and management [Internet], 2015. Available from https://www.nice.org.uk/guidance/ng17. Accessed 2 February 2018

2. American Diabetes Association. 6. Glycemic targets: *Standards of Medical Care in Diabetes—2018*. Diabetes Care 2018;41(Suppl. 1):S55–S64

3. Borot S, Benhamou PY, Atlan C, et al.; Société francophone du diabète (SFD); Société française d'endocrinologie (SFE); Évaluation dans le diabète des implants actifs Group (EVADIAC). Practical implementation, education and interpretation guidelines for continuous glucose monitoring: a French position statement. Diabetes Metab 2018;44:61–72

4. Danne T, Nimri R, Battelino T, et al. International consensus on use of continuous glucose monitoring. Diabetes Care 2017;40:1631–1640

5. Fonseca VA, Grunberger G, Anhalt H, et al.; Consensus Conference Writing Committee. Continuous glucose monitoring: a consensus conference of the American Association of Clinical Endocrinologists and American College of Endocrinology. Endocr Pract 2016;22:1008–1021

6. Peters AL, Ahmann AJ, Battelino T, et al. Diabetes technology-continuous subcutaneous insulin infusion therapy and continuous glucose monitoring in adults: an Endocrine Society clinical practice guideline. J Clin Endocrinol Metab 2016;101:3922–3937

7. Lind M, Polonsky W, Hirsch IB, et al. Continuous glucose monitoring vs conventional therapy for glycemic control in adults with type 1 diabetes treated with multiple daily insulin injections: the GOLD randomized clinical trial. JAMA 2017;317:379–387

8. Beck RW, Riddlesworth T, Ruedy K, et al.; DIAMOND Study Group. Effect of continuous glucose monitoring on glycemic control in adults with type 1 diabetes using insulin injections: the DIAMOND randomized clinical trial. JAMA 2017; 317:371–378

9. Beck RW, Riddlesworth TD, Ruedy K, et al.; DIAMOND Study Group. Continuous glucose monitoring versus usual care in patients with type 2 diabetes receiving multiple daily insulin injections: a randomized trial. Ann Intern Med 2017;167:365–374

10. Heinemann L, Freckmann G, Ehrmann D, et al. Real-time continuous glucose monitoring in adults with type 1 diabetes and impaired hypoglycaemia awareness or severe hypoglycaemia treated with multiple daily insulin injections (HypoDE): a multicentre, randomised controlled trial. Lancet 2018;391:1367–1377

11. Polonsky WH, Hessler D, Ruedy KJ, Beck RW; DIAMOND Study Group. The impact of continuous glucose monitoring on markers of quality of life in adults with type 1 diabetes: further findings from the DIAMOND randomized clinical trial. Diabetes Care 2017;40:736–741

12. Pickup JC, Freeman SC, Sutton AJ. Glycaemic control in type 1 diabetes during real time continuous glucose monitoring compared with self monitoring of blood glucose: meta-analysis of randomised controlled trials using individual patient data. BMJ 2011;343:d3805

13. van Beers CA, DeVries JH, Kleijer SJ, et al. Continuous glucose monitoring for patients with type 1 diabetes and impaired awareness of hypoglycaemia (IN CONTROL): a randomised, open-label, crossover trial. Lancet Diabetes Endocrinol 2016;4:893–902

14. Hirsch IB, Abelseth J, Bode BW, et al. Sensor-augmented insulin pump therapy: results of the first randomized treat-to-target study. Diabetes Technol Ther 2008;10:377–383

15. Tamborlane WV, Beck RW, Bode BW, et al.; Juvenile Diabetes Research Foundation Continuous Glucose Monitoring Study Group. Continuous glucose monitoring and intensive treatment of type 1 diabetes. N Engl J Med 2008;359:1464–1476

16. Edelman SV, Polonsky WH. Type 2 diabetes in the real world: the elusive nature of glycemic control. Diabetes Care 2017;40:1425–1432

17. van Onzenoort HA, Menger FE, Neef C, et al. Participation in a clinical trial enhances adherence and persistence to treatment: a retrospective cohort study. Hypertension 2011;58:573–578

18. Rothwell PM. External validity of randomised controlled trials: "to whom do the results of this trial apply?". Lancet 2005;365:82–93

19. Šoupal J, Petruželková L, Flekač M, et al. Comparison of different treatment modalities for type 1 diabetes, including sensor-augmented insulin regimens, in 52 weeks of follow-up: a COMISAIR study. Diabetes Technol Ther 2016;18:532–538

20. World Medical Association Declaration of Helsinki: recommendations guiding physicians in biomedical research involving human subjects. JAMA 1997;277:925–926

21. Nathan DM, Genuth S, Lachin J, et al.; Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977–986

22. Gilmer TP, O'Connor PJ, Rush WA, et al. Predictors of health care costs in adults with diabetes. Diabetes Care 2005;28:59–64

23. Khosla S, White R, Medina J, et al. Real world evidence (RWE) - a disruptive innovation or the quiet evolution of medical evidence generation? F1000Res 2018;7:111

24. Food and Drug Administration (FDA). Use of real-world evidence to support regulatory decision-making for medical devices [Internet]. Available from https://www.fda.gov/downloads/medicaldevices/deviceregulationand-guidance/guidancedocuments/ucm513027.pdf. Accessed 5 April 2017; 30 March 2019

25. Resnic FS, Matheny ME. Medical devices in the real world. N Engl J Med 2018;378:595–597

26. Katkade VB, Sanders KN, Zou KH. Real world data: an opportunity to supplement existing evidence for the use of long-established medicines in health care decision making. J Multidiscip Healthc 2018;11:295–304

27. Wan W, Skandari MR, Minc A, et al. Cost-effectiveness of initiating an insulin pump in T1D adults using continuous glucose monitoring compared with multiple daily insulin injections: the DIAMOND Randomized Trial. Med Decis Making 2018;38:942–953

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Diabetes, Cardiovascular Disorders and 2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin Body Burden in Czech Patients 50 Years After the Intoxication

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Abstract: The correlation between 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) intoxication and the parameters of metabolic impairment was examined in the last eight male survivors of 80 workers exposed to TCDD during the production of herbicides in a chemical factory in 1965–1967. Their median TCDD blood level was 112 (46–390) pg/g lipids, and the median TCDD body deposit was 3.9 (0.8–11.7) μg. This puts these patients into the most severely intoxicated group of subjects, according to back-calculated levels of TCDD. The median TCDD blood level in eight controls was 12 pg/g (<0.10 to 22.2 pg/g). Markers of metabolic impairment – diabetes, dyslipidaemia, arterial hypertension, carotid artery plaque, skin microvascular reactivity, eye fundus hypertensive angiopathy and history of coronary heart disease – were assessed and compared to a general male population of comparable age. Measured parameters compared with a population of comparable age were as follows: prevalence of diabetes (62.5% *versus* 17.6%), arterial hypertension (87.5% *versus* 71.8%), dyslipidaemia (87.5% *versus* 88.8%), history of coronary heart disease (62.5% *versus* 26.0%) and eye fundus hypertension angiopathy (50% *versus* 14%). All eight patients (100% *versus* 43%) developed plaques in carotid arteries, six had stenosis >50% and two had a carotid intervention (stenting or endarterectomy). Total cholesterol levels decreased compared to the earlier study this patient group in 2008, most likely due to a more intensive use of lipid-lowering drugs. Several metabolic parameters were higher (diabetes as much as 3.5-fold) in the group of severely TCDD-intoxicated subjects than in a general population of comparable age. This suggests that TCDD plays a role in the development of metabolic impairment and vascular changes.

Cardiovascular and metabolic diseases attributable to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) are associated with aryl hydrocarbon receptor (AhR) activation and subsequent induction of metabolic changes, and inflammation in blood vessels which is combined with premature cell senescence mediated by produced reactive oxygen species [1,2]. The association of environmental exposure to TCDD with increasing incidence of diabetes mellitus is a topic of interest; however, it remains unanswered [3], as do similar questions on coronary heart disease, hypertension and on dyslipidaemia [4–6].

From this point of view, the follow-up study of the severely intoxicated Czech chemical workers brings new data to bear on these questions. We examined the last eight survivors of 80 workers, who became seriously intoxicated during the production of herbicide 2,4,5-trichlorphenoxyacetic acid from the unintentional by-production of TCDD between the years 1965 and 1968.

The first TCDD blood analysis was possible in 1996 [7] when a median of 305 pg/g fat (74–760 pg/g fat) was found in these workers. The back-calculated TCDD plasma level at the time of exposure (time zero) using the physiologically based model [8] might have reached about 35,000 and 350,000 pg/g fat in patient No. 8 and No. 1, respectively [9]. This made them one of the most severely intoxicated groups of subjects in the world among herbicide producers and users in several countries, including veterans of the war spraying TCDD-contaminated Agent Orange in Vietnam and the associated affected Vietnamese population [8,10]. The half-life of TCDD during the first months after exposure is about 3 months. However, the half-life prolongs 50 years after exposure to more than 10 years, in agreement with the physiologically based model [8].

Due to the long elimination half-life, TCDD stays in the human body, bound on the lipids, for decades, and there is no antidote that would increase the elimination of this toxic agent or mitigate its toxic effect.

At the time of exposure, the mean age of the group of the male workers was 35 (20–58) years. All subjects developed chloracne during this occupation, 36% of the subjects had skin hyperpigmentation and/or hypertrichosis, and 50% had dyslipidaemia. During the first 2 years after exposure, 15% of the workers were diagnosed with type 2 diabetes [10]. The levels of cholesterol, total plasma lipids and triglycerides (TG) in the past correlated well with the TCDD levels [11,12]. Vascular dysfunction was observed in these subjects in 2004 [13]. In addition, this group of patients showed severe neurological

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and neuropsychological impairment throughout the following years [9,14,15].

Our objective was to evaluate the long-term metabolic and vascular changes in the last survivors of the TCDD intoxication and TCDD elimination half-life, as, to the best of our knowledge, no such data exist for humans 50 years after such exposure.

Methods

An examination of eight men (72.4 ± 1.3 years) in 2016 included TCDD in blood using high-resolution gas chromatography and mass spectrometry, serum cholesterol and TG, fasting blood glucose, HbA$_{1C}$, duplex sonography of carotid arteries, eye fundus examination, laser Doppler flowmetry for microvascular reactivity evaluation and clinical examination. The total body fat mass was determined using dual-energy X-ray absorptiometry (QDR 4500 A; Hologic, Inc., Waltham, MA, USA).

Hypertension was defined as systolic blood pressure of 140 mm Hg and/or diastolic blood pressure 90 mm Hg, or higher, or use of antihypertensive medication. Dyslipidaemia was defined according to total cholesterol levels (above upper reference limit of 5.2 mmol/l), TG levels (above reference limit of 1.7 mmol/l) or use of lipid-lowering drugs. Diagnosis of diabetes mellitus was based on a level of fasting plasma glucose (above 7.1 mmol/l), HbA$_{1c}$ (above 6.5%/42 mmol/mol) or use of glucose-lowering drugs.

Carotid duplex Doppler ultrasonography was performed by a linear probe 6–15 MHz on the General Electric Vivid E9 (General Electric, Boston, MA, USA) device. The presence of plaque was noted.

Skin microvascular reactivity was measured using laser Doppler flowmetry (instrument PeriFlux PF 4001 manufactured by Perimed, Järfalla, Sweden) on the finger and forearm of the non-dominant upper arm during post-occlusive reactive hyperaemia (PORH) and thermal hyperaemia (TH). Basal perfusion, maximal perfusion (PORH$_{max}$), time to reach maximal perfusion (PORH$_t$), velocity of the perfusion increase (PORH$_{max}$/t) and post-occlusion relative hyperaemia (PORH%) were measured. For TH, parameters TH$_{max}$, TH$_t$, TH$_{max}$/t and PORH% were obtained similarly as to those in the post-occlusion tests. All the data were collected using the same methodology used in the previous study [13].

The results were compared with the finding of these subjects in 2001 and 2004 [13]. The prevalence of diseases was compared with the data of the Czech male population of a comparable age [16,17] or of a non-Czech male population of comparable age [18,19].

Results

The median TCDD level was 112 (46–390) pg/g of blood lipids. For comparison, a median of 12 pg/g (<0.10 to 22.2 pg/g) TCDD was found in eight controls. The median plasma half-time prolonged from 8.5 (5.9–9.0) years 30–35 years after exposure [20], and 50 years after exposure reached 10.2 (5.5–24.1) years, which is in agreement with the physiologically based model.

The median body fat content was 31.6 (27.8–44.6) %, or 25.6 (17.8–69.7) kg, and the median TCDD body deposit was 3.9 (0.8–11.7) μg, (average 4.95 ± 3.65 μg). Individual levels and characteristics of the subjects are shown in table 1. Mean metabolic parameters are as follows: total cholesterol 4.38 ± 0.68 mmol/l, TG 1.56 ± 0.54 mmol/l, fasting glucose 6.76 ± 2.3 mmol/l and HbA1c 47 ± 13 mmol/mol.

All patients had residues of chloracne. All eight patients had atherosclerotic plaques on carotid arteries as can be seen in table 1. Six patients (No. 1, 4, 5, 6, 7 and 8) had stenosis >50% and among them, subjects No. 1 and No. 4 had a history of surgery or stenting due to significant carotid stenosis. Eye fundus examination showed stable hypertensive angiopathy in four of the subjects and physiological age-relevant vascular findings with a mild improvement since 2004 in the remaining four subjects. The patient with the highest TCCD burden (No. 1) had developed all observed characteristics (diabetes mellitus, dyslipidaemia, hypertension, coronary heart disease, carotid artery stenting) during his life.

For microvascular reactivity, a mean basal perfusion showed increase in comparison with the results from 2004 on the forearm (10.8 ± 4.9 versus 7.1 ± 2.4 perfusion units (PU), $p < 0.05$), while a decrease in basal perfusion was observed on the finger (132 ± 45 versus 257 ± 110 PU, $p < 0.05$). Improvement in microvascular reactivity on the forearm was observed both in PORH and in TH (PORH$_{max}$: 42 ± 24 versus 26 ± 7 PU, $p < 0.02$; PORH time-to-max 6 ± 4 versus 13 ± 4 sec., $p < 0.02$; TH$_{max}$ 80 ± 32 versus 59 ± 18 PU, $p < 0.02$, TH time-to-max 76 ± 24 versus 89 ± 20 sec., NS). Microvascular reactivity did not improve on the finger. There was no improvement in parameters of microvascular reactivity when the results were compared with the findings in 2001.

Discussion

Recent TCDD level classifies this group in those with the highest exposure – patient No. 1 is comparable with individuals with the highest ever documented levels of TCDD as measured a few months after exposure [21,22] and shows multiple signs of metabolic impairment, more severe than patients with lower TCDD levels.

The prevalence of diabetes in these chronically TCDD-exposed patients is more than 3.5-fold higher compared to the male population of a comparable age [15]. Also, the prevalence of hypertension and hyperlipidaemia exceeds general male population values, as shown in table 1. Atherosclerosis was highly prevalent, based on carotid ultrasonography and on a substantial history of cardiovascular disease.

In 2004, four patients were diagnosed with diabetes and two of them were treated with glucose-lowering drugs. By 2016, already five patients used glucose-lowering drugs. The higher proportion of diabetes as compared with the population is also in accordance with experimental studies [23–25]. Activators of peroxisome proliferator-activated receptor alpha (PPAR-α), a key regulator of systemic insulin sensitivity, delay the onset of type 2 diabetes by lowering plasma TG. The inhibition of PPAR-α through the Ah receptor could explain TCDD-mediated diabetes [26]. Dioxin has been recognized as an environmental endocrine disruptor. A study in Taiwan that evaluated the association between exposure to dioxin and diabetes in TCDD-contaminated areas found exposure to TCDD is a risk factor for diabetes, independent of age and body mass index (BMI) [27]. Also, cross-sectional studies of populations with low-level TCDD exposures (serum

Table 1.

Characteristics of the patients.

Subject no.	Age (years)	Smoker	TCDD level, pg/g fat	TCDD body depot, µg	BMI, kg/m²	Diabetes	Atherosclerotic plaques on carotid arteries	Atherosclerosis (CHD, AMI, IS)	Hypertension	Dyslipidaemia	Eye hypertensive angiopathy
1	72	Yes	390	11.71	29.7	Yes	Yes	CHD 2010 AMI 2011	Yes	Yes	No
2	73	No	320	7.11	25.6	No	Yes	No	Yes	Yes	Yes
3	77	No	300	7.71	29.4	No	Yes	CHD 1995	Yes	Yes	Yes
4	73	Yes	140	3.15	27.0	Yes	Yes	CHD 1997 AMI 1997 IS 2007	Yes	Yes	No
5	72	Yes	84	2.13	28.7	Yes	Yes	No	Yes	Yes	Yes
6	73	No	83	2.32	28.4	Yes	Yes	CHD 1995 AMI 1999	Yes	Yes	No
7	73	No	66	4.60	44.1	Yes	Yes	CHD 1975 IS 1991 IS 1993	Yes	Yes	Yes
8	77	No	46	0.82	23.9	No	Yes	No	No	No	No
Mean/ median/%	72.4 ± 1.3	37.5%	180 ± 110 (112 (46–390))	4.95 ± 3.65 (3.9 (0.8–11.7))	28.2 ± 5.2	62.5%	100%	CHD 62.5%	87.5%	87.5%	50%
Prevalence in comparable male population of similar age						17.6% [17]	43% [19]	CHD 26% [18]	71.8% [17]	88.8% [17]	14% [20]

AMI, acute myocardial infarction; BMI, body mass index; CHD, coronary heart disease; IS, ischaemic stroke.

concentrations <10 pg/g lipid) found positive dose–response. Heterogeneous results were seen in studies of subjects with high TCDD body burdens [3]; however, our study of very high exposure supports the association.

Oxidative stress is the crucial pathogenic mechanism inducing impairment of vascular function, which can be accelerated by dyslipidaemia and both oxidative stress and dyslipidaemia. Elevated markers of oxidative stress have been found in these TCDD-exposed patients [12].

We found a rather unexpected improvement in the microvascular reactivity as compared with the findings in 2004. This may be explained by a more efficient hypolipidaemic treatment in these patients and their lower cholesterol level. This lowering can be observed in the whole group already from 1991. At that time, the mean total cholesterol and TG levels reached 7.7 and 3.7 mmol/l, respectively [28], which follows the total trend in the general population [16]. Another positive finding is that the last clinically relevant coronary heart disease, acute myocardial infarction and/or ischaemic stroke were diagnosed in 2011. These positive results may also be associated with about 50% lowering of TCDD levels.

Obviously, a limitation of this study is the low number of highly exposed survivors of TCDD intoxication, which complicates the generalization of the findings and the comparison with the general population. The advantage, on the other side, is the long-term follow-up of highly exposed subjects using the same methods.

Conclusion

The results in the last eight surviving men who have been severely intoxicated with TCDD for 50 years suggest that TCDD may promote metabolic impairment. The proportion of diabetes, hypertension, carotid plaques, eye fundus angiopathy and coronary heart disease in this group of patients is higher than in the general population. These data support the associations between this persistent organic pollutant and diabetes [6].

On the other hand, cholesterol and TG levels further decreased due to more efficient pharmacological treatment. Dyslipidaemia treatment may therefore have a beneficial effect both on the plasma lipid profile and on the vascular activity, even if there is no causal treatment and increased elimination of TCDD from the human body.

Conflict of Interests

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the manuscript.

References

1 Hara F, Tatebe J, Watanabe I, Yamazaki J, Ikeda T, Morita T. Molecular hydrogen alleviates cellular senescence in endothelial cells. Circ J 2016;**80**:2037–46.

2 Mulero-Navarro S, Fernandez-Salguero PM. New trends in aryl hydrocarbon receptor biology. Front Cell Dev Biol 2016;**4**:45.

3 Goodman M, Narayan KM, Flanders D, Chang ET, Adami HO, Boffetta P *et al.* Dose–response relationship between serum 2,3,7,8-tetrachlorodibenzo-p-dioxin and diabetes mellitus: a meta-analysis. Am J Epidemiol 2015;**181**:374–84.

4 Sergeev AV, Carpenter DO. Increased hospitalizations for ischemic stroke with comorbid diabetes and residential proximity to sources of organic pollutants: a 12-year population-based study. Neuroepidemiology 2010;**35**:196–201.

5 Sergeev AV, Carpenter DO. Residential proximity to environmental sources of persistent organic pollutants and first-time hospitalizations for myocardial infarction with comorbid diabetes mellitus: a 12-year population-based study. Int J Occup Med Environ Health 2010;**23**:5–13.

6 Ruiz P, Perlina A, Mumtaz M, Fowler BA. A systems biology approach reveals converging molecular mechanisms that link different POPs to common metabolic diseases. Environ Health Perspect 2016;**124**:1034–41.

7 Pelclova D, Fenclova Z, Dlaskova Z, Urban P, Lukas E, Prochazka B *et al.* Biochemical, neuropsychological, and neurological abnormalities following 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) exposure. Arch Environ Health 2001;**56**:493–500.

8 Emond C, Michalek JE, Birnbaum LS, DeVito MJ. Comparison of the use of a physiologically based pharmacokinetic model and a classical pharmacokinetic model for dioxin exposure assessments. Environ Health Perspect 2005;**113**:1666–8.

9 Pelclova D, Urban P, Fenclova Z, Vlckova S, Ridzon P, Kupka K *et al.* Neurological and neurophysiological findings in the workers with chronic 2,3,7,8-TCDD intoxication 50 years after exposure. Basic Clin Pharmacol Toxicol 2018;**122**:271–7.

10 Manh HD, Kido T, Okamoto R, Xianliang S, le Anh T, Supratman S *et al.* Serum dioxin levels in Vietnamese men more than 40 years after herbicide spraying. Environ Sci Technol 2014;**48**:3496–503.

11 Pelclova D, Fenclova Z, Preiss J, Prochazka B, Spacil J, Dubska Z *et al.* Lipid metabolism and neuropsychological follow-up study of workers exposed to 2,3,7,8- tetrachlordibenzo- p-dioxin. Int Arch Occup Environ Health 2002;**75**:60–6.

12 Pelclova D, Navratil T, Fenclova Z, Vlckova S, Kupka K, Urban P *et al.* Increased oxidative/nitrosative stress markers measured non-invasively in patients with high 2,3,7,8-tetrachloro-dibenzo-p-dioxin plasma level. Neuro Endocrinol Lett 2011;**32**:71–6.

13 Pelclova D, Prazny M, Skrha J, Fenclova Z, Kalousova M, Urban P *et al.* 2,3,7,8-TCDD exposure, endothelial dysfunction and impaired microvascular reactivity. Hum Exp Toxicol 2007;**26**:705–13.

14 Urban P, Pelclova D, Lukas E, Kupka K, Preiss J, Fenclová Z *et al.* Neurological and neurophysiological examinations on workers with chronic poisoning by 2,3,7,8-TCDD: follow-up 35 years after exposure. Eur J Neurol 2007;**14**:213–8.

15 Pelclova D, Fenclova Z, Urban P, Ridzon P, Preiss J, Kupka K *et al.* Chronic health impairment due to 2,3,7,8-tetrachloro-dibenzo-p-dioxin exposure. Neuro Endocrinol Lett 2009;**30**:219–24.

16 Cifkova R, Bruthans J, Adámkova V, Jozifova M, Galovcova M, Wohlfart P *et al.* The prevalence of major cardiovascular risk factors in the Czech population in 2006–2009. The Czech post-MONICA study. Cor Vasa 2011;**53**:220–9.

17 Ischaemic heart diseases – morbidity and mortality in the Czech Republic in 2003–2010, actualization UZIS. www.uzis.cz/system/files/24_12.pdf (accessed on 30.11.2017).

18 Josse MO, Touboul PJ, Mas JL, Laplane D, Bousser MG. Prevalence of asymptomatic internal carotid artery stenosis. Neuroepidemiology 1987;**6**:150–2.

19 Wong T, Mitchell P. The eye in hypertension. Lancet 2007;**369**:425–35.

20 Pelclova D, Urban P, Preiss J, Lukas E, Fenclova Z, Navrátil T *et al.* Adverse health effects in humans exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). Rev Environ Health 2006;**21**:119–38.

21 Geusau A, Abraham K, Geissler K, Sator MO, Stingl G, Tschachler E. Severe 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) intoxication: clinical and laboratory effects. Environ Health Perspect 2001;**109**:865–9.

22 Sorg O, Zennegg M, Schmid P, Fedosyuk R, Valikhnovskyi R, Gaide O *et al.* 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) poisoning in Victor Yushchenko: identification and measurement of TCDD metabolites. Lancet 2009;**374**:1179–85.

23 Reichard JF, Dalton TP, Shertzer HG, Puga A. Induction of oxidative stress responses by dioxin and other ligands of the aryl hydrocarbon receptor. Dose Response 2006;**3**:306–31.

24 Wu D, Nishimura N, Kuo V, Fiehn O, Shahbaz S, Van Winkle L *et al.* Activation of aryl hydrocarbon receptor induces vascular inflammation and promotes atherosclerosis in apolipoprotein E−/− mice. Arterioscler Thromb Vasc Biol 2011;**31**:1260–7.

25 Fracchiolla NS, Todoerti K, Bertazzi PA, Servida F, Corradini P, Carniti C *et al.* Dioxin exposure of human CD34+ hemopoetic cells induced gene expression modulation that recapitulates its in vivo clinical and biological effects. Toxicology 2011;**283**:18–23.

26 Remillard RB, Bunce NJ. Linking dioxins to diabetes: epidemiology and biologic plausibility. Environ Health Perspect 2002;**110**:853–8.

27 Huang CY, Wu CL, Yang YC, Chang JW, Kuo YC, Cheng YY *et al.* Association between dioxin and diabetes mellitus in an endemic area of exposure in Taiwan: a population-based study. Medicine (Baltimore) 2015;**94**:1730.

28 Pazderova-Vejlupkova J, Lukas E, Nemcova M, Pickova J, Jirasek L. The development and prognosis of chronic intoxication by tetrachlordibenzo-p-dioxin in men. Arch Environ Health 1981;**36**:5–11.

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ORIGINAL PAPER

CrossMark

Exhaled breath condensate biomarkers reflect systemic changes in patients with chronic dioxin intoxication

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is highly toxic and affects the cardiovascular system, brain, and skin by AhR-dependent and other mechanisms, as well as causing metabolic impairments and cancer. The involvement of the respiratory system has not yet been studied. TCDD in the blood was measured and biomarkers of oxidative stress and inflammation were analysed in 2016 in the exhaled breath condensate (EBC) of the last eight male survivors (mean age 72.4 ± 1.3 years) from 80 workers intoxicated with TCDD during the production of herbicides from 1965 to 1968. The results were compared with their findings in 2010 to evaluate a trend. Malondialdehyde, 4-hydroxy-*trans*-nonenale, and 8-isoprostaglandin in F2α (8-isoprostane), in addition to markers of the oxidation of nucleic acids and proteins 8-hydroxy-2-deoxyguanosine, 8-hydroxyguanosine, 5-(hydroxymethyl)uracil, *o*-tyrosine, and 3-nitrotyrosine, as well as markers of inflammation leukotrienes and anti-inflammatory lipoxins, were analysed in EBC by liquid chromatography–electrospray ionisation–tandem mass spectrometry. In addition, the patients underwent chest X-ray, spirometry and fractional exhaled nitric oxide (FeNO) examinations. The control group included 7 men (66 ± 16 years) with comparable lifestyle factors. The median plasma TCDD level lowered from 155 (28–553) ng/kg fat in 2010 to 112 (46–390) ng/kg fat in 2016, i.e., 50 years after exposure. The mean TCDD body deposit was 5.0 ± 3.7 µg. Serum TCDD level in the pooled sample of the controls was 12 ng/kg fat. All markers of oxidative stress, LTB4 and LTC4, remained overexpressed in patients and anti-inflammatory lipoxins were under-expressed compared to controls (all $p < 0.01$). The mean FeNO and spirometry results were within the reference values. Borderline X-ray findings and combined lung function impairments were seen in the patients with the lower TCDD plasma levels. Differences in the expression of the biomolecular markers in EBC as compared to controls were not associated with lung impairments and the respiratory parameters measured. Therefore, these EBC markers can be used to evaluate systemic oxidative stress and inflammation in tissues and the endovascular, atherosclerotic, neurotoxic, and metabolic effects of TCDD.

Graphical abstract
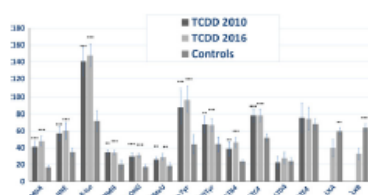
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Introduction

Chlorinated dibenzo-*p*-dioxins (CDDs) are a family of 75 compounds commonly referred to as chlorinated dioxins, with varying harmful effects [1–3]. Among them, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD) is one of the most toxic CDDs to mammals. It damages the cardiovascular system, brain, liver, and skin, induces metabolic impairments [4, 5], and has been classified as a human carcinogen [6, 7]. The elimination half-life of TCDD is about 10 years five decades after exposure [8]. This very long persistence in the plasma provides an opportunity to study both the chronic health impact and the mechanism of action even 50 years later.

The group of Czech chemical workers belongs to the most severely exposed groups of subjects among German, Austrian, and USA chemical workers, US Ranch Hand veterans spraying Agent Orange, populations in Vietnam and populations exposed during an industrial accident in Seveso in 1976 [9–14]. 55 Czech chemical workers got ill during pesticide production in 1965–1968; however, the TCDD concentration in the blood could only be measured for the first time in 1996, i.e., about 30 years after exposure, in 13 remaining subjects. The median level of 210 (14–760) ng/kg fat detected confirmed a very high level of exposure [8].

A correlation was found for hyperlipidaemia, atherosclerosis, hypertension, and diabetes with the level of TCDD in the plasma fat in the patients [15, 16]. Signs of central nervous system damage, including neuropsychological impairment, and single-photon-emission computed tomography scans of the brain correlated with TCDD levels [17, 18]; 50 years after intoxication, these findings were present in 100% of individuals examined [13].

Several diseases attributable to TCDD might be associated with macrophage activation through aryl hydrocarbon receptor activation in blood vessels and with premature cell senescence mediated by the production of reactive oxygen species; however, other, not fully defined mechanisms may play a significant role [19]. These effects can be studied using several biomarkers, especially unsaturated fatty acids derivatives, arising from arachidonic acid. Leukotrienes (LT) are synthesised by leukocytes through the enzymatic catalysis of 5-lipoxygenase and released from the cell wall by phospholipase A_2. LTB4 is a potent inducer of inflammation due to the activation of leukocytes and is increased in patients with chronic airway inflammation, as well as in patients after cardiothoracic surgery. Cysteinyl LTs (LTC4, LTD4, and LTE4) contract airway smooth muscles and increase vascular permeability [20–22].

8-Isoprostaglandin F2α (8-isoprostane) is produced by free-radical lipid peroxidation of arachidonic acid and represents an in vivo specific biomarker of oxidative stress. Oxidative modification of lipids occurs during aging and in certain disease conditions. Lipid peroxides are unstable indicators of oxidative stress in cells that transform into more complex and reactive compounds such as malondialdehyde (MDA) and 4-hydroxy-*trans*-nonenale (HNE) which can form covalent adducts with biomolecules including DNA and proteins, and are, therefore, regarded as genotoxic and cytotoxic [23]. These markers have been found in the exhaled breath condensate (EBC) of patients with pneumoconiosis caused by carcinogenic asbestos and silica [24, 25].

3-Nitrotyrosine (3-NOTyr) is a stable product of peroxynitrite ($ONOOO^-$), with tyrosine residues of proteins, which may lead to a functional relationship with neutrophilic inflammation. Both 3-NOTyr and *o*-tyrosine (*o*-Tyr) have been found in patients with interstitial lung diseases, but other studies are limited. The predominant form of free-radical-induced lesions used as a biomarker for oxidative stress and carcinogenesis is 8-hydroxy-2-deoxyguanosine (8-OHdG) and 5-(hydroxymethyl)uracil (5-OHMeU) in DNA and 8-hydroxyguanosine (8-OHG) in RNA. Chronic elevation of these markers in blood was associated with systemic inflammation [23].

Lipoxin (LX) A4 and LXB4 are also arachidonic acid-derived eicosanoids; however, their activity is anti-inflammatory and neuroprotective [26]. They belong to the group of pro-resolving chemical mediators that enact resolution programs in response to injury, infection or allergy. They turn off acute inflammatory responses and restore tissue homeostasis [27].

In 2010, we analysed the EBC of TCDD-intoxicated patients, and 10 out of 12 measured markers of oxidative stress and inflammation were elevated: MDA, HNE, 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, *o*-Tyr, 3-NOTyr, LTC4, and LTD4 [28]. In the last follow-up in 2016, we repeated the collection of EBC samples to analyse the trend in the level of these markers and increased the spectrum of markers to include LXA and LXB. In addition, we focused on the respiratory tract and examined chest X-rays, spirometry results, and fractional exhaled nitric oxide (FeNO) levels to determine whether the lungs are affected in these severely intoxicated patients or if the increased expression of biomarkers can be explained by their systemic disorders. To the best of our knowledge, the

information concerning respiratory findings in TCDD intoxication is not currently available.

Results and discussion

TCDD, chest X-ray, FeNO, and pulmonary functions

The mean TCDD blood level of the patients decreased from 208 ± 178 ng/kg fat in 2010. The characteristics and findings in the patients are given in Table 1 in descending order of TCDD blood level. The median body fat mass of the patients was 25.6 kg (17.8–69.7 kg) and their TCDD body deposit decreased from mean 5.4 ± 5.0 to 5.0 ± 3.7 μg.

TCDD levels from all years, when they were available, are shown in Table 2. The median TCDD plasma level dropped from 155 (28–553) ng/kg fat in 2010 to 112 (46–390) ng/kg fat and the median TCDD half-life 50 years after exposure reached 10.2 (5.5–24.1) years. The declining level of TCDD is clearly seen, in spite of some missing data and body weight changes during the years (mostly body weight increase resulting in dilution of TCDD concentration in fat), similar to the findings of Neuberger [14]. Serum TCDD level in the pooled sample of the seven male controls was 12 ng/kg fat, and toxic equivalency quotient (TEQ) was 20.5 ng/kg. All other congeners were under detection limit of 8 ng/kg fat.

Individual dioxin-like congeners and their median World Health Organization Toxic Equivalency Factor (TEF) (WHO-TEF 2005 [29] in the patients and three wives of the patients, living in the distance 1.5–10 km from the factory are shown in Table 3. Median TEQ in the

patients in 2016 was 146 (74–418) ng/kg fat. Rather surprisingly, the levels of two congeners in the wives (OCDD and 2,3,7,8-PeCDF) were higher than in the control, in addition, OCDD was higher than in the group of patients. Three further congeners were elevated in the patients, as can be seen.

2,3,4,7,8-PeCDF with TEF 0.3 may be the most important among the congeners found; its median level dropped from 56 (19–63) ng/kg lipids in 1996 to about 50%. However, during this period, it increased 1.5–2-fold in patient No. 1 and patient No. 4. Surprisingly, 2,3,7,8-TCDF was ninefold higher comparing with median 3.7 (1.1–4.3) ng/kg in 1996. Eventually, 1,2,3,6,7,8-HxCDF in patient No. 1 increased 11-fold from 1996. Therefore, other sources, such as food with a higher fat content may have contributed. Because of a low level of these congeners in the wives of the patients, local contamination with these congeners due to living in the proximity of a chemical plant does not seem to be the cause [30].

Patients 5 and 6 declared chronic bronchitis symptoms, i.e., the presence of a chronic productive cough for 3 months during each of 2 consecutive years. 50% of patients showed a normal chest X-ray pattern. Borderline X-ray findings, described as interstitial pattern, were seen in patients 4, 5, 7, and 8, as shown in Table 1. Body plethysmography found obstructive ventilatory impairment of mild grade in patients 1, 4, 5, and 6; patient 7 had medium and patient 8 mild combined obstruction and restriction.

Two patients had borderline FeNO values, while the results in the remaining patients were within the normal range. The average FeNO in the patients (18.1 ± 7.8 μg/kg) corresponded to the median FeNO of healthy male

Table 1 Characteristics of the patients and their X-ray and spirometry findings

Subject no.	Age	TCDD level/ng/kg fat	Smoking	Pack-years	Chest X-ray	FEV1 %p	FVC %p	FEV1/FVC	TLC %p	RV % TLC
1	71	390	Smoker	13	N	**59.3**	55.9	0.81	96.2	152
2	72	320	Ex-smoker	1	N	116	119	0.74	134	98
3	73	300	No	0	N	104.3	107.9	0.74	121.5	107
4	72	140	Smoker	45	B	**76**	62.9	0.93	102.1	130
5	71	84	Smoker	36	B	**66.4**	77.3	**0.67**	107	143
6	72	83	Ex-smoker	28	N	**75.2**	74.1	0.77	91	104
7	71	66	Ex-smoker	35	B	**61.7**	56.5	0.83	**58.6**	135
8	76	46	Ex-smoker	22	B	**75.8**	63.5	0.90	**73.6**	108
Mean/ %	72.4 ± 1.3	180 ± 110	38% Smokers	23 ± 14	50%	79 ± 17	77 ± 20	0.80 ± 0.07	98 ± 20	122 ± 17

FEV1/FVC ratio was considered low if it was less than 0.70, the other parameters under 80% of the predicted values (lower levels are in bold)
TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin; N, normal; B, borderline interstitial pattern; FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; TLC, total lung capacity; RV, residual volume; p, predicted

Table 2 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) in the serum of the patients from 1996 to 2016

TCDD plasma level ng/kg fat	1996	2001/2003	2008	2010	2016
Patient 1	760	517	756	553	390
Patient 2	600	401	415	221	300
Patient 3	400	264	364	380	320
Patient 4	230	NA	272	182	140
Patient 5	NA	128	179	28	66
Patient 6	220	NA	137	128	83
Patient 7	NA	123	264	129	84
Patient 8	74	NA	236	45	46
Mean	381	287	328	208	179
SD	259	172	195	178	136
Median	315	264	268	155.5	112

NA not available

Table 3 Plasma concentration (ng/kg) of polychlorobenzodioxins (–CDD) and polychlorodibenzofurans (–CDF) in the group of patients in 2016

Patient	Congener	TEF WHO 2005	Patients ($N = 8$)			Wives (of patient 1, 2, 4)		
			Median	Mean	Maximum	Median	Mean	Maximum
1	2,3,7,8-TCDD	**1**	**112**	**180**	**390**	< 8	**6**	**22**
2	1,2,3,7,8-PeCDD	**1**	< 30	< 30	< 30	< 8	< 8	< 8
3	1,2,3,4,7,8-HxCDD	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
4	1,2,3,6,7,8,9-HxCDD	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
5	1,2,3,7,8,9-HxCDD	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
6	1,2,3,4,6,7,8-HpCDD	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
7	OCDD	**0.0003**	< 30	< 30	< 30	**99**	**89**	**134**
8	2,3,7,8-TCDF	**0.1**	**33**↑	**42**	**150**	< 8	< 8	< 8
9	1,2,3,7,8-PeCDF	**0.03**	< 30	**16**	**130**	< 8	< 8	< 8
10	2,3,4,7,8-PeCDF	**0.3**	**25**	**49**	**160**	**12**	**13**	**18**
11	1,2,3,4,7,8-HxCDF	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
12	1,2,3,6,7,8-HxCDF	**0.1**	< 30	**5**	**42**↑	< 8	< 8	< 8
13	1,2,3,7,8,9-HxCDF	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
14	2,3,4,6,7,8-HxCDF	**0.1**	< 30	< 30	< 30	< 8	< 8	< 8
15	1,2,3,4,6,7,8-HpCDF	**0.01**	< 30	< 30	< 30	< 8	< 8	< 8
16	1,2,3,4,7,8,9-HpCDF	**0.01**	< 30	< 30	< 30	< 8	< 8	< 8
17	OCDF	**0.0003**	< 30	< 30	< 30	< 8	< 8	< 8

< 8/< 30 = under detection limit = under detection limit". Patient 2 lives at the 1.5 km distance from the factory, patient 1 at 2 km, patient 4 at 10 km distance. ↑ elevation since 1996

subjects [31]. No correlation of spirometry parameters with plasma TCDD was seen.

Markers of oxidative stress and inflammation

All markers of oxidation of lipids, proteins and nucleic acids in EBC were overexpressed in the patients compared to healthy control males, as shown in Fig. 1.

Among the inflammatory markers, LTB4 and LTC4 remained overexpressed in the EBC of patients and anti-inflammatory LXA4 ($p < 0.003$) and LXB4 ($p < 0.001$) were under-expressed. LXA4 correlated with LXB4 ($p < 0.001$). No trend was seen in the levels and no significant elevation in 2016 compared to 2010 was seen. No correlation of EBC markers with the impairment in lung function parameters was seen. Maximal α-amylase activity in all samples did not exceed 0.1% of the saliva activity. No significant differences in conductivity or EBC pH were found. No correlation of EBC markers with TCDD level or TCDD body burden in the same year was seen.
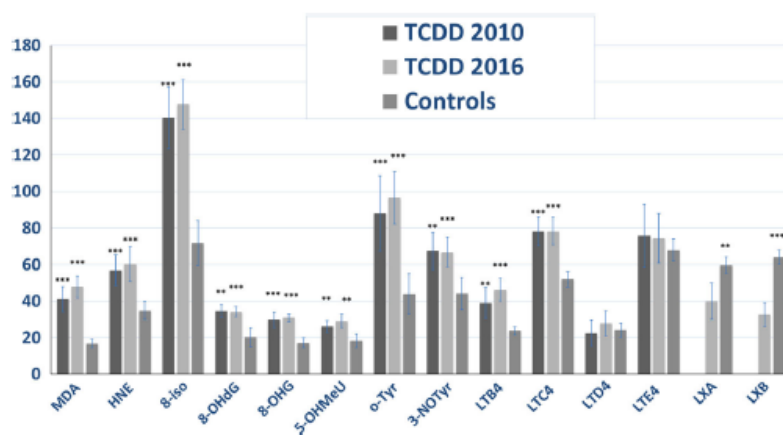
Fig. 1 Markers of oxidative stress and inflammation in 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)-exposed patients (in 2010 and 2016) and controls (2010) in exhaled breath condensate. MDA, malondialdehyde; HNE, 4-hydroxy-*trans*-nonenale; 8-iso, 8-iso-prostaglandin F2α; 8-OHdG, 8-hydroxy-2-deoxyguanosine; 8-OHG, 8-hydroxyguanosine; 5-OHMeU, 5-(hydroxymethyl)uracil; *o*-Tyr, *o*-tyrosine; 3-NOTyr, 3-nitrotyrosine; LT, leukotriene; LX, lipoxin. MDA and HNE are expressed in pg/m^3, all other markers in ng/m^3. The symbols denote the significance levels of data equivalency gained from the workers and controls. ***$p < 0.001$, **$p < 0.01$. The bars denote the confidence levels ($p = 0.05$)

The elevated biomolecular markers of oxidative stress and inflammation in EBC are commonly related to respiratory disorders, as pro-inflammatory LTs, 8-isoprostane, and other markers of oxidative stress are important in the pathophysiology of many lung diseases. The high proportion of elevated EBC markers in our TCDD-intoxicated group of patients in 2010 inspired us to consider a potential association with respiratory disorders [27].

The results of the examinations of the respiratory system in eight patients with the severe chronic TCDD intoxication did not display any pathological findings related to TCDD. The impairments in the chest X-ray and lung functions were more pronounced in those patients with a lower TCDD level and could possibly be attributed to smoking. No correlation of these findings with TCDD body burden was found.

Obviously, the origin of EBC markers is not limited to the lungs [32]. As has already been mentioned, most of our patients have hyperlipidaemia, atherosclerosis, hypertension, and diabetes [18, 33]. It has been shown that induction of oxidative stress plays a key role in cardiovascular and neurotoxic damage, aging and cancer development, and elevated markers can be found in the blood [34–36]. It was also shown that the level of EBC markers can be reduced by blood purification using extracorporal methods [37].

Therefore, our results do not point to individual organs. Arachidonic acid, the precursor of eicosanoids, is stored within membrane phospholipids of numerous cells, such as granulocytes, macrophages, eosinophils, differentiated T cells, dendritic cells, and osteoclasts. Isoprostanes in various body fluids are elevated by conditions related to oxidative stress, which may accelerate the development of vascular disease. 8-Isoprostane and HNE were associated with hyperlipidaemia and cerebral microbleeds. Reactive oxygen species-based biomarkers appear excellent integrators for total cardiovascular risk [38]. Peripheral blood lipid peroxidation markers may also indicate small vessel disease in the brain [39] and impaired brain perfusion which was documented in these TCDD chronically exposed patients [13, 17].

Likewise, LTA4 hydrolase, metabolising LTA4–LTB4, is ubiquitously expressed, which can explain the production of LTB4 in many tissues at the site of inflammation, which is called the "transcellular biosynthesis of LTs" [40, 41]. This involves various inflammatory diseases, cardiac dysfunction, and cardiac apoptosis [42, 43] in addition to endoluminal gastrointestinal diseases, oesophagogastric cancer, colorectal cancer, and inflammatory bowel disease [44].

EBC analysis confirmed the prominent level of oxidative and inflammation markers, which had already been detected in 2010; no significant differences from the 2010 results were seen. This supports the use of EBC analysis in the evaluation of toxic environmental exposures. In disorders where oxidative stress disturbs several organ systems, and especially when the toxic agent is highly elevated in the blood, markers from lipids, proteins, and nucleic acids can be found in EBC and reflect systemic effects. This appears to be the case in TCDD intoxication and agrees with the experimental data [45]. The mechanism of TCDD-induced damage results in changes in the expression of

genes of a broad spectrum of drug-metabolising enzymes which play a key role in TCDD toxicity and has close links to cardiovascular, immune, metabolic diseases and other systemic effects [5, 46].

The main limitation is the low number of patients examined in this study; unfortunately, due to the 50-year delay, no other patients are available for follow-up. The results are non-specific and do not point to specific organs being affected. The strength, on the other hand, is the availability of actual TCDD plasma levels, enabling the results to be compared with the exposure level.

Conclusion

Markers of oxidative stress and inflammation are stable in the EBC of patients with severe chronic TCDD intoxication and reflect systemic damage due to this toxic agent. They appear useful for the monitoring of systemic intoxication, including atherosclerosis, vascular, metabolic, and neurotoxic impairments. TCDD does not importantly affect the lungs and the elevated markers of oxidative stress and inflammation in eight patients with severe chronic TCDD intoxication were not associated with pathological X-ray findings, lung function impairments or FeNO elevation.

Methods

Follow-up examination of eight men (72.4 ± 1.3 years) included the analysis of TCDD in the blood using high-resolution gas chromatography and mass spectrometry after clean-up of the silica and carbon columns, as previously described [13]. The patients answered questions on their symptoms and medication, in addition to their family, personal and occupational history. Then, they underwent physical examination, chest X-ray, EBC collection, FeNO analysis, and body plethysmography. TCDD content in the body fat was calculated from their total body fat mass measured by dual-energy X-ray absorptiometry and multiplied by TCDD concentration in the fat, as previously described [33].

The control group included 7 men (66.0 ± 16 years), five were smokers (71.4%), and two non-smokers. They had physical examination, blood, and EBC collection. In addition, blood of three wives of the patients was analysed for TCDD and other dioxin-like contaminants to eliminate a possible local effect of the living location in the 1.5–10 km distance from the factory, from which, however, the old TCDD-contaminated herbicides residues have been removed more than a decade ago. All subjects gave written consent for the examination according to the Helsinki Declaration. The results in 2016 were compared with their findings during the previous examinations in 2010 [28].

EBC samples were collected using the Ecoscreen Turbo (DECCS, Jaeger, Germany). All subjects breathed tidally for about 15 min through a mouthpiece connected to a condenser (− 20 °C) while wearing a nose clip. A minimum volume of exhaled air of 120 dm³ was maintained using the EcoVent device by Jaeger (Germany), and time of collection was 15 min. All samples were immediately frozen and stored at − 80 °C [37, 47]. MDA, HNE, 8-isoprostane, 8-OHdG, 8-OHG, 5-OHMeU, o-Tyr, and 3-NOTyr were measured in the EBC of the subjects [48]. Furthermore, LTs LTB4, LTC4, LTD4, and LTE4 were detected using a pre-treatment step, solid-phase extraction and a detection method using liquid chromatography–electrospray ionisation–tandem mass spectrometry (LC–ESI–MS/MS), consisting of a quaternary pump, Accela 600, and an Accela autosampler linked with a triple quadrupole mass spectrometer TSQ Vantage equipped with heated electrospray ionisation (Thermo Fisher Scientific), as previously described [49, 50]. To exclude contamination of EBC by saliva, α-amylase concentration was determined [47]. The measurement of acidity of EBC (pH value) was done by standardized pH-meter S220, Mettler Toledo (Switzerland). The conductivity was measured directly by CyberScan CON 11, Cond/TDS Meter, Moris Technology (USA) as a reference indicator in the EBC dilution [51].

Pulmonary function testing was performed by body plethysmography, Jaeger, Germany. The measurements were carried out according to the standard protocols of the American Thoracic Society and European Respiratory Society Guidelines [52] and the results were expressed as a % of the predictive value. The best of three consecutive measurements was chosen. The measurement included forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC), total lung capacity (TLC), and residual volume (RV). FEV1 and FVC were used to calculate the FEV1/FVC ratio. The parameters were considered low if they were less than 80% predicted and if the FEV1/FVC ratio was less than 0.70.

Prior to lung function examinations, fractional exhaled nitric oxide (FeNO) was measured by a portable Hypair FeNO analyser (Medisoft, Belgium). According to ATS/ERS recommendations, values above 25 µg/kg were evaluated as borderline, and above 50 µg/kg as highly probably indicating eosinophilic inflammation [53, 54].

Basic descriptive statistics (mean, median, confidence interval, standard deviation, skewness, and kurtosis) were computed for all variables, which were subsequently tested for normality using the Kolmogorov–Smirnov test. The independent-groups t test was used for workers versus controls. The bivariate relationship between variables under study was assessed using the correlation coefficient.

Statistical significance was set at $p < 0.05$. All analyses were conducted using QCExpert (Trilobyte, Czech Republic) and using MS Excel (Microsoft CZ, Czech Republic).

References

1. Cerna M, Kratenova J, Zejglicova K, Brabec M, Maly M, Smid J, Crhova S, Grabic R, Volf J (2007) Chemosphere 67:S238
2. Iszatt N, Stigum H, Govarts E, Murinova LP, Schoeters G, Trnovec T, Legler J, Thomsen C, Koppen G, Eggesbo M (2016) Environ Int 94:399
3. Trnovec T, Jusko TA, Sovcikova E, Lancz K, Chovancova J, Patayova H, Palkovicova L, Drobna B, Langer P, Van den Berg M, Dedik L, Wimmerova S (2013) Environ Health Perspect 121:886
4. Saurat JH, Kaya G, Saxer-Sekulic N, Pardo B, Becker M, Fontao L, Mottu F, Carraux P, Pham XC, Barde C, Fontao F, Zennegg M, Schmid P, Schaad O, Descombes P, Sorg O (2012) Toxicol Sci 125:310
5. Hwang HJ, Dornbos P, Steidemann M, Dunivin TK, Rizzo M, LaPres JJ (2016) Toxicol Appl Pharmacol 304:121
6. IARC (1997) Polychlorinated dibenzo-para-dioxins and poly-chlorinated dibenzofurans. IARC Monogr Eval Carcinog Risks Hum 69:227
7. Dziubanek G, Marchwinska E, Hajok I, Piekut A (2016) Cent Eur J Public Health 24:115
8. Pelclova D, Fenclova Z, Dlaskova Z, Urban P, Lukas E, Prochazka B, Rappe C, Preiss J, Kocan A, Vejlupkova J (2001) Arch Environ Health 56:493
9. Landi MT, Bertazzi PA, Baccarelli A, Consonni D, Masten S, Lucier G, Mocarelli P, Needham L, Caporaso N, Grassman J (2003) Carcinogenesis 24:673
10. Pelclova D, Urban P, Preiss J, Lukas E, Fenclova Z, Navratil T, Dubska Z, Senholdova Z (2006) Rev Environ Health 21:119
11. Manh HD, Kido T, Okamoto R, Sun XL, Anh LT, Supratman S, Maruzeni S, Nishijo M, Nakagawa H, Honma S, Nakano T, Takasuga T, Nhu DD, Hung NN, Son LK (2014) Environ Sci Technol 48:3496
12. Jeanneret F, Boccard J, Badoud F, Sorg O, Tonoli D, Pelclova D, Vlckova S, Rutledge DN, Samer CF, Hochstrasser D, Saurat JH, Rudaz S (2014) Toxicol Lett 230:234
13. Pelclova D, Urban P, Fenclova Z, Vlckova S, Ridzon P, Kupka K, Meckova Z, Bezdicek O, Navratil T, Rosmus J, Zakharov S (2018) Basic Clin Pharmacol Toxicol 122:271
14. Neuberger M, Rappe C, Bergek S, Cai H, Hansson M, Jager R, Kundi M, Lim CK, Wingfors H, Smith AG (1999) Environ Res 81:206
15. Pelclova D, Fenclova Z, Preiss J, Prochazka B, Spacil J, Dubska Z, Okrouhlik B, Lukas E, Urban P (2002) Int Arch Occup Environ Health 75(Suppl):S60
16. Pelclova D, Prazny M, Skrha J, Fenclova Z, Kalousova M, Urban P, Navratil T, Senholdova Z, Smerhovsky Z (2007) Hum Exp Toxicol 26:705
17. Urban P, Pelclova D, Lukas E, Kupka K, Preiss J, Fenclova Z, Smerhovsky Z (2007) Eur J Neurol 14:213
18. Pelclova D, Fenclova Z, Urban P, Ridzon P, Preiss J, Kupka K, Malik J, Dubska Z, Navratil T (2009) Neuro Endocrinol Lett 30:219
19. Sorg O (2014) Toxicol Lett 230:225
20. Peters-Golden M (2008) Curr Allergy Asthma Rep 8:367
21. Pelclova D, Fenclova Z, Vlckova S, Lebedova J, Syslova K, Pecha O, Belacek J, Navratil T, Kuzma M, Kacer P (2012) Ind Health 50:299
22. Pelclova D, Fenclova Z, Vlckova S, Klusackova P, Lebedova J, Syslova K, Belacek J, Kuzma M, Navratil T, Zakharov S, Kacer P (2014) Int J Occup Med Environ Health 27:206
23. Antus B (2016) Oxid Med Cell Longev 2016:2930434
24. Pelclova D, Fenclova Z, Kacer P, Kuzma M, Navratil T, Lebedova J (2008) Ind Health 46:484
25. Pelclova D, Fenclova Z, Kacer P, Navratil T, Kuzma M, Lebedova J, Klusackova P (2007) Ind Health 45:766
26. Chandrasekharan JA, Sharma-Walia N (2015) J Inflammation Res 8:181
27. Duvall MG, Bruggemann TR, Levy BD (2017) Mol Aspects Med 58:44
28. Pelclova D, Navratil T, Fenclova Z, Vlckova S, Kupka K, Urban P, Ridzon P, Zikan V, Landova L, Syslova K, Kuzma M, Kacer P (2011) Neuro Endocrinol Lett 32:71
29. Van den Berg M, Birnbaum LS, Denison M, De Vito M, Farland W, Feeley M, Fiedler H, Hakansson H, Hanberg A, Haws L, Rose M, Safe S, Schrenk D, Tohyama C, Tritscher A, Tuomisto J, Tysklind M, Walker N, Peterson RE (2006) Toxicol Sci 93:223
30. Cerna M, Bajgar L, Crhova S, Grabic R, Kozak J, Kratenova J, Maly M, Volf M, Smid J, Zejglicova K (2004) Organohalogen Compd 66:3637
31. Toren K, Murgia N, Schioler L, Bake B, Olin AC (2017) BMC Pulm Med 17:118
32. Syslova K, Bohmova A, Demirbag E, Simkova K, Kuzma M, Pelclova D, Sedlak V, Cap P, Martasek P, Kacer P (2013) J Pharm Biomed Anal 81–82:108
33. Pelcl T, Skrha J, Prazny M, Vlckova S, Fenclova Z, Navratil T, Malik J, Diblik P, Zikan V, Pelclova D (2018) Basic Clin Pharmacol Toxicol. https://doi.org/10.1111/bcpt.13013
34. Syslova K, Bohmova A, Mikoska M, Kuzma M, Pelclova D, Kacer P (2014) Oxid Med Cell Longev 562860
35. Go YM, Jones DP (2017) Clin Sci 131:1669
36. Mastalerz-Migas A, Reksa D, Pokorski M, Steciwko A, Muszynska A, Bunio A, Drobnik J, Pokorna-Kalwak D (2007) J Physiol Pharmacol 58:363
37. Ahmadzai H, Huang SY, Hettiarachchi R, Lin JL, Thomas PS, Zhang Q (2013) Clin Chem Lab Med 51:1343
38. Ho E, Galougahi KK, Liu CC, Bhindi R, Figtree GA (2013) Redox Biol 1:483
39. Swardfager W, Yu D, Scola G, Cogo-Moreira H, Chan P, Zou Y, Herrmann N, Lanctot KL, Ramirez J, Gao FQ, Masellis M, Swartz RH, Sahlas DJ, Chan PC, Ojeda-Lopez C, Milan-Tomas A, Pettersen JA, Andreazza AC, Black SE (2017) Neurobiol Aging 59:91
40. Saeki K, Yokomizo T (2017) Semin Immunol 33:30
41. Gelfand EW (2017) Semin Immunol 33:44
42. Yokomizo T (2015) J Biochem (Tokyo) 157:65
43. Sun M, Wang R, Han QH (2017) Scientific Rep 7:44352
44. Markar SR, Wiggins T, Kumar S, Hanna GB (2015) J Clin Gastroenterol 49:1
45. Takeda T, Komiya Y, Koga T, Ishida T, Ishii Y, Kikuta Y, Nakaya M, Kurose H, Yokomizo T, Shimizu T, Uchi H, Furue M, Yamada H (2017) J Biol Chem 292:10586
46. Seok SH, Lee W, Jiang L, Molugu K, Zheng AP, Li YT, Park S, Bradfield CA, Xing YN (2017) Proc Natl Acad Sci USA 114:5431
47. Horvath I, Barnes PJ, Loukides S, Sterk PJ, Hogman M, Olin AC, Amann A, Antus B, Baraldi E, Bikov A, Boots AW, Bos LD, Brinkman P, Bucca C, Carpagnano GE, Corradi M, Cristescu S, de Jongste JC, Dinh-Xuan AT, Dompeling E, Fens N, Fowler S,

Hohlfeld JM, Holz O, Jobsis Q, Van De Kant KV, Knobel HH, Kostikas K, Lehtimaki L, Lundberg J, Montuschi P, Van Muylem A, Pennazza G, Reinhold P, Ricciardolo FLM, Rosias P, Santonico M, van der Schee MP, van Schooten FJ, Spanevello A, Tonia T, Vink TJ (2017) Eur Resp J 49:1600965

48. Syslova K, Kacer P, Kuzma M, Pankracova A, Fenclova Z, Vlckova S, Lebedova J, Pelclova D (2010) J Breath Res 4:017104

49. Neprasova M, Maixnerova D, Novak J, Reily C, Julian BA, Boron J, Novotny P, Suchanek M, Tesar V, Kacer P (2016) Dis Markers 2016:3650909

50. Syslova K, Kacer P, Vilhanova B, Kuzma M, Lipovova P, Fenclova Z, Lebedova J, Pelclova D (2011) J Chromatogr B 879:2220

51. Effros RM, Peterson B, Casaburi R, Su J, Dunning M, Torday J, Biller J, Shaker R (2005) J Appl Physiol 99:1286

52. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, Crapo R, Enright P, van der Grinten CPM, Gustafsson P, Jensen R, Johnson DC, MacIntyre N, McKay R, Navajas D, Pedersen OF, Pellegrino R, Viegi G, Wanger J (2005) Eur Resp J 26:319

53. Dweik RA, Boggs PB, Erzurum SC, Irvin CG, Leigh MW, Lundberg JO, Olin AC, Plummer AL, Taylor DR (2011) Am J Respir Crit Care Med 184:602

54. American Thoracic Society and European Respiratory Society (2005) Am J Respir Crit Care Med 171:912

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